



145
046
THS

PENICILLIN MILK LEVELS IN COWS
FOLLOWING INTRAMAMMARY
INFUSIONS

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

W. F. Jackson

1949

This is to certify that the

thesis entitled

Penicillin Milk Levels in Cows
Following Intramammary Infusions

presented by

W. F. Jackson

has been accepted towards fulfillment
of the requirements for

Masters degree in Science

C. S. Bryan
Major Professor

Date July 26, 1949

PENICILLIN MILK LEVELS IN COWS
FOLLOWING
INTRAMAMMARY INFUSIONS

PENICILLIN MILK LEVELS IN COWS
FOLLOWING
INTRAMAMMARY INFUSIONS

by
W. F. JACKSON

A THESIS

Submitted to the Graduate School of Michigan
State College of Agriculture and Applied
Science in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

Department of Surgery and Medicine

1949

ACKNOWLEDGMENT

The author wishes to express his gratitude to Dr. C. S. Bryan for making available the facilities needed in this investigation, for helpful counsel in solving many of the technical problems, and for valuable suggestions in regard to the arrangement of this thesis.

Thanks is also expressed to the Commercial Solvents Corporation of Terra Haute, Indiana, for the grant-in-aid which made this work possible.

To Dr. Frank Thorp, Jr. goes the credit for encouraging suggestions during the course of this study and for contributions to the final arrangement of this manuscript.

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

INTRODUCTION

One of the major problems confronting the veterinary practitioner is the paucity of basic knowledge underlying the use of the newer antibiotics. In recent years penicillin has been the drug of choice in treating most susceptible bacterial infections in the mammary gland of the bovine. If penicillin is to be used to the best advantage in the treatment of mastitis caused by these organisms, basic studies of its sojourn in the mammary gland are essential.

The following study was limited to certain observations on penicillin levels in the bovine udder. The main objectives upon which this investigation was based were: (1) the determination of the height and duration of penicillin levels obtained following the intramammary instillation of various dosages in different vehicles; (2) the effect of the stage of lactation (pounds of milk produced) upon the levels of penicillin in the foremilk of bovines.

An attempt was made to correlate the above with present available data on the treatment of mastitis.

REVIEW OF LITERATURE

In recent years the literature has contained many reports regarding the efficacy of penicillin as a satisfactory agent in the treatment of streptococcic mastitis. However, only a few reports have been given indicating how long an adequate therapeutic level was maintained in the milk of treated cows by the various dosages given. There was an indication in these reports that the levels vary from one quarter to another and within individual animals.

Gerber et al. (13) found that in spite of the fact that penicillin is readily diffusible, it does not penetrate from the blood stream into the normal spinal fluid, tears, breast milk, saliva, or into the cornea, lens, brain, dura, veins, and bone marrow unless huge amounts are administered parenterally. In the presence of an inflammatory process with increased vascularization, only slight penetration in these tissues can be accomplished. Seeley et al. (36) tested milk samples for 24 hours after an intravenous injection of 500,000 units and found no measurable penicillin level in the milk. Barker et al. (2) treated an infected udder with a total of 2,681,000 units over a period of four days and concluded that the lactating mammary gland was nonpermeable to penicillin administered subcutaneously. Watts et al. (45) used the intramuscular route for injection of 1,000,000 units of penicillin and were unable to detect the presence of penicillin in the milk. Welsh et al. (47) administered doses of 16,250,000 and 17,250,000 units to two different cows that were in milk production.

The dosages were administered subcutaneously in amounts of: 5,000 units per pound of body weight initially, and 25,000 units per pound at the sixth, twelfth and eighteenth hours. The milk level obtained in both animals was less than 0.05 units per ml. at 15 minutes. At the fifth hour a level of 0.84 units per ml. was demonstrated, and at 30 hours the level in the animal receiving 17,250,000 units was 0.1 while the one receiving 16,250,000 units of penicillin showed a level of 0.06 units per ml. of milk. The penicillin level disappeared from the blood in both subjects at 23 hours, but was present in the milk for a few hours longer. This work did not suggest the parenteral administration of penicillin for the treatment of mastitis but did demonstrate that there was diffusion of small quantities of penicillin from the blood to the milk.

It was obvious that if an infected quarter was to be cured, complete destruction of the viable organisms must be accomplished. Since penicillin has a fissibactericidal action, it was to be expected that multiplying streptococci will be killed quickly and those in a dormant or nondividing state will be unaffected. A great amount of penicillin above the effective therapeutic level for streptococci has no apparent advantage, since death of the bacteria was dependent upon the susceptibility of the organism, which in turn was dependent upon the phase of growth of the organism. Packer (24) found that 220 of 256 strains of streptococci and staphylococci present in the udder were sensitive to penicillin levels of 0.125 units per ml. in vitro. Heishman (14) found 129 strains sensitive to levels from 0.03 to 0.125 units of penicillin per ml. in vitro.

