ANTIBIOTICS IN MILK – A REVIEW* 

I. RECENT DEVELOPMENTS

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INTRODUCTION

The presence of antibiotic residues in milk and milk products continues to concern people in the dairy industry, and in regulatory agencies. Consumers have become aware of the problem through the popular press and, in a few instances, through personal experience of an allergic reaction after consuming contaminated dairy products. A previous review by Marth and Ellickson (108) discussed the presence of antibiotics in milk and milk products. Another review by the same authors (109) considered problems which were created by contamination of milk and milk products with antibiotics. The present series of papers summarizes information on (a) recent developments in regard to antibiotic contamination of milk, and (b) methods for detecting antibiotics in milk and milk products.

Recently published information on various aspects associated with antibiotic contamination of milk is summarized in this section of the present review. The reader who desires a summary of earlier information is referred to previous reviews (108, 109).

SURVEYS OF MILK SUPPLIES FOR ANTIBIOTIC CONTAMINATION

A recent limited survey was made by Martin (111) of penicillin contamination in raw milks produced in Elkhart County, Indiana. Eleven out of 105 samples tested were found to contain penicillin. Most contaminated samples had about 30 units of penicillin per ml. of milk although two contained as much as 300 units per ml.

The Food and Drug Administration, in 1959, conducted its fourth nationwide survey of antibiotic contamination in milk (77, 121). Raw milks (1,170 samples) were taken from tank trucks after milk pick-up at dairy farms in 16 areas of the U. S. and tested for antibiotic residues. Penicillin, in the range of 0.006 to 1.22 units per ml., was found in 3.7% of the samples. Streptomycin (1.40 µg. per ml.) was detected in one of the samples which also contained penicillin. Tetracyclines were noted in 22 samples at a concentration of 0.006 to 0.465 µg. per ml. One sample contained 0.155 unit per ml. of bacitracin.

Three surveys on incidence of penicillin in milk supplies of New South Wales, Australia were conducted in 1954, 1957 and 1958 (145). The 1954 survey showed penicillin (range: <0.2 to 0.32 unit per ml.) present in 69 out of 242 samples. Eight out of 470 milk samples in the 1957 and 1958 surveys contained from 0.05 to 2.25 units of penicillin per ml.

TRENDS IN TYPES OF MASTITIS

Antibiotic therapy has resulted in a shift from streptococci to staphylococci as major etiological agents of mastitis. Other forms of mastitis (bacterial, fungal) also have been recently reported, with some frequency.

Streptococcal Mastitis

Although Streptococcus agalactiae still causes some mastitis, one survey showed that its incidence dropped from 34 to 10.5% during the period of 1949 to 1957 (88). Other work (115) showed similar results.

A recent study in Australia (120) indicated S. agalactiae could not be completely eliminated from udders of well-managed dairy cattle through use of penicillin. Reinfection from the environment was blamed for the failure.

Research by Das (38) showed that penicillin-resistant streptococci caused mastitis among cows in two different herds. These streptococci were more resistant to the antibiotic than staphylococci isolated from other mastitic cows.

S. pneumoniae has been found to cause mastitis (105, 147). Some cases responded to penicillin (177) but well established infections did not (105). Mastitis was experimentally produced by inoculating udders of healthy cows (via the teat canal) with broth cultures of S. pneumoniae (147).
Staphylococcal Mastitis

The decrease, in recent years, of mastitis caused by \textit{S. agalactiae} has been accompanied by an increase in staphylococcal mastitis. McCoy (115) reported that by 1954 most cases of mastitis were caused by staphylococci. She estimated around 70% of dairy herds to be carrying such infections which, in some instances, could not be eliminated by treatment. A recent three-year survey of more than 2,000 cows around Madison, Wisconsin showed: (a) one out of four cows was shedding staphylococci from at least one quarter, (b) there was 20 times more mastitis than herd owners recognized, (c) many first-calf heifers were shedding staphylococci; sometimes from all four quarters, (d) no correlation existed between numbers of staphylococci in milk and Standard Plate Count or methylene blue test results.

Research on mastitis in a dairy herd (57), was concerned with 254 cases over a seven year period and showed a reduction in milk production of ten to 28 pounds per day when cows were normally producing about 38 pounds daily. This reduction preceded appearance of clinical symptoms by 24 hours. Bacteriological studies of milks from 15 of the mastitic cows showed \textit{Staphylococcus aureus} was most frequently present.

The incidence of staphylococcal mastitis increased from 20.5 to 48% during the period of 1949 to 1957 according to results reported from investigations of 7,009 clinical cases (88). The frequency of mastitis was highest during the months of July through September.

Wallmark and Thorne (188) isolated 246 strains of coagulase-positive staphylococci from 165 cows distributed among 56 herds. Further work showed 13.4% of the isolates, all of which produced penicillinase, to be resistant to penicillin.

