THE EFFECT OF ANTIBIOTICS IN MILK: A REVIEW*

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The advances made in the use of antibiotics during recent years have done much to aid the farmer in controlling and curing mastitis in dairy herds. Such antibiotics as penicillin, aureomycin, streptomycin, chloromycetin, bacitracin, tyrothricin, and subtilin have been used for the treatment of mastitic cows. Today, penicillin probably is used more widely than the other antibiotics for this purpose. Some investigators have reported using as high as 100,000 to 400,000 Oxford units of penicillin for each infected quarter. In other cases, where the infection has been quite serious, whole herds have been treated in the "flash method."

In a number of cases the antibiotics have been found in sufficient quantities in mixed herd milk to cause arrested acid development of lactic cultures when such milk is used for cheese or other cultured dairy products. The presence of antibiotics has resulted either in the inability to make the product or in a greatly lowered product quality due to abnormalities. The mode of action of antibiotics on the sensitive bacterial cells is not clearly known; however, several investigators have summarized evidence suggesting that the action is essentially an intracellular one.

Although the presence of antibiotics in milk has been associated primarily with treatments of udder infections, Foley and Byrne suggested that penicillin or other antibiotics should not be used as a substitute for cleanliness or accepted sanitary practice.

Whitehead, although he had not actually observed this type of difficulty, warned patrons that milk from mastitic cows under treatment with penicillin should not be sent to a cheese factory during the treatment, since penicillin has an effect upon starter action in the factory. Another early warning appeared in a German trade journal, Anonymous. Beale warned producers to withhold milk from antibiotic treated cows for 48 hours after treatment. Others who have warned producers against antibiotic-treated milk are Anonymous, Donn, Hansen et al., Jørgensen, Krienke, Petersen, and Yahraes.

AMOUNT OF ANTIBIOTICS FOUND IN MILK FROM TREATED UDDER QUARTERS

Numerous workers have dealt with the treatment of bovine mastitis by antibiotics. Many have determined the amount of antibiotic remaining in the milk after treatment. A few of these findings are presented here.

Schalm and Casselberry injected 20,000 units of penicillin per quarter into udders of cows averaging 30–35 pounds and 48–68 pounds of milk per day. At the end of 12 hours the milk from the two groups averaged 14 units and 5 units per ml respectively. When 100,000 units were injected into eight "dry" quarters, there were from 5.5 to 29 units per ml of milk 24 hours afterwards. Stevenson found 14 units of penicillin per ml of milk 24 hours after treatment with 25,000 Oxford units. Murnane found 0.25 unit of penicillin per ml 24 hours after treatment with 25,000 Oxford units. Hunter estimated that approximately 5 units of penicillin per ml was the concentration of penicillin in milk 12 hours after treatment with 25,000 Oxford units. Weirather and others injected 30,000 units per quarter and found 0.5 unit per ml after 24 hours. Packer found that penicillin is evenly distributed throughout the quarter after injection. The average penicillin content of foremilk in 15 quarters was 26.4 units per ml, and of strippings 21.7 units per ml. He also reported that the amounts of penicillin remaining in the udder 24 hours after injection of 25,000 or 200,000 units were similar. Watts and McLeod determined that 0.04 unit of penicillin per ml inhibited 56 strains of Streptococcus agalactiae and 4 strains of Streptococcus aureus.

McCullock et al. reported that the normal aureomycin treatment for mastitis was from 100 to 200 mg per infected quarter. They found that 48 hours after treatment, the milk contained from 0.64 to 5.1 µg per ml of milk. After 72 hours (six milkings), enough aureomycin was present to inhibit S. agalactiae.

Jackson and Bryan found that the vehicle used in penicillin treatments helps determine the time it may take to reduce the high milk levels of penicillin in the udder. They found that procaine penicillin in oil with aluminum monostearate maintained the highest milk level of penicillin for the longest period of time. If the penicillin was injected in a water solution, the penicillin level was reduced in 24 to 36 hours to a level ineffective against the mastitis streptococci; if in oil, approximately 36 hours were required and when in an ointment base, approximately 48 hours. The total number of Oxford units injected into the infected quarter also influences the time to reduce the penicillin level in the milk. The larger the volume of water used in the treatment, the sooner the penicillin content was reduced to a non-inhibiting level. Cows at the beginning of their lactation periods, because of their higher production levels, excreted the penicillin quicker than the cows in the middle or at the end of their lactation periods.