This has also been confirmed in reports by Burnane (20), Seeley et al. (35) and Foley et al. (10). Streptococcus dysgalactiae is the most sensitive, while some strains of Staphylococcus aureus will grow in concentrated solutions containing 7 to 50 units per ml. of penicillin. No resistant strains of mastitis streptococci were found. A strain of E. Coli isolated from an acute case of mastitis by Seeley et al. (36) was refractory to 99 units per ml. of penicillin. This was to be expected since coliform bacteria were not considered susceptible to penicillin.

Schalm et al. (32) used sterile distilled water, 0.9 per cent saline solution, 0.4 per cent pectin, 10 per cent dextrose and mineral oil, as vehicles for the intramammary infusion of penicillin. Their results for all vehicles were similar, and so distilled water was suggested for routine use. Petersen (27) stated that injections of saline produced greater reaction in udder tissues than did distilled water, but the reverse was true in the case of other body tissues. Milk withdrawn and immediately reinjected into the udder also produced similar reactions to saline. Foley et al. (11) studied three types of vehicles for udder infusion. They were: 1) distilled water, 2) vehicle A*, 3) vehicle B*. When 100,000 units of penicillin was administered in 20 mls. of each vehicle the water solution yielded measurable levels for 24 hours, vehicle A for 48 hours, and vehicle B for 72 hours. The levels at 72 hours for vehicle B varied from 0.7 to

* Vehicle A contained mineral oil, water and lanolin derivatives.

* Vehicle B contained the above materials plus a small amount of propylene glycol and a nonionic wetting agent.

to 4 units per ml. of milk. Peanut oil did not prolong the penicillin level when compared to distilled water by Murmane (21). The oil served as a vehicle to carry the penicillin into the milk cistern. The penicillin was then available for solution in the milk and was not combined in any way to the oil. Some oils have been reported to exert a slight bacteriostatic effect; this may explain why slightly better results have been obtained by penicillin suspended in oil vehicles. If this is true penicillin in oil may be of more value in dry cows in maintaining a longer therapeutic level of penicillin.

Solid vehicles are represented by bougies (Mastics and Penstixs). These are 25 to 35 mm. long and 4 to 5 mm. in diameter. In these forms the penicillin was carried in a minimum of foreign material, usually weighing about 0.25 grams. The solid base was milk and water soluble, disintegrating within 30 minutes after insertion through the teat canal. Bougies retain their potency after being stored for several months at room temperature. Their therapeutic value was comparable to other vehicles according to reports by Bryan (5) and Barnum (3). Barnum obtained levels of from 0.3 to 1.2 units of penicillin per ml. of milk at 24 hours after the insertion of a single bougie containing 25,000 units of penicillin. The penicillin was maintained at a higher level by the bougie than when 200 ml. of water was used as the vehicle for the same amount of penicillin. There were no published reports on crystalline procaine penicillin G in oil or water, soluble repository penicillin or penicillin of various particle sizes; infused in the udders of cows.

Weirether et al. (46) found that the rate of decline of

penicillin in the milk was greater during the first ten hours, after which it disappeared more slowly. Also, if penicillin was carried in a large volume of diluent, the concentration of the penicillin per ml. of milk increases temporarily because the liquid is quickly absorbed and leaves the penicillin in the udder. Schofield (34) took samples from the mammary vein and the urine, and found that penicillin left the udder by way of the blood stream and was excreted in the urine. Stevenson (40) reported that the nature of the bacterial flora of the udder determined, to a considerable degree, the length of time that penicillin remained after udder infusion. Weirether et al. (46) found that the rate of absorption from an infected gland, except for coliform where it is much faster, was essentially the same as from a normal gland. Murnane (21) found the penicillin concentration in the milk to be inversely proportional to the volume of milk secreted. The rate of absorption was higher in high producers (96 per cent in 12 hours) than from udders in the late stage of lactation (69 per cent in 12 hours) or in the dry state. Schalm et al. (32) stated that the volume of milk secreted by the quarter receiving treatment has a greater influence on the efficacy of the penicillin infusions than the extent of tissue damage. Packer (25) in his data showed that much more penicillin actually left the udder in a low producing cow than in a high producing one, with the result that the same level of penicillin was found in either case at 12 and 24 hours after injection of various amounts. This contradicts the usual recommendation that a larger number of units of penicillin per dose be administered to cows in high production to maintain a high concentration of penicillin in the milk.