Examination of 208 composite morning milk samples taken at nine weekly intervals from 24 farms revealed that 61% contained coagulase-positive staphylococci (189). Penicillin-resistant, coagulase-positive staphylococci, were recovered from 10% of the samples.

The incidence of penicillin-resistant \textit{Staphylococcus aureus} in herd milks increased from 9 to 37% during the 1954 to 1956 period according to Tee (179). It was suggested that widespread use of penicillin for treatment of bovine mastitis caused emergence of resistant staphylococci.

Penicillin-resistant staphylococci were isolated from mastitic cows on two farms in studies by Das (38). He reported the staphylococci were less resistant to penicillin than streptococci also isolated from mastitic cows.

The increase in staphylococcal mastitis may give rise to food poisoning outbreaks if contaminated milk is improperly handled (115). Recent cases of food poisoning which involved dried milk, Colby and Cheddar cheeses, could have resulted from such contamination (115). Consumption of four to eight month old Cheddar cheese, made from raw milk, caused a recent outbreak (200 cases) of food poisoning (68). Coagulase-positive, beta-hemolytic \textit{Staphylococcus aureus} of similar phage type was isolated from the cheese and milk from two out of eight herds which supplied the factory where toxic cheese was made. A survey (51) of 207 samples of pasteurized dairy products (milk, low-fat milk, chocolate drink, cultured buttermilk, half-and-half, coffee and whipping cream) obtained from 42 plants over a two-month period showed potentially pathogenic staphylococci to be present in some samples of all products tested. Over 3% of all samples checked contained coagulase-positive staphylococci. The bacteriophage pattern of staphylococci isolated from some samples of buttermilk and half-and-half was similar to that of staphylococci involved in hospital outbreaks of enteritis and abscesses.

Coliform Mastitis

Mastitis caused by coliform bacteria has long been recognized. Recent outbreaks have been reported and additional information recorded. \textit{Escherichia coli} caused 25 cases of mastitis in a study by Bellani (20) of 1400 cows. The infection lasted from one to eight days with treatment. Mild cases were successfully treated with streptomycin and mepyramine maleate while severe cases required supplementary treatment with sulfonamides, antihistamines and a glucose solution. Mastitis caused by \textit{E. coli} appeared to be either chronic or acute in nature (138). Histological examinations showed necrotic udder tissue in 22 out of 30 cows with mastitis caused by \textit{E. coli} (48). \textit{Aerobacter aerogenes} caused an outbreak of acute mastitis in a Normandy dairy herd (59). Treatment with streptomycin was successful and good results were also obtained with a vaccine. Chloramphenicol, administered intravenously, was reported successful for treatment of coliform mastitis (130).

\textit{Pseudomonas} Mastitis

Two recent reports (95, 106) indicate that mastitis caused by pseudomonads (\textit{Pseudomonas aeruginosa} and \textit{Ps. pyocyanea}) failed to respond to antibiotic therapy. Another investigation (155) showed milking machines cleaned with chloramine compounds to be constant reservoirs of organisms in herds infected with \textit{Ps. aeruginosa}. 

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Mastitis Caused by Other Bacteria

A variety of bacteria have recently been implicated in outbreaks of mastitis. Organisms reported as responsible include: Bacillus cereus (127), Serratia marcescens (15), Bacteroides fundiformis (170), Corynebacterium pyogenes (52), Leptospira pomona or hyos (69) and Listeria monocytogenes (187).

Mastitis caused by S. marcescens was successfully treated with three doses of neomycin (15). Early mastitis caused by C. pyogenes responded after five to seven days of treatment with a mixture of penicillin, streptomycin, and crystalline trypsin (52). Sometimes treatment of lactating animals for periods up to 12 days was not very successful. B. fundiformis infections resisted treatment with oxytetracycline plus polymyxin-B-sulfate plus penicillin, streptomycin, nitrofurazone, and tyrothricin. It was believed capsular material produced by the organism during growth in udder tissue caused the high level of resistance (170).

Fungal Mastitis

A number of fungi have recently been reported as etiological agents in outbreaks of mastitis. Included are: Candida krusei (13), Candida sp. (35), Candida tropicalis (104, 140), Candida albicans (137), Candida pelliculosa (140), Cryptococcus neoformans (137, 139, 143), Saccharomyces fragilis (137), Hansenula anomala (137), Torulopsis sp. (140), Pichia sp. (140), Nocardia sp. (132), Nocardia asteroides (36, 133) and Nocardia brasiliensis (40). Candida sp. infections were encountered after treatment with penicillin (35, 104). Actidione and nystatin were effective for treatment of mastitis caused by C. neoformans. Infections of N. asteroides were successfully treated with a mixture of novobiocin and nitrofurazone. Penicillin, polymixin, neomycin and streptomycin were ineffective (133). Mastitis caused by N. brasiliensis failed to respond to antibiotic therapy (40). N. asteroides survived milk pasteurization treatments which suggests possible public health problems (133).