Foley et al. found that a penicillin vehicle containing water, mineral oil,
lanolin, propylene glycol and a non-ionic wetting agent maintained approximately 5 units of penicillin per ml of milk, even after 72 hours (six milkings), when 100,000 units of penicillin were used as the treatment.

**Recommendations for Handling Milk from Treated Quarters**

Hunter calculated that it would take from 45 to 171 treated quarters to provide a critical level of 0.10 unit of penicillin per ml in a 900-gallon cheese vat. Hunter determined also that this critical level could be reached when 90 gallons of milk containing 5 units of penicillin per ml is diluted up to 4,500 gallons with penicillin-free milk. He estimated that approximately 30 treated cows or 120 treated quarters would be necessary to produce the 90 gallons. These 30 treated cows would constitute about 2 percent of the cows on a milk shed. Johnson and Bryan found that approximately 22 percent of the cows had mastitis; hence, it would be possible to have 2 percent of the animals on a milk shed being treated simultaneously for mastitis.

Krienke and Bryan pointed out that the milk of one average cow, which had been treated in all quarters, would provide a critical level of 0.10 unit per ml when diluted with the milk from 80 non-treated cows. Katznelson and Hood calculated that if the milk of one treated cow, 12 hours after infusion, is pooled with the milk of ten non-treated cows, the resulting milk would contain approximately 1.4 units of penicillin per ml of milk. Petersen recommended that the first milking after treatment, with a "strong injection" of penicillin should be diluted over 200 times before using the milk for cheesemaking.

Hunter, Jørgensen, Krienke and Petersen recommended that the milk from treated cows should be discarded for the first two or three milkings after treatment. Whitehead and Doan recommended that the milk from treated cows be withhold from the market for three milkings. Ruehe recommended that the milk be withheld from the market the first 24 hours after treatment. Of somewhat different opinion were Hansen et al., who found that it took from five to six milkings before the milk was back to normal, as determined by its ability to support the growth of lactic acid bacteria, after an injection with 100,000 units of penicillin in a physiological salt solution. They found there was no diffusion of the antibiotics from one quarter of the udder to another; hence, they recommended that the milk from only the treated quarters should be discarded. Kastli also recommended longer withholding periods. He thought milk should be withheld from the market for 4 days before it was used for manufacturing purposes.

**Action of Antibiotics on Lactic Dairy Cultures**

Probably the first worker to deal with the effect of penicillin on Streptococcus lactis was Bornstein. He found that of 27 strains of enterococci and 6 strains of *S. lactis*, all were resistant to 8 units of penicillin in 1 percent glucose broth. These units were not Oxford units.

Kastli first reported that milk from a penicillin treated quarter would impair the manufacture of butter and cheese. He found that from 0.1 to 1 unit penicillin per ml was sufficient to inhibit the growth of *Streptococcus cremoris* and *S. lactis*.

In his early work, Hunter investigated the effect of penicillin on the sensitivity of 10 single strains of stock cultures of *S. cremoris* and *S. lactis*. All of his trials used 1 percent starter, and the production of acid (calculated as lactic acid) was observed in sterile milk containing various amounts of penicillin. Then strains of *S. lactis* and *S. cremoris* were tested and all showed decreasing acid production with increasing amounts of penicillin. As a group, the *S. cremoris* strains were less resistant than the *S. lactis* strains. Strain K of the *S. cremoris* group was affected by an amount of 0.025 unit of penicillin per ml of milk. The other strains were seriously affected by either 0.10 or 0.15 unit per ml of milk. Strain ML-1 of the *S. lactis* group, was affected by 0.10 unit of penicillin, while the others were not affected until the penicillin concentration reached 0.25 to 0.30 unit per ml. Hunter found that when the concentration of penicillin approached full inhibiting strength, the cocci gave place to elongated rods, apparently because of difficulty experienced in the division and splitting off of the daughter cells, a phenomenon also observed by Trebath. The power of *S. cremoris* strains to reduce methylene blue in milk containing 0.15 unit of penicillin per ml was inhibited to a marked degree. The *S. lactis* strains were not affected in this manner by 0.3 unit per ml. Trebath found that one of two *S. lactis* starters were partially inhibited by 0.11 unit penicillin per ml and the other by 0.32 unit penicillin per ml but not by 0.16 unit per ml. Scott working with an active multi-strain commercial starter, found approximately the same acidities after various incubation periods when the milk contained from 0.02 to 0.08 unit penicillin per ml, although the acidities were slightly lower than the control.