After treatment, Murnane (20) found slightly higher levels of penicillin persisted in streptococcus mastitis than in staphylococcus mastitis. This may have been due to different degrees of tissue damage caused by the two organisms. The persisting levels in both varied considerably 14 hours after injection of 50,000 units of penicillin in 100 ml. of sterile distilled water. The levels in the staphylococcus infected quarters varied from 1.5 to 7.5 units per ml. of milk, and in the streptococcus infected quarters from 0.33 to 10 units per ml. of milk. This indicated that there was a definite variation from quarter to quarter and from cow to cow. This was especially true in clinical cases owing to the difference in infections and resultant tissue damage. Assays of the foremilk, mid-milk and strippings by Murnane (20) showed an individual but not an average difference, whereas, Packer (26) and Stevenson (40) found that the level obtained in the foremilk was slightly higher than the strippings. These reports indicated that after the udder was filled with milk the distribution of the penicillin in the udder was fairly uniform. Penicillin was well distributed following the insertion of bougies, as it was following aqueous vehicles, according to a study by Barnum (3).

Murnane (21) reported that the leucocyte counts of the milk rose sharply immediately after penicillin administration, but fell very rapidly thereafter. This was attributed to the saline vehicle and not to the penicillin itself. In normal cows there was a 13 to 37 per cent transient decrease in milk production when compared to the previous 24 hours. In one case there was swelling of the gland and alteration of

the milk. Packer (26) reported an increase in body temperature from 1.0 to 1.7 degrees F. 7 hours after penicillin administration. The amount of vehicle varied from 10 ml. to 200 ml. of saline solution, and the amount of penicillin from 50,000 to 200,000 units. Some swelling occurred in the quarters receiving 200 ml. of saline but none was present in the quarters receiving 10 ml. of saline, although the unitage of penicillin was the same. The swelling disappeared after four hours. Checking the production of 9 cows for 7 days previous to, 7 days during, and 7 days after treatment he found no significant change in the amount of milk produced as a result of the penicillin therapy. The results of work by Schalm et al. (32) confirm these observations. Following infusion of 59 quarters with 100,000 units of penicillin in 50 ml. of sterile distilled water, there were observable clots, shreds and flakes for 24 hours, but no swelling. This action was attributed to the vehicle, which was distilled in an old copper apparatus and passed through a length of lead pipe. Sterile water from other sources did not produce any reaction, indicating that there may have been pyrogens or other irritating substances in the first water used. Barnum (3) compared the cell counts after inserting 25,000 units of penicillin in bougie form with the same amount in 200 ml. of water. At no time after inserting the bougie did the leucocyte count rise above one million cells per ml. of milk, this is usually considered to be in the normal range. The count varied from a high of 5 to 25 million within the first 60 hours after water instillation. There was no decrease in the milk flow. However, flakes did occur after the water infusion along with the

increased cell counts. Byrne et al. (7) observed that the udder secretions consisted largely of cells and serum rapidly began to assume a normal milk appearance, following penicillin therapy for mastitis. Barker (1) mentioned cases showing swelling and hardness of the udder, a slight rise in temperature, and one case of slight anorexia following intramammary instillation of penicillin in distilled water.

Most investigators used the agar cup and plate methods for the assay of milk samples for penicillin. Packer (26) found that wells made with OO rubber stoppers in an agar plate would hold 0.2 ml. of milk. The readings thus obtained were similar to those obtained when porcelain cups were used. Chilling the plates a few hours before using gave a clear zone of inhibition. Watts et al. (45) used the serial dilution technique in litmus milk, employing a strain of Streptococcus agalactiae (606) with a known sensitivity to penicillin as the test organism. Readings were made after incubation at 37 degrees C. for three days. A definite pink coloration was taken as evidence of growth. In order to prevent penicillinase activity of certain bacteria and contaminating organisms, found in the milk, they heated the milk to 100 degrees C. for varying lengths of time. No destruction of the penicillin could be detected within 15 minutes, but after 30 minutes 50 per cent was destroyed, and after 60 minutes there was a reduction of 75 per cent in the penicillin present. Barnum (3) modified the above technic and used Difco Purple milk instead of Litmus milk, making the reading after

48 hours incubation at 37 degrees C. The results of this technic compared favorably with those from the deep well plate method. Trussell et al. (43) cooled the samples overnight, adjusted the pH to about 4.6 and allowed the milk samples to clot. A 15 ml. sample was then centrifuged and the whey further clarified by passing the supernatant through a small celited Buchner filter. The filtrate was then adjusted to a pH of 6.0. For routine assays, large plates were used, the test organism was Staph. aureus (209). In checking with known standards, approximately 75 per cent of the penicillin present could be recovered by this procedure.