Viral Mastitis

No reports were found of naturally occurring viral mastitis. It is possible that such infections have occurred in the past, and have gone undetected. Several recent reports described mastitis which was experimentally produced by means of viruses. Severe mastitis resulted after an emulsion which contained viruses of the psittacosis-lymphogranuloma group was infused into the udder of a cow via the teat canal (12). Results indicated that viruses multiplied in the udder and were excreted with milk. Inflammatory reactions appeared after udders of lactating and non-lactating cows were inoculated with vesicular stomatitis, Newcastle disease or vaccinia viruses (45). Viruses generally stimulated production of neutralizing antibodies.

Mastitis Treatment

Some specific information on treatment of various infections has been discussed previously in this review. General information and new developments will be considered here.

Five different commercial penicillin preparations were administered to cows by intramammary infusion in doses of 100,000 units (169). Six to twelve days had to elapse before penicillin no longer appeared in milk.

Autoradiographic methods were used by Ulberg, et al. (184) to examine diffusion of aqueous solutions and oil suspensions of penicillin in the udder. In healthy udders, penicillin was found primarily in ducts and parenchyma and none or only insignificant amounts in interstitial tissues. Penicillin was also found in ducts and parenchyma of mastitic udders but a great portion had penetrated into interstitial tissues especially where they were acutely inflamed and where fibrous tissue was not deposited.

The use of proteolytic enzymes in conjunction with antibiotics for treatment of mastitis has been investigated by Jordan (83). Streptokinase, streptodornase and crystalline trypsin produced noticeable improvements in some mastitis cases which were unaffected by antibiotics alone. Papain, however, had no noticeable effect.

Neomycin ointments were used to treat 84 cows with coliform, staphylococcal or streptococcal mastitis (44). The treatment gave “poor” results for chronic staphylococcal, “fair” for acute, and “good” for subacute mastitis.

Bakshi (10) prepared a mixture of calcium dioxytetracycline and polymyxin-B-sulfate to treat 20 cases of staphylococcal mastitis (11). Infected udders responded well after treatment for three consecutive days.

Recently it was reported (4) that a staphylococcal-toxoid vaccine has been used successfully to maintain dairy herds relatively free from staphylococcus-induced mastitis. Initially cows received two inoculations spaced one month apart. After that, it is claimed, one vaccination per year maintained immunity.

The use of antibiotics injected intramurally for treatment of mastitis has been suggested (6, 50). Barr, et al. (16) found cases of mastitis caused by antibiotic-resistant staphylococci responded well when the cows were injected intramurally with one gm. of neomycin and one million units of polymyxin-B-sulfate on two consecutive days. Foley (50) reported...
streptococcal mastitis responded to intramuscular injection of prednisone (metiicorten) and penicillin.

**Antibiotics in Milk from Injected Cows**

Limited information was presented in a previous review (108) on the presence of antibiotics in milk after injection of cows for treatment of disease. Additional data have become available and are summarized below.

Penicillin G was injected intravenously or intramuscularly into healthy and mastitic cows at levels of one, three and ten million units. The antibiotic appeared in the milk of healthy and infected cows for 12 to 24 hours after injection according to additional data have become available and are summarized below.

In additional studies 0.056 and 0.044 units of penicillin per ml. of milk were found six and 12 hours after three million units were injected into cows intravenously or intramuscularly (142).

The intramuscular injection into cows of 5,000 units of penicillin G per pound of body weight resulted in a blood-serum level of 0.03 units per ml. after four days and a concentration in milk of 0.005 units per ml. after three days (185). When the same level of penicillin G in oil with two per cent aluminum monostearate was injected, 0.022 units per ml. was in blood serum after six days and .003 units per ml. in milk after five days.

Penicillin G in aqueous solution was injected into cows intramuscularly at a level of 5,000 units per pound of body weight three times at 24 hour intervals. An oil base suspension with added aluminum monostearate was injected once at the same rate (185). The highest level of penicillin in milk from cows treated with the aqueous solution was 0.52 unit per ml. while in milk from cows treated with the oil suspension it was 0.15 unit per ml. Penicillin persisted in milks from cows treated with the aqueous solution for 48 hours after the final injection and for 96 hours when the oil base suspension was used.