Later the same year, Hunter added known amounts of penicillin to lots of raw milk and, after pasteurization and cooling, made cheddar cheese in 500 lb lots. The cheese was made according to the steps in acidity. His results show that 0.15 unit of penicillin per ml of milk stopped acid production of starter IP at 0.64 percent. When concentrations of penicillin ranging from 0.07 to 0.13 unit per ml were added, the time to make the cheese was extended by 5 to 55 minutes. When starter K was used, only 0.07 unit per ml was necessary to stop the growth of the organisms at 0.38 percent titratable acidity. It also was shown that a low concentration of penicillin will cause slow acid formation with most starters used for cheddar cheese manufacture. The cheese made from vats which were 30 to 40 minutes slow were weak in body and possessed off-flavors. The defects of the final cheese could be correlated with the adverse effect of penicillin on the rate and extent of acid development by the starter cultures in the vat during the manufacture.

Hood and Katznelson worked with mixed and single strains of starter cultures and found partial inhibition of acid production with the mixed strain with 0.5 unit of penicillin per ml and complete inhibition by 50 units. The single strain culture was partially inhibited by 1 unit per ml and completely by 50 units. In their trials a 2 or 3 percent inoculum was used. Katznelson and Hood studied the action of different antibiotics on lactic streptococcus starter cultures used
in cheddar cheese making. Penicillin was the most active of six antibiotics, with aureomycin and subtilin equal in regard to dilutions giving complete inhibition of growth. However, both penicillin and subtilin caused inhibition over a wider range of concentrations than did bacitracin, aureomycin, cloramphenicol, and bacitracin. They also found that 45 strains of S. lactis all were completely inhibited by 0.2 to 0.4 unit of penicillin per ml of skim milk. Complete inhibition was obtained with 0.5 unit of penicillin per ml with six of the starter (mixed) cultures.

Krienke studied the effects of penicillin on buttermilk starters. When milk contained as little as 0.10 unit of penicillin per ml, the acidity developed slowly with incubation at 68-70°F for 16 hours. When the entire milking (8.6 lb) of one cow was taken 12 hours after one quarter had been treated with 75,000 units of penicillin and a portion of this milk diluted with 95 percent milk not treated with penicillin, the resulting titratable acidity was reduced, compared to a control containing untreated milk.

Hansen et al. encountered occasional trouble in cheddar cheese plants, and traced this to antibiotics. Their study was made to determine the amount of milk from treated quarters required to prevent the growth of lactic acid bacteria when mixed with drug-free milk. They also were interested in the effect on the cream used for cultured butter and the milk for powder, when the milk contained drugs used for the treatment of mastitis. They used penicillin, streptomycin, and aureomycin, as well as two sulfa drugs. Eight different lactic starters were used in the experiments. The results showed that when 100,000 units of penicillin were injected into a quarter, 1 percent of milk from that quarter mixed with drug-free milk, would show restrictive action to cultures after three milkings. When 1 gm of streptomycin was injected into the quarter, 1 percent of the treated milk inhibited the cultures after six milkings. When 200 mg of aureomycin were injected, 1 percent of the treated milk inhibited the cultures for the first two milkings. All milk was pasteurized and 2 percent starter was used. The results also showed that penicillin and streptomycin interfered with the acidity development of cream, when 1 percent of the treated cream was added to drug-free cream. When the same two antibiotics were added to skim milk, pre-heated, condensed and spray dried, the reconstituted milk restricted the growth of lactic bacteria.