METHODS AND MATERIALS

Collection of Samples. The first few streams of milk from each quarter were discarded into a strip cup. This was done to flush out the streak **canal** and to note the physical appearance of the milk. The udder and teats were thoroughly disinfected with a clean cloth saturated with a 1-1000 solution of a quaternary ammonium compound or a 1 to 5 dilution of a liquid germicidal detergent to prevent the entrance of contaminating bacteria to the sample at the time of collection. Milk samples were collected by milking directly into sterile tubes. All tubes were sterilized by dry heat. Particular **care** was taken to prevent contamination of the corks and plugs. Five ml. vials were used for smaller quantities to facilitate storage, and standard 20 ml. test tubes were used for larger quantities. Immediately after collection of the milk samples, they were placed in a flowing steam cabinet for twenty minutes. This was to **destroy** the natural udder flora, and to insure inactivation of any penicillinase that might be present. They were stored in a refrigerator (45 degrees F.) until assayed, at no time for more than eight hours. Ordinarily about 16 to 20 samples were assayed at one run. If the sample was likely to be above 100 units of penicillin per ml., dilutions of 1:10 or 1:100 were made and assayed. Sterile distilled water was used as the diluent. The water had a pH of 6. The optimum pH for stability in aqueous solution is 6 to 6.5, (while a practical range is from a pH of 5.5 to 7.5). A drop in pH to 4.0 will cause rapid inactivation of the penicillin, as reported by Fleming (9). This dilution of the samples

saved considerable time and glassware. Its accuracy was determined by making determinations on known solutions of penicillin.

Assay of the Milk Samples: The serial dilution method of assay for penicillin in body fluids as described by Randall et al. (3) was employed in this study.

One-half ml. amounts of sterile yeast beef broth were placed into standard test tubes covered with one inch glass caps. All liquid media were sterilized at 15 lbs. of pressure for 20 minutes. Serial dilutions by halves were made by adding one-half ml. of the fluid being tested to one of the tubes containing broth, mixing, and then carrying one-half ml. amount in serial dilution for as many tubes as necessary. The first tube in the series contained one-half ml. of the unknown sample under test only. A standard was prepared for comparison by diluting a known potency penicillin (reference standard) to one unit per ml. in distilled water. The pH of the distilled water was slightly plus or minus six at weekly readings. This one unit standard was diluted exactly as above in serial dilution by halves. One and one-half ml. of a 1:100 dilution of the test organism (Bacillus subtilis) in sterile yeast broth was then added to each tube. All tubes were then incubated at 37 degrees C. for 18 hours. The last tube in which no growth occurs was taken as the end point. This was usually sharp, inasmuch as one tube was clear while the next one in the series had the typical pellicle of B. subtilis on the surface of the medium.

The concentration of penicillin in the unknown was then determined by comparing the end-point of the unknown with that of the

standard. Ordinarily the test as described here was sufficiently sensitive to determine potencies as low as 0.03 units of penicillin per ml. of blood plasma or milk serum.

This method of assay was not satisfactory with whole milk if the potency was below one unit per ml. The turbidity and the cream line of the milk made it impossible to accurately determine pellicle formation. If it was demonstrated that the potency was lower than one unit per ml. of milk, the agar cup method was used. Seventeen ml. of sterile, melted, tryptose agar were poured into standard petri dishes and allowed to cool. After the agar had hardened, a five ml. quantity of inoculated, melted agar was pipetted onto the top of the agar and rotated to insure uniform distribution. This inoculated agar consisted of one ml. of a 24 hour broth culture of Staph. aureus 313 to 50 ml. of melted and cooled tryptose agar. Six sterile porcelain cups were warmed and dropped on the plates. The plates were chilled before the cups were filled. Into each cup was pipetted 0.02 ml. of the material to be assayed. With each group of samples, a standard curve was determined by making dilutions of the standard containing: 1, 0.5, 0.25, 0.125, 0.06, 0.03 unit per ml. of penicillin. On each plate that was run one or two of the standard dilutions were included to make sure that there were no individual plate variations. The two standards that were likely to be the closest to the unknown potency were used. The plates were incubated at 37 degrees C. for eighteen hours. The zone of inhibition was measured in mm. and the standard curve was plotted. The unknowns were compared to the curve and a reading was recorded.

It was difficult to accurately determine potencies of 0.03 and 0.06 units of penicillin per ml. on the inoculated plates. If the unknown sample fell into this range it was assayed by a filtered, serum dilution method. This consisted of taking 15 ml. of skimmed milk, obtained by the centrifugation of the sample and removal of the cream layer, and adding 0.4 ml. of rennet to clot the milk. These tubes were slanted and incubated for two to four hours at 37 degrees C. If enough serum could not be collected from the top of the slanted tubes, they were centrifuged at high speed for a short time.

The milk serum obtained was passed through a sterile Swinney Filter Adapter with filter disc made by Becton, Dickinson & Co., Rutherford, N. J. A blue plunger precision type syringe made by Becton, Dickinson & Co., Rutherford, N. J. of one or two ml. capacity was found better adapted than larger syringes. More effective pressure could be obtained with a small plunger than with a syringe with a larger plunger, thereby lessening the danger of breaking the syringe tip. The filtered samples were assayed by the serial dilution method previously described. If the standard, which was run with each group was negative in the sixth tube, samples with a potency of as little as 0.03 units per ml. of milk could be determined.