Investigations by Hollister, et al. (72) on the concentration of penicillin in blood and milk after intramuscular injection showed: (a) penicillin appeared longer in milk from distinctly atrophied quarters than from normal ones, (b) fibrotic quarters yielded milk with higher levels of penicillin than nonfibrotic ones, (c) udder secretions of dry cows contained no penicillin, and (d) inflammatory processes, interfered with appearance of penicillin in milk, presumably because of disturbances in secretory function.

When chlorotetraacycline was injected intravenously into cows at the rate of 6.6 mg. per kg., demonstrable levels (0.034 µg. per ml.) were found in milk after two days (136).

**Antibiotics Fed to Dairy Cattle**

Dairy cattle may ingest antibiotics orally if they are: (a) present as residues on feed such as silage, (b) administered to control bloat, or (c) fed to control diseases, increase milk production or for other reasons. Such uses of antibiotics will be discussed below.

**Preservation of Silage with Antibiotics**

Bacitracin was suggested by several workers (3, 153, 154, 194) for use in preservation of silage, especially grass silage. In one study (3) legume silage was prepared with 4, 40 or 400 g. of bacitracin per ton. Satisfactory pH levels in silages were attained with the first two levels of bacitracin, but only if ground corn or molasses was added. The pH never dropped to 5.0 in bacitracin-treated silages to which corn or molasses was not added nor in the silages to which corn or molasses and 400 g. of bacitracin per ton were added. The addition of bacitracin had no effect on composition of silage at the end of 90 days.

Silages, in another investigation (153, 154), were prepared with the addition per ton of: (a) 5, 10, or 15 g. of bacitracin, (b) 40 to 80 pounds of molasses, (c) eight pounds of sodium metabisulfite or (d) combinations of bacitracin and molasses. All treated silages had a good odor. When all silages were fed to steers, free-choice, for two weeks, they consumed about twice as much silage preserved with 5 g. of bacitracin per ton as of the others. Similar silages were prepared and fed to dairy cattle (153). No differences were noted in quantity or efficiency of milk production when the different silages were fed. Consumption of untreated silage was highest, followed in order by molasses-, bacitracin-, and bisulfite-treated silages.

Wing and Wilcox (194) treated Pearl millet with bacitracin, chlortetraacycline, oleandomycin, streptomycin, oxytetracycline or penicillin at the time of ensiling. Silages were analyzed and fed to four lactating cows for a period of three days. Consumption of untreated and oleandomycin-treated silages were equal and highest followed in order by penicillin-, bacitracin-, streptomycin-, oxytetracycline- and chlortetraacycline-treated silages. There was slight proteolytic activity in the chlortetracycline-treated silage. Other bacterial activity appeared light in the oleandomycin-treated silage and moderate in all others. Oleandomycin was the only antibiotic which appeared to be present in milks of cows fed the different silages.

Alfalfa silages were prepared over a three year period with several antibiotics at concentrations of one to 200 p.p.m. (39). The initiation of fermentation...
was greatly delayed by antibiotic treatment in 1954 but no differences were noted in 1955 and 1956.

Antibiotics for Bloat Control

Feeding penicillin to grazing cattle has been suggested for prevention of bloat (17, 46, 78, 81, 82, 198). Tetracycline, oxytetracycline, chlortetracycline, bacitracin, streptomycin and penicillin were fed to steers grazing on Ladino clover in attempts to prevent bloat (17). Penicillin was the only antibiotic of those studied which appeared useful. Single doses of 25 to 75 mg. of procaine penicillin or potassium penicillin protected steers from bloat for about two days.

Penicillin was fed with grain at the rate 100 mg. per animal (daily or every third day) to 739 cows over a 150 day period (46). The incidence of bloat was reduced by two-thirds and a slight decrease in milk production was noted.

Three pair of identical twin dairy cows were used to study effects of administering penicillin orally every third day at a rate of 500,000; 100,000; or 200,000 units per dose for 12 days, 15 days or five months, respectively (78). No penicillin was found in milk and there was no adverse effect on health or weight of cattle, milk or butterfat production, composition of milk or butterfat constants. Suggested doses of penicillin up to 500,000 units every third day appeared safe for use in New Zealand to control bloat in dairy cattle.

Two groups of lactating dairy cows were pastured on alfalfa six hours daily for 55 days (81). Cows in one group (23 cows) were fed 62,500 units of penicillin daily while the other group (19 cows) served as a control. Eight treated cows bloated 15 times and 11 of the control cows bloated 27 times. Studies by Wooldridge and Bellings (198) showed the incidence of bloat to be reduced by a daily feed-supplement of 10 mg. of procaine benzyl-penicillin per cow. The less frequent administration of 40, 50 or 100 mg. of penicillin was also less effective.