Kastli reported that from 0.1 to 1 unit penicillin per ml was sufficient to inhibit the growth of Lactobacillus lactis, and Lactobacillus helveticus. A concentration of 0.1 unit penicillin completely inhibited Streptococcus thermophilus. Hargrove et al. reported inhibition of Swiss cheese starters by penicillin and streptomycin in the milk supply. They found that S. thermophilus was inhibited markedly by 0.01 unit penicillin or 5 µg streptomycin per ml, and Lactobacillus bulgaricus and Propionibacterium shermani by 0.1 unit penicillin or 5 µg streptomycin per ml.

Katenelson and Blood found one Lactobacillus casei culture to be completely inhibited by 1.6 units penicillin per ml. Contrary to this report was the work of Petersen who found that Leuconostoc sp. were especially sensitive to penicillin. The penicillin impaired the development of flavor and aroma in the cream used for butter.

Herrler noted that aureomycin and penicillin were more active against Gram-positive organisms than were streptomycin and chloramphenicol. Aureomycin also was active against Gram-negative organisms, and thus could be used against both types of organisms with a great deal of success in the treatment of mastitic cows. This is especially true when the causative organisms are resistant to penicillin. While organisms will build up a resistance to penicillin, no evidence has been shown that bacteria will develop resistance to aureomycin.

Krienke studied the effect of aureomycin in two ways: (a) when added to the milk and then pasteurized, and (b) when five cows had been treated with aureomycin in an ointment base. Milk containing 0.0005 mg of aureomycin per ml inhibited the acid production of cheese starters, while 0.0005 mg of aureomycin did not. Inocula of 1 and 3 percent starter were used. The milk of three of the five cows treated contained enough aureomycin in 12 milkings after treatment (2X per day) to retard acid production. When mixed with 90 percent aureomycin-free milk the acid production was inhibited until the sixth milking.

On the basis of his work, Krienke recommended that careful consideration be given to the acceptance of milk from aureomycin-treated cows. Johnston found that most cultures were inhibited by 0.05 µg of aureomycin. However, he had found some resistance strains that could be used for cheese starters. When mastitic cows were treated with aureomycin, he found large enough quantities of the antibiotic 4 days after treatment to inhibit starter activity.

Hansen et al. found that aureomycin did not inhibit eight lactic acid cultures when 1 percent milk from a treated quarter was mixed with drug-free milk, separated, the skim milk powdered and reconstituted and the cream made into cultured butter.

A comparison of the effectiveness of four antibiotics against bovine mastitis has been made by Benson. He found that penicillin was more satisfactory for treatment of mastitis than tyrothricin, bacitracin, and streptomycin. Streptomycin was more effective than penicillin against Escherichia coli. In his experiments he used 20 to 40 ml of the tyrothricin; however, he found that the milk from the treated quarters had an increased viscosity. When the bacitracin was used, about one-tenth the number of units of penicillin was used. In the treatment of the E. coli infection, he used 500,000 units of streptomycin.

Tests for Antibiotics in Milk

If antibiotics are going to be used for treatment of udder infections, they will be found in the milk supply at a critical level from time to time. Several assay methods are available; however, these take considerable equipment and time and frequently do not detect accurately the small amounts that would provide a critical level in dairy plants.

Krienke, Ruehe and Stolz and Hankinson have outlined tests for detecting the presence of antibiotics in raw milk. Krienke and Ruehe both added an active starter culture to previously-heated raw milk samples. Krienke used 3 percent starter, while Ruehe used only 1 percent. Ruehe incubated the samples 10 hours and noted the coagulation of the samples, while Krienke tested the...
titratable acidity at the end of 2, 3, and 4 hours. Both incubate the samples at 98° F. Stoltz and Hankinson 39 have modified the Scharer field test for phosphatase activity. The test is based on the fact that the activity of the phosphatase enzyme is retarded in the presence of antibiotics. They show that as little as 0.01 unit of penicillin, 0.01 mg streptomycin, 0.01 mg aureomycin, and 0.01 mg of tyrothrin can be detected by use of this test. A gray color indicates complete phosphatase inactivation, while a green color indicates only partial inactivation. None of these tests indicate the amount or kind of antibiotic present in the milk.

One of the greatest needs at the present time is an accurate, simple, and rapid test for antibiotics in milk.