Materials Used: Cow A was a six year old Jersey, in apparent good health, weighing 650 pounds. Injections were begun while the cow was in the "drying off" period of lactation, giving an average of two pounds of milk per day. Cow B, five years of age, weighed 700 pounds. She was in the fifth month of lactation, giving 15 pounds of milk each day. Cow C also was producing 15 pounds of milk a day. She

was highly nervous, so was used only in comparison with cow B since both were in the same stage of lactation. Cow D was a 1000 pound Holstein, giving an average of 45 pounds of milk per day. Injections were begun soon after her third parturition.

The cows were confined throughout the experiment in well bedded stalls in the barn. The barn was kept at a constant temperature. They were fed an ample grain ration with good quality hay, and fresh water before them at all times. All cows were hand-milked at seven in the morning and five in the evening. They were fed, milked and handled by the same caretaker. The animals were in excellent physical condition, gaining weight throughout the experiment. The milk was examined at weekly intervals for the presence of any infectious organisms; all such tests were negative.

The distilled water used as a vehicle was double distilled through a standard coil copper apparatus. The distilled water was sterilized in the autoclave and stored in 100 and 200 ml., rubber, diaphragm stoppered bottles until use. To make the necessary dilutions, the water was withdrawn with a sterile needle and syringe for injection into the vials of crystalline penicillin.

The necessary precautions were taken to prevent contamination of the vial tops from the time the diluent was added until the dose was withdrawn. The udder was prepared for injection by the same procedure as was used in taking the milk samples. Little difficulty was experienced in making the infusions.

Quantities of penicillin solution, 50 ml. or less, were instilled by sterile glass syringes and milk tubes. For 100 ml. of solution, a gravity flow intravenous outfit and sterile teat tubes

were used.

The diluent for aqueous penicillin solution in water-in-oil (Penicle) was obtained in 100 ml. bottles made by Wallace Laboratories, Inc. N. Y., N. Y. The desired volume of the diluent was added to a two ml. quantity of an aqueous solution containing the desired amount of penicillin.

The syringes, teat tubes and needles were sterilized by autoclaving in a covered metal tray and remained there until they were removed with forceps at the time of injection.

Quantities of one ml. or less to be instilled were injected with a one ml. precision type blue barrel syringe. Instead of using a standard teat tube, a twenty gauge needle was filed off to a blunt end, leaving a one-half inch shank. This allowed a minimum of loss when injecting small quantities of penicillin solution, and worked well with either oil or the heavier aqueous suspensions of procaine penicillin.

The solid forms of vehicle came in the bougie form. They were obtainable in 25,000, 50,000, and 100,000 units per bougie. They were used as recommended by the manufacturers. Some of the bougies were too large in diameter to enter the streak canal of the smaller Jersey. All penicillin administrations were made within one hour after milking.

The sodium crystalline penicillin G used in this experiment was obtained in sterile, rubber stoppered vials containing either one or two hundred thousand units per vial. The potency was expressed in terms of units, 60 mg. of crystalline sodium salt being

equivalent to one hundred thousand units. One unit was defined as the penicillin activity contained in 0.6 microgram of the Food and Drug Administration Master Standard, and is approximately equivalent to the earlier Oxford Unit. These vials were stored at room temperature until needed. All solutions were made up just before injection; no aqueous solutions were used after they had been made up for more than twelve hours, and during the 12 hour period they were kept under refrigeration. Sodium crystalline penicillin G also came in several bougie (Mastic) forms* containing 25,000, 50,000 and 100,000 units. The bougies were both milk and water soluble, usually disintegrating within thirty minutes. Crystalline penicillin G sodium in ointment form also was used; each tube contained 50,000 units in one-twelfth ounce of an ointment base. These tubes had a special nozzle adaptable to enter the streak canal with sealed caps to prevent contamination prior to use.

Crystalline procaine penicillin G was also obtained in sterile vials in dry form containing a small amount of the effective dispersing agent, aluminium monostearate. Each vial contained 1,500,000 units for aqueous suspension. When 4.4 ml. of sterile water were added to the vial and shaken to dissolve there resulted 5 ml. of solution each ml. containing 300,000 units. Crystalline procaine penicillin G was the water insoluble procaine salt of benzyl penicillin. It contained one molecule of penicillin G combined with one molecule of procaine base, which amounts to approximately 40.1 per cent of the total. The addition of aluminium monostearate acted as a dispersing agent and maintained a property known as "thixotropy" which aids in keeping the small particles of crystalline procaine penicillin G well and thoroughly

* Made by F. E. Martin Laboratory, West Chester, Penna.

dispersed, and thus in maintaining the mixture in an easy flowing fluid state. Aluminium monostearate also acted to repel water in the tissues and by virtue of this characteristic delayed absorption and prolonged the effects of the penicillin.