Milks from cows fed penicillin were tested for residues by Skaggs and Miller (171, 172). They found no penicillin in milk when cows were fed 88.9 mg. per day. When the rate of feeding was increased to 177.8 mg. daily, approximately 0.05 unit of penicillin was present per ml. of milk. The level of antibiotic in milk increased to between 0.10 and 0.15 unit per ml. when 277.8 mg. of penicillin was fed daily.

Steers on alfalfa pasture were protected from bloat for nine days by means of 75 mg. of penicillin fed daily after which its effectiveness diminished rapidly (82). A subsequent increase in dosage to 125 mg.

per day reduced bloat for two additional days, after which incidence again increased sharply.

Antibiotics as Dietary Supplements

The addition of antibiotics to feed of dairy cattle has been suggested for: (a) reduction of bacterial diarrhea, (b) reduction of losses from respiratory infections, and (c) prevention of foot rot (165). Some authors (96, 107, 178) claimed an increase in milk production through feeding antibiotics while others (27, 28, 64, 74, 148, 149, 152) found no difference.

Haq, et al. (64) fed 130 mg. per cow daily of either chlortetracycline or tyrothricin. Numbers of bacteria in milks from treated cows were determined initially and after holding at 35°C, for 12 hours. All milks developed lactic acid and produced a normal curd. It was concluded that antibiotics, as used in this study, probably did not appear in milk or, if they did, were present in concentrations which did not interfere with acid production. Similar results were also reported by Rusoff and Haq (152). Chlortetracycline was fed (240 mg. per heifer daily) to 17 heifers for 15 months prior to freshening and during the first lactation (74). No differences in milk or fat production were observed between controls and heifers which received the antibiotic.

Another investigation was conducted in which chlortetracycline was fed to 60 Jersey and Holstein cows (0.1 mg. per pound of body weight per day) for 16 weeks (148, 149). No effect was noted on milk yield or on incidence of mastitis, bloat, or foot rot. The antibiotic could not be detected in milk from cows to which it was fed.

Eighteen dairy cows daily received 40 g. per 1,000 pounds of body weight of a residual fermentation product (Aurofac-2A) which contained 8 mg. of chlortetracycline per gram (18). Studies continued for 18 weeks showed that cows which received the antibiotic produced an average of 0.15 pounds more fat-corrected milk daily, consumed from 0.2 to 0.4 pounds more alfalfa hay daily, and consumed 0.15 pounds less water daily than controls. These differences were considered not significant. No effect was noted on body weight, rumination, grain and silage consumption, pulse rate, body temperature or general health and well being. It was concluded from results, that neither beneficial or deleterious effects resulted when 300 to 500 mg. of chlortetracycline were fed per cow daily.

Shor, et al. (166) fed chlortetracycline to lactating dairy cattle at levels of 0.1, 0.5 and 1.0 mg. per pound of body weight per day for two weeks. A depressed appetite was noted initially in two of four cows in each of the two groups which received high-
est doses. Appetites returned to normal after a brief adjustment period. Milk production was also reduced during the adjustment period but returned to normal within a few days. No marked clinical effects were noted in the group which received the 0.1 mg. dosage nor could the antibiotic be detected in milk from these cows. Low levels of chlortetracycline were found in milks from cows fed 0.5 mg. (0.01 to 0.21 μg per ml.) and 1.0 mg. (0.03 to 0.23 μg per ml.) doses of antibiotic. Shor (175) also reported that feeding antibiotics to dairy cattle was accompanied by an adjustment in rumen microflora. This difficulty was prevented by gradual introduction of antibiotics into feed.

Two experiments were conducted in which 0.1 mg. of chlortetracycline per pound of body weight was fed daily to dairy cattle for 22 or 29 weeks (96). Milk production was increased by 0.21 pound per cow per day (not significant) through feeding the antibiotic in the first investigation which involved 18 herds and approximately 1,000 cows. Results of the second study, based on only 11 of the 18 herds, showed an increase in milk production of 1.81 pound per cow per day. Bloating appeared to be associated with feeding chlortetracycline since eight per cent of treated cows were affected.

The response of 62 pairs of cows to feeding chlortetracycline (10 mg. per 100 pounds of body weight daily) was investigated by Boyd, et al. (27, 28). Feeding the antibiotic to different groups continued for 12, 17 or 25 weeks. No significant differences were found between treated and control cows in milk production, body weight, resistance to mastitis, foot rot or other bacterial disorders.

Oxytetracycline was fed to 2,370 lactating cows in field trials in six states (107). The dosage ranged from 75 to 100 mg. per cow per day for an average of 186 days. Cows fed the antibiotic produced an average of 0.87 pounds more per day of milk than controls. Feeding oxytetracycline did not affect bacterial content of the milk, growth of cheese cultures in milk or result in its presence in milk. Socini (178) fed oxytetracycline to seven cows and reported increases in total milk yields and in protein and lactose contents of the milk.