**Effect of Antibiotics on Reductase or Quality Tests**

Many plants use a reductase test as an indication of the relative numbers of organisms in raw milk; however, if a critical level of antibiotic is present, difficulty may be experienced in interpreting the results of these tests. Hunter 18 and Johns and Katznelson 19 have studied the effect of penicillin on dye reduction tests for milk quality. Hunter worked with methylene blue, while Johns and Katznelson were working with resazurin. Both investigators found that presence of antibiotics may permit acceptance of poor quality milk as high quality milk when tested with either the resazurin or methylene blue tests. Hunter concluded that the presence of penicillin or other antibiotics sometimes gives an improvement in the quality of the milk by inhibiting contaminating organisms and thus may have a variable effect in the reductase test for milk quality.

**Penicillin Added to Pasteurized Milk**

Two workers have determined the effect of adding penicillin to raw milk before pasteurization. Foley and Byrne 7 added penicillin to raw milk at the rate of 3 units per ml, pasteurized the milk, and noted the effect on growth of organisms. There seemed to be no effect with or without the penicillin when the milk was held at 7° C. The penicillin inhibited growth of the Gram-positive organisms in the milk held at 27° C for about 24 hours. Pasteurization efficiency was increased by the addition of penicillin. However, on high grade milk there seemed to be no advantage to its addition. Krienke and Pouvez 3 added 0.25 unit per ml to raw milk and pasteurized it. The addition of the penicillin kept the growth of *S. lactis* down for 10 days at refrigeration temperatures. They also carried out studies on evaporated milk, condensed whole milk, condensed skim milk, and non-fat dry-milk-solids. "When penicillin was added at the rate of 1 unit per ml before pasteurization or the heat treatment, only the processing of the evaporated milk gave any reduction in antibiotic activity.

**Stability to Heat Treatment of Antibiotics in Milk**

Foster and Woodruff 8 stated that pasteurization of penicillin in liquid broth at 60° C for 30 minutes resulted in a definite, though variable, deterioration in activity ranging from 5 to 30 percent. Watts and McLeod 41 found that penicillin activity in raw, full-cream milk and sterilized skim milk heated to 100° C for 30 minutes was reduced 50 percent; holding these penicillin solutions for 60 minutes resulted in 75 percent reduction of activity. No destruction was found when the milk-penicillin solutions were heated 15 minutes. Hunter 19 found that when two lots of milk containing 1.3 and 4.5 units of penicillin per ml were steamed for 15 minutes, 73 percent decrease in the activity resulted. It appeared to him that the penicillin was more heat stable in the presence of milk than in an aqueous solution. However, he did find that steaming for one hour resulted in approximately 50 percent reduction. Hood and Katznelson 12 also found no inactivation of penicillin by pasteurization temperatures. Krienke 34, 35 found that neither penicillin nor aureomycin was inactivated by heating the milk to 143° F for 30 minutes. The penicillin was not inactivated by heating in milk to 190° F for 60 minutes or when autoclaved at 15 pounds pressure for 15 minutes. Doan 9 stated that pasteurization temperatures did not inactivate antibiotics in milk nor did oxidizing and reducing agents and surface active agents. Autoclaving the milk has only a slight effect in reducing the potency. Scott 37 found that autoclaving milk for 30 minutes at 15 pounds pressure, with penicillin levels from 0.025 to 1 unit per ml almost entirely destroyed the antibiotic activity. When the penicillin levels were increased to 5 and 10 units per ml, no acid development was noticed in the heat-treated milk after 4 days' incubation at 75° F.

Trembath 40 reported that the popular belief in Australia was that the heat treatment given the milk by cheese factories largely inactivated the residual penicillin that would be present in the milk supply. In testing two mixed starter cultures, he found that autoclaving the milk for 10 minutes at 115° C destroyed the potency of the penicillin; however, heating the milk at 155° F for 10 minutes, 185° F for 10 minutes, or 190° F for 30 minutes, did not materially affect the potency of the penicillin.

Hansen et al. 10 found no decrease in activity of penicillin or streptomycin when 1 percent of antibiotic-treated milk was mixed with drug-free milk, preheated to 175° F, condensed to 35 percent total solids, spray dried, and then reconstituted and pasteurized at 195° F for 30 minutes. Aureomycin, on the other hand, did not inhibit the growth of the lactic acid cultures under these conditions.