Another form of procaine penicillin used was a sterile suspension of micronized (particle size of less than five micra) crystalline procaine penicillin G suspended in refined peanut oil with a two per cent (w/v) aluminium monostearate added. Each ml. contained the equivalent of 120 mg. of procaine base. Crystalline procaine penicillin G* has a potency of 900 units per mg. It was also available in bougie (Penstix)^{xx} form containing 25,000 units.

Soluble-Repository penicillin^x was available in standard sterile vials containing two million units in a dry form. Sterile distilled water was used as a diluent. The addition of 4.2 ml. of water produced a total volume of five ml. of solution. Each ml. contained 400,000 units of penicillin (300,000 units of procaine penicillin and 100,000 units of sodium crystalline penicillin G.) The suspension had to be thoroughly shaken before withdrawal and immediately before injection, or there was a tendency for the heavier particles to settle out. This would give an improper ratio of the two penicillins. The two penicillins were also of different particle size. This penicillin was injected with a small precision syringe with a special twenty gauge needle in 0.25, 0.5, and 1.0 ml. amounts.

x As quoted in literature from manufacturer (Parke, Davis and Co., Detroit, Michigan).

xx Made by Wyeth Incorporated, Philadelphia, Penna.

* As quoted in literature from manufacturer.

Throughout the remaining discussion crystalline sodium penicillin G will be referred to as penicillin, crystalline procaine penicillin G as procaine penicillin and soluble repository penicillin as S-R penicillin.

DISCUSSION AND RESULTS

Of inevitable significance in the assay of milk samples was the inhibitory action of normal milk on the B. subtilis pellicle formation.

The B. subtilis strain used in this work was obtained from the Northern Regional Research Laboratory, Peoria, Illinois. It grew luxuriantly at 30 degrees C. with diffuse turbidity, and at 37 degrees C. the growth consisted of a definite pellicle with a clear medium. The resistance to penicillin was of the same order as some strains of hemolytic streptococcus which required as little as 0.0085 units per ml. to inhibit a 1:100 dilution of a broth culture of the organism. This sensitivity was maintained over a long period of time without the necessity of repeated transfers.

All unknown milk samples were first assayed by the serial dilution method as previously described. The concentration of penicillin in the unknown was then determined by comparing the end point of the unknown with that of the standard. The following outline presents this in graphic form:

Fluid	Tube Numbers									
	1	2	3	4	5	6	7	8	9	10
Standard	0	0	0	0	0	0	+	+	+	+
Unknown #1	0	0	0	0	0	0	0	+	+	+
Unknown #2	0	0	0	0	0	+	+	+	+	+

In this instance, tube six was clear in the case of the standard, and tubes number seven on had the typical E. subtilis pellicle. Since this represents one unit per ml., the unknown #1 contained this amount or two units per ml. and unknown #2 contained 0.5 unit per ml., the end points being seven and five, respectively. In this way it was usually possible to determine potencies as low as 0.03 units per ml.

Milk samples from each cow were taken weekly before any penicillin was injected. These samples were set up by the serial dilution method as previously described. Upon reading the sample after 18 hours incubation it was always difficult to ascertain pellicle formation in tubes one to four or five, usually five. In none of the above experiments was there any indication of inhibition of pellicle formation at the higher dilutions.

Another problem of importance was to determine whether whole milk or milk serum obtained by rennet coagulation inactivated any of the penicillin present in the assay samples. To solve this problem, sterile whole milk was used to make a solution of one unit of penicillin per ml. using the standard penicillin. This was divided into five tubes: 1) whole milk, 2) skimmed milk (by centrifugation), 3) cream (from #2), 4) serum from rennet coagulated whole milk, 5) serum from rennet coagulated skimmed milk. These samples were assayed by the plate method. In the final reading there was only a very slight variation of one or two mm. in the diameter of the zone of bacterial inhibition. This was repeated obtaining similar results, also equal results, also equal to the 1.0 unit control standard which was run with all plates. Thus one would assume that there was no appreciable

inactivation of penicillin by normal, sterile, whole milk or serum obtained by the rennet coagulation of milk. These results indicate that removing the cream layer would not appreciably alter the penicillin content of the remaining milk sample.

It was necessary to determine the action of rennet in a milk sample for penicillin assay. A series of 15 ml. milk samples (1.0 units of penicillin per ml.) were set up to determine the amount of rennet required to produce coagulation with the necessary serum production, during a given time period. Amounts of 0.1, 0.2, 0.3, 0.4, 0.6, 1.0, 1.5 and 2.0 ml. (of the commercial rennet solution of rennin) were added to the samples. The samples were incubated for 3 hours at 37 degrees C. The serum was; 0.15, 0.25, 0.5, 1.0, 1.0, 1.2, 1.5, and 1.6 ml. respectively. The optimum yield was obtained by the use of 0.4 ml. of rennet per tube containing 15 ml. of milk.