The following were listed by Grashuis (61) as disadvantages of feeding antibiotics: (a) resistant pathogenic bacteria may develop, (b) infected animals may not be recognized at time of slaughter, (c) favorable effects on growth and feed utilization diminish, and (d) antibiotics may lose their therapeutic value. It was felt a stimulation in the rate of growth similar to that obtained by use of antibiotics would result from addition to feed of 0.1 per cent copper sulfate.

**Effect of Antibiotics on Rumen Function and Bacteria**

Wiseman, et al. (195) fed daily doses of 50 and 150 mg. penicillin to two fistulated steers for two weeks. Bacteriological analyses showed no pronounced change in numbers of rumen lactobacilli, streptococci or paracolon bacteria while penicillin was administered. This apparent lack of penicillin activity could not be correlated with the appearance of resistant lactobacilli or streptococci. Since paracolon bacteria can inactivate relatively high concentrations of penicillin in vitro, it was thought they may be responsible for its inactivation in the rumen.

The in vitro effects of various antibiotics on cellulolytic rumen bacteria was investigated by Hardie, et al. (65). Little effect was produced by polymyxin-B-sulfate and chloramphenical while bacitracin and dihydrostreptomycin showed slight inhibition. Oxytetracycline and chlortetracycline inhibited bacteria moderately while penicillin did so markedly.

**Effect of Antibiotics on Starter Cultures**

The level of antibiotic in milk which inhibits most starter cultures has been discussed previously (109). Additional information of this type will be presented here. Other recent work to be discussed deals primarily with starter culture resistance and physiology.

The effect of low penicillin concentrations in milk on acid production by different cheese cultures was investigated by Richards (144). It was found that 0.1 unit per ml. inhibited *Streptococcus durans* and *S. thermophilus*; 0.25 unit per ml. inhibited *S. diacetilactis* and *S. cremoris* and 0.5 unit per ml. inhibited
mixtures of S. cremoris plus Leuconostoc sp. and S. diacetylactis plus S. cremoris.

Gelsey and Hagen (56) examined the effect of seven different antibiotics on three different strains of Propionibacterium shermanii and on four strains of propionibacteria isolated from Samsoe cheese. All strains were completely inhibited by the presence per ml. of: 0.6 unit penicillin, 10 to 30 μg. streptomycin, 0.5 to one μg. oxytetracycline, 0.5 to more than one μg. tetracycline, 0.1 to five μg. chlorotetracycline, less than one to 10 μg. erythromycin and less than 0.1 to one μg. chloramphenicol.

Data obtained from making cheese with penicillin-contaminated milk (100, 250, and 500 units per l.) suggested that lactic acid bacteria are less sensitive to penicillin today than ten years ago (76). Secretion of penicillinase or formation of resistant mutants were suggested explanations.

Verlinski (186) reported a commercial starter was made resistant to penicillin over a period of four to five weeks by addition, at each daily transfer, of penicillin in increasing quantities from 0.05 to 0.1 unit per ml. The starter, when fully adapted to the antibiotic and incubated at 28°C., retained normal flavor and developed acid in milk with up to one unit per ml. of penicillin. Attempts were made, in another study, to produce penicillin-resistant cultures (146). A selection procedure for isolation of naturally resistant mutants and a training program for induction of resistance were employed. Cultures were obtained which grew in the presence of 0.5 unit of penicillin per ml. of milk although acid production by these strains was reduced 50 per cent compared to sensitive cultures.

Mikolajcik, et al. (118) found a strain of Streptococcus lactis which was resistant to oxytetracycline, produced low levels of acid and possessed the ability to "bind" the antibiotic. It was possible to obtain normal acid development by sensitive cultures in milks with 1 or 10 p.p.m. oxytetracycline if sufficient resistant cells were added first, given time to "bind" the antibiotic and thereby neutralize its effects.

In other investigations (86) attempts were made to improve acid production of the oxytetracycline-resistant culture through addition of various nutrients to milk. Peptide-rich extracts, when added to milk at the rate of 0.1 to 0.5%, permitted normal acid production by resistant cultures. Effective additives were yeast extract, liver fraction L, neopeptone, trypitone, milk-protein hydrolyzate, casein hydrolyzate, pancreas extract, tryptose and peptonized milk. Sensitive cultures, however, did not produce acid in antibiotic-contaminated milk when the extracts were added.

Differences between cells sensitive and resistant to oxytetracycline were examined by Mikolajcik (117). He found: (a) sensitive cells able to tolerate higher concentrations of sodium chloride and alkaline substances than resistant cells, (b) sensitive cells had a more diffuse cell wall and more prominent electron dense cytoplasm than resistant cells, (c) sensitive cells were less exacting in requirements for anaerobiosis, and (d) the medium (containing oxytetracycline) in which sensitive cells grew showed an accumulation of glutamic acid, leucine, acetone and alanine while aspartic and oxaloacetic acids accumulated after growth of resistant cells. Mikolajcik felt that the bacterial cell wall was the probable site of oxytetracycline action. This observation lead to development of the method for antibiotic "binding" previously described.