**Means of Overcoming Difficulties in Milk**

**Increased Inoculum**

It can be concluded from the above information that pasteurization temperatures and times are not effective in inactivating the antibiotics. Therefore other means must be used to reduce the difficulties resulting from the antibiotics. Maas and Johnson 22, 23 reported that 0.8 unit of penicillin was firmly bound to each milliliter of *Staphylococcus aureus* cells collected by centrifugation, or that 750 moles of penicillin were specifically absorbed by each bacterial cell and could not be washed out but remained tightly bound during subsequent multiplication of the cells in a penicillin-free medium. Radioactive penicillin was used in the latter experiment. Krienke 36 concurred with Maas and Johnson. Thus the bacterial cells for the initial inoculum will have absorbed
practically all of the antibiotic and are so weakened that acid production does not progress at a normal rate. When a second inoculation is added, the penicillin is tightly bound to the original inoculum and will not act upon the new cells; consequently they can function normally. It should be pointed out that this is only practical at low concentrations of antibiotics and will not yield a product of quality equal to that resulting from antibiotic-free milk.

Katznelson and Hood reported the use of 3 percent starter to overcome the effect of the penicillin. Ruehe recommended that cheese factories suspected in their experiments and found this to be effective in inactivating penicillin. Lawrence studied the effect of 18 enzyme systems upon penicillin and found two, taka-diastase and clarase, to inactivate solutions of sodium penicillin. Both of these enzymes are derived from Aspergillus oryzae.

**Summary**

Antibiotics used in the treatment of mastitic cows will be found in the milk coming to the dairy plants and may likely be the cause of slow acid production by streptococcus starters. On the other hand, all cases of slow acid production should not be considered due to antibiotics.

The amount of antibiotics present in the milk from animals treated for mastitis will depend on the number of quarters treated, the number of cows in the herd, the number of units or milligrams of antibiotic used in the treatment, the vehicle carrying the antibiotic, the time elapsing after treatment, the level of the production and many others. In general, if farmers would not send the milk to market from treated cows for 24 to 48 hours much of the trouble of the dairy plant operators would be solved. If the farmers would mark cans that contain milk from treated cows, that would help in control of this difficulty.

As low as 0.07 unit of penicillin or 0.0005 mg of aureomycin per ml of milk may be detrimental to the most sensitive lactic streptococci. A concentration of 5 μg of streptomycin or 0.01 to 0.1 unit of penicillin will markedly affect the acid production of Swiss cheese starters. Other antibiotics have not been fully tested against lactic starter cultures.

There seems to be agreement that the normal pasteurization temperatures employed will not inactivate the antibiotics. Autoclaving and prolonged steaming of milk have a slight effect on penicillin and an appreciable effect on streptomycin.

The use of a heavy inoculum seems to be one method of overcoming to some degree the action of small amounts of antibiotics in milk supplies. Development of cultures somewhat resistant to penicillin, aureomycin, and streptomycin has been reported. Penicillinase has been reported to inactivate penicillin but the cost has been reported by two investigators as being prohibitive. Another factor is that use of this enzyme would have to be permitted by the Federal Food and Drug Administration.

A test is available which has been reported to detect the presence of antibiotics in the milk supply rather quickly; however, it does not determine the type of antibiotic. Two other tests have also been outlined and have taken from 4 to 10 hours to complete.
The Efficacy of the Microscopic Examination of the Incubated Producer Milk Samples in Detecting Streptococci Mastitis in Dairy Herds. J. Milk Technol., 10, 197-201 (1946).

Lye Solution for Rubber Parts

(Continued from page 154)

8. Less fat is absorbed by the rubber inflations than when other recommended compounds are used.11
9. The slipperiness of the solution affords a simple method of determining that it is up to strength.
10. It is much more effective than chlorine compounds in the destruction of coliform organisms.
11. Periodical boiling in a 2 percent solution is extremely effective in the control of thermocic organisms,11 while also prolonging the life of the rubber parts.
12. Lye does not injure the metal parts (except aluminum or aluminum alloys).
13. Even a 5-minute treatment with lye solution is sufficient to maintain surfaces in good sanitary condition.

CONCLUSION

Lye solution maintained milking machine rubber parts in a satisfactory sanitary condition even when the residual milk was permitted to dry on the surfaces.

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REFERENCES