Another series of tubes was set up to determine the optimum time for incubation at 37 degrees C. to yield the milk serum required. It was found that two hours incubation yielded the amount of serum necessary for the test. Samples of milk incubated for periods longer than six hours showed a marked reduction in the penicillin content. If enough serum could not be collected from the slanted tubes, they were centrifuged at high speeds for a short period of time. If insufficient serum resulted the slants were broken up and the tube was **recentrifuged**. The milk serum obtained on the unbroken slants was clear while that obtained after recentrifugation was frequently turbid. If the serum obtained was very turbid, it was siphoned off and again centrifuged, thereby obtaining a clearer fluid. Since the milk and rennet were not sterile the milk

serum was sterilized by filtration. The Swinney filter adapter used in virus isolation was found to be adaptable to filtering the serum. The filter removed the organisms and particles of curd from the milk serum. This was especially important in samples containing less than one unit of penicillin per ml. Although samples were always assayed within less than eight hours after collection it was interesting to note that some did not lose their potency during storage periods of from three to eight days.

The main objectives upon which this investigation was based were; (1) The determination of the height and duration on penicillin levels obtained following the intramammary instillation of various dosages in different vehicles; (2) The effect of the stage of lactation (pounds of milk produced) upon the levels of penicillin in the foremilk of bovines.

These objectives were initiated by the infusion of an aqueous solution penicillin in cow A, this cow was prevented from drying off by milking twice daily. This milking maintained a flow sufficient for the collection of samples at regular intervals. This cow was withdrawn from the experiment before complete data on all dosages and vehicles could be compiled. The penicillin levels of milk obtained in cow A (near end of lactation period giving 2 pounds of milk daily) taken at specified intervals following the infusion of 25,000, 50,000 and 100,000 units per quarter each in 10, 50, and 100 ml. of sterile distilled water were presented in Table I. Higher levels were obtained when the penicillin was infused in 10 ml. of sterile water when compared to 50 and 100 ml. of water as diluent. In example 100,000 units of penicillin in 10 ml. produced a level of 0.06 units of peni-

cillin per ml. or higher for 84 hours, whereas, the same amount of penicillin in 50 ml. resulted in a level of 0.06 units per ml. or higher for 72 hours. The level of 0.06 units per ml. persisted for 60 hours after 100 ml. water was used as the diluent.

These data compare favorably with those obtained by Packer (25) following the injection of 25,000, 50,000 and 100,000 units per quarter in 50 ml. of saline in cows producing an equal quantity of milk. He found levels at 24 hours of 1.0 (in 12.5 ml. saline) 2.2, and 2.9 units per ml. respectively of saline injected. The findings of Murnane (20) were 81 and 21 units per ml. at 12 and 24 hours respectively following the infusion of 15,000 units in 100 ml. of saline. The levels recorded in Table I are 1, 4, and 16 units per ml. respectively. Schofield (34) reported an average of 6 units per ml. at 24 hours following the infusion of 25,000 units in 200 ml. water, also 100,000 units in same amount of water gave a level of 10 units per ml. Twenty-four hours after infusion of 50,000 units 50 ml. of water in a dry cow Thorp et al. (42) had data that showed a level of 18 units per ml. The data reported by all of these workers was very uniform, the various levels obtained may have been due to different technics employed in collection and assay for penicillin and to the different individuals. Errors inherent to technic may have given rise to the variations in the levels; but the author knows of no tests that produced more accurate results.

The data presented in Table II present the levels of penicillin in cow A following the insertion of 25,000 units of procaine penicillin in bougie form, 35,000 units (tube broken) penicillin in ointment base and 100,000 units of penicillin in bougie form. The

TABLE I. The penicillin milk level in cow A (end of lactation) following the infusion of 25,000, 50,000, and 100,000 units per quarter of crystalline sodium penicillin G* in varying amounts of sterile distilled water as vehicle.

Ml. of water infused	Units of penicillin used	<u>Hours after udder infusion</u>								
		6	12	24	36	48	60	72	84	96
<u>Units of penicillin per ml. of milk</u>										
50	25,000	256	64	1	0.12	-0.06*				
100	"	128	64	0.5	0.1	-0.06				
10	50,000	200	64	16	2	0.5	0.06	-0.06	-0.06	
50	"	128	32	4	0.5	0.1	0.06	-0.06		
10	100,000	1600	400	32	8	1	0.5	0.25	0.06	-0.06
50	"	800	200	16	2	0.5	0.25	0.06	-0.06	
100	"	800	128	8	1	0.5	0.06	0.03	-0.03	

* hereafter referred to a penicillin.

* -0.06 or -0.03 designates less than that amount per ml. of milk.

TABLE II. The penicillin milk level in cow A (end of lactation) following the infusion of 25,000 units (procaine penicillin) and 100,000 units penicillin per quarter in a bougie form. Approximately 35,000 units (tube broken) in ointment form, were inserted.