Shahani (159) studied differences in phosphorous compounds present in oxytetracycline-sensitive and resistant cells of S. lactis. More cellular material was consistently produced by the sensitive strain. Young cells of sensitive strains generally contained higher levels of protein and nucleic acid phosphorus than did similar resistant cells. The nucleic acid content of both types of 24-hour old cells was nearly equal.

Galactokinase and galactosidase were detected in S. lactis although lactase was not found (162). Penicillin and streptomycin had no effect on galactokinase but it was completely inhibited by chlorotetracycline and oxytetracycline. Penicillin was found to inhibit 33 to 50 per cent of the galactosidase activity.

**Effect of Low Temperature Storage on Antibiotics**

Studies on storage of antibiotics in milk showed the following were relatively stable for seven days at 8 to 10°C.: penicillin, streptomycin, oxytetracycline, chlorotetracycline, magnamycin, bacitracin and polymyxin (87). Penicillin, streptomycin and a combination of both were checked in another study (97) for their ability to retard growth of bacteria in milk during storage in a refrigerator and at 22°C. A combination of the two was found most effective at both temperatures.

Hibbs and Boyd (70) reported samples of milk which contained penicillin, oxytetracycline, neomycin, streptomycin or chlorotetracycline could be held frozen for at least 12 weeks without appreciable losses of antibiotic activity.

**Interaction of Antibiotics and Milk Components**

Different workers have shown antibiotics to affect
enzymes in milk and also that they themselves are affected by other milk constituents.

**Enzymes**

The effect of chlortetracycline and penicillin on the phosphatase enzyme system in milk was investigated by Morr (119). He found phosphatase was unaffected by chlortetracycline (up to 25 μg. per ml.) and only slightly by penicillin (up to 25 units per ml.). Enzyme inhibition produced by penicillin was not enough to alter reliability of the phosphatase test. The degree of enzyme reactivation in milk heated to 210°F. for 10 seconds or 145°F. for 30 minutes was not changed by either of the antibiotics studied.

In work on effect of different antibiotics (up to 50 p.p.m.) on milk lipase (33) it was seen that the enzyme was inhibited 9 to 42% by chlortetracycline, 7 to 49% by penicillin, 9 to 39% by streptomycin and 7 to 44% by oxytetracycline. The antibiotics were not selective in their inhibitory effects on sites of enzyme action.

**Salts and Proteins**

The influence of milk components on antibiotic activity was investigated by Price, et al. (135) and Martin and Harper (110). Price, et al. (135) showed the antagonistic influence of milk on oxytetracycline, chlortetracycline and polymyxin-B could be attributed to the presence of a high concentration of inorganic ions (principally calcium and magnesium). Readily ionizable salts of calcium and magnesium were more inhibitory than poorly ionized ones. Dihydrostreptomycin and neomycin were inhibited by the inorganic ions and also by casein. Magnesium salts were found more antagonistic to oxytetracycline and chlortetracycline and calcium salts were more antagonistic to polymyxin-B, dihydrostreptomycin and neomycin.

The loss of antibiotic activity in milk was markedly different for penicillin than for tetracyclines, according to Martin and Harper (110). The decrease in penicillin activity immediately after its addition to milk or whey was small but considerable inactivation occurred during refrigeration storage. Pasteurization of milk or whey prior to addition of penicillin reduced losses in activity during storage. The major loss of oxytetracycline and chlortetracycline activity occurred immediately after addition to either raw or pasteurized skim milk with only slight losses during cold storage of the milk–antibiotic mixture. The immediate loss of oxytetracycline and chlortetracycline activity was much less in whey than in skim milk although losses during refrigeration storage were considerable. Dialysis experiments showed no interaction between penicillin and the protein systems of either whey or skim milk. There was interaction between the protein systems of both whey and skim milk and chlortetracycline with a resultant loss of antibiotic activity in skim milk only. Radioactive tracer studies showed activity of penicillin was decreased by alpha-casein and especially so by aged solutions. Calcium ions together with alpha-casein caused considerable decreases in activity of oxytetracycline and chlortetracycline. Purified beta-lactoglobulin had no significant effect on penicillin or chlortetracycline activity and no chemical interaction was observed between this protein and the antibiotics.

Experiments on the influence of penicillin on hydrogen peroxide added to milk showed the bactericidal efficiency of peroxide to be reduced by 30 to 70 per cent. The interference with the preservative was proportional to the concentration of penicillin but hydrogen peroxide was not directly decomposed by the antibiotic (9).

**Allergic Reactions to Penicillin in Dairy Products**

The majority of a representative group of allergists, whose opinions were sought regarding the current status of the problem of allergic reactions from penicillin-contaminated milk agreed: (a) such reactions have occurred and probably have gone undetected in many instances, (b) that reactions occurred after ingestion of milk although some felt the small amounts of penicillin in milk were harmless to penicillin-sensitive people except in rare, extremely sensitive individuals, (c) an oral dose two to three times as great as an intravenous one was necessary to produce reactions in sensitive individuals, and (d) reactions most commonly encountered were chronic or recurrent urticaria (6). The Council on Drugs of the American Medical Association has confirmed the occurrence of allergic reactions in sensitive persons who have consumed milk with small amounts of penicillin. Other antibiotics in milk have not caused similar reactions (5).

Zimmerman (199) reported case studies of four patients who suffered urticaria after ingestion of dairy products. All patients had had previous allergic reactions to penicillin. Reactions were cleared up rapidly by injection of penicillinase, an enzyme which catalyzes the hydrolysis of penicillin to penicilloic acid (150), and subsequent prophylactic injections of the enzyme enabled patients to eat dairy products without allergic reactions. Zimmerman suggested that dairy products (including ice cream and cheeses containing molds of the genus *Penicillium*) should be eliminated from the diets of patients with chronic urticaria.

Penicillinase was used to treat 50 other patients
with penicillin reactions \((54)\). Rapid improvement after intramuscular administration was observed in some cases although others failed to improve. Local pain, swelling and systemic febrile reactions were commonly encountered after intramuscular injections of the enzyme. The antigenicity of penicillinase is such that sensitization may result from its repeated administration.

Common tests for sensitivity to penicillin use the skin-scratch, intradermal inoculation and conjunctival methods \((47)\). A new serological procedure has recently been reported \((47)\). The antigen for the test is prepared by coupling the antibiotic to erythrocytes with bis-diazotized benzidine. Hemagglutination occurred when cells prepared in this fashion were mixed with serum from sensitive individuals. The pattern of agglutination resembled that of the virus hemagglutination test.

### STUDIES ON THE USE OF SEWAGE EFFLUENT FOR IRRIGATION OF TRUCK CROPS\(^1,2\)

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*Salmonella, Ascaris ova and Endamoeba coli cysts were recovered from more than 50 per cent of irrigation water samples contaminated with either raw sewage or primary-treated, chlorinated effluents. Only one of 97 samples of vegetables irrigated with this water yielded *Salmonella*, but *Ascaris ova* were recovered twice from 34 of the vegetable samples. The public health implications of these results are discussed.*

The importance of irrigation for the economic and agricultural development of many parts of the world is well recognized. In Colorado, for example, approximately 3,000,000 acres are irrigated, of which over 40,000 acres are devoted to truck crops. Most of this land is irrigated from streams which contain either raw or partially treated sewage. Thus, when the irrigated crops include vegetables or fruits which may be consumed raw, a possible public health hazard is apparent.

Tanner \((8)\) and more recently Rudolfs, Falk, and Ragotzkie \((6)\) have reviewed the literature on the occurrence and survival of pathogenic enteric microorganisms in soil, water, sewage, and sludges, and on vegetation irrigated or fertilized with these materials. It would appear from these reviews that fruits and vegetables growing in infected soil can become contaminated with pathogenic microorganisms, and that these organisms may survive for periods of a few days to several weeks or more in the soil and on vegetation.

Rudolfs and his co-workers \((7)\) have carried on extensive experiments in which growing tomatoes and lettuce, in some cases, were sprayed with suspensions of *Salmonella, Shigella, Ascaris* ova, and *Endamoeba* cysts. The *Salmonella* and *Shigella* could not be recovered one week after spraying. *Endamoeba histolytica* cysts likewise died out within a few days, particularly when the weather was dry. The *Ascaris* ova, however, were still found on the surfaces of the vegetables one month after application, although their numbers had been reduced and the eggs were incapable of development into the infective stage. These authors concluded that if sewage irrigation or night soil application is stopped one month before harvest, the fruit or vegetables would not be likely vectors for the transmission of human enteric disease. In dry climates, however, such as the Western United States, it is common practice to irrigate some crops several times weekly right up to the day of harvest.

The studies reported here were designed to assess the public health hazard of the use of sewage-contaminated irrigation water under actual field conditions.

**Experimental**

The development of methods for the quantitative estimation of *Salmonella* and animal parasites from sewage, irrigation water, and vegetables has been reported in previous publications \((1, 2, 3, 10)\). In 1959, the methods used for isolation of *Salmonella* were as follows:

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