Vehicle containing the penicillin	Units of penicillin inserted	<u>Hours after udder insertion</u>									
		6	12	24	36	48	60	72	84	96	
Units of penicillin per ml. of milk											
Penstix	25,000	512	128	8	0.5	0.5	0.06	0.03	-0.03		
Ointment	25,000	64	64	16	1	0.5	0.5	0.1	not run		
Mastic	100,000	1600	800	16	1	0.5	0.25	0.03	0.03	-0.03	

levels of penicillin 24 hours after infusion were 8, 16 and 16 units per ml., respectively, the same at 48 hours, while at 72 hours the levels existing in the udder were 0.03, 0.1 and 0.03 units per ml. respectively. These data showed that the bougie of 25,000 units of the procaine penicillin produced a level of similar height and duration as did 100,000 units of penicillin in bougie form. Although the 84 hour assay of the penicillin ointment was not run there was a trend of a higher level with the ointment than was produced by either of the two bougies. Some recent work by Bolton et al. (4) reported a 6 hour level of 46.6 units per ml. following the insertion of 25,000 units of procaine penicillin in a bougie. The stage of production of the cows used was not given. This may be compared with a level of 512 units per ml. in cow A (Table II).

Determinations in Cow B: Cow B was a Jersey, giving 15 pounds of milk daily. Results in Table III were concerned with the levels of penicillin in the milk of cow B following the infusion of 25,000, 50,000 and 100,000 units. Each of these amounts was dissolved in 10, 25 and 100 ml. of sterilized distilled water. In the amounts of water mentioned 25,000 units, at 24 hours a level persisted of 1, 0.5 and 0.06 units per ml. of milk. A dosage of 50,000 units produced a level of 1, 0.5 and 0.12 units per ml., while 100,000 units yielded levels of 4, 2 and 1 units per ml. respectively at 24 hours. The above unitages in 10 ml. of water usually gave a measurable level twelve hours longer and somewhat higher than did the same unitages in 50 and 100 ml. of water. Fifty thousand units in 10 ml. of water produced the same level (0.03 units per ml.) at 48 hours as did 100,000 units

in 50 ml., the same held true of 50,000 units in 50 ml. and 100,000 units in 100 ml.. Packer (25) infused 100,000 units of penicillin in 50 ml. and found a level existed at 12 and 24 hours of 25 and 1.3 units per ml. respectively. These compare favorably with the levels presented in Table III of 8 and 2 units per ml. at the 12 and 24 hour examination periods.

Thorp et al. (42), Foley et al. (11) and Schofield (34) either did not state the production record or did not have any data in this range (15 pounds per day).

Bougies were inserted into the udder of cow B (middle of lactation). Penicillin determinations were made of 6, 12, 24, 36, 48, 60, 72, and 84 hours, the cow was milked twice each day; those data are presented in Table IV. Levels obtainable at 24 and 48 hours following the administration of 25,000, 50,000, 100,000, and 200,000 units per quarter of penicillin bougies were: at 24 hours, 1, 1, 2, and 8 units per ml., and at 48 hours less than 0.06, 0.03, 0.03 and 0.12 units per ml. respectively. Terminal levels of 0.03 and 0.06 units per ml. of milk were found at 60 hours in the quarters that received 100,000 and 200,000 units respectively. In 9 cows averaging twice the production (34.8 pounds of milk per day) of cow B, at 12 hours Trussell et al. (43) found an average of 5.4 units per ml. after inserting a 25,000 unit bougie in each quarter. In a cow giving 10 pounds of milk per day Barnum found 2.4 and 0.06 units per ml. and in a cow giving 15 pounds of milk per day a level of 1.2 units per ml. was found 24 hours after inserting a 25,000 unit bougie of sodium penicillin G.

Table III. The penicillin milk level in cow B (middle of lactation) following the infusion of 25,000, 50,000 and 100,000 units per quarter of penicillin in varying amounts of sterile water as the vehicle.

Ml. of water infused	Units of penicillin used	<u>Hours after udder infusion</u>							
		6	12	24	36	48	60	72	84
Units of penicillin per ml. of milk									
10	25,000	200	8	1	0.06	-0.06			
50	"	100	4	0.5	-0.06				
100*	"	64	2	0.06	-0.06				
10	50,000	400	16	1	0.25	0.06	-0.06		
50	"	400	8	0.5	0.1	0.03	-0.03		
100*	"	200	4	0.12	0.06	-0.06			
10	100,000	400	32	4	1	0.25	0.12	-0.06	
50	"	400	8	2	1	0.06	-0.06		
100*	"	200	4	1	0.12	0.03	-0.03		

*Slight swelling was noted at the 6 hour period, the secretion contained a few flakes.

TABLE IV. The penicillin level in cow B following the insertion of 25,000, 50,000, 100,000 and 200,000 units of penicillin in solid (Mastic) form. Also 25,000 and 50,000 units per quarter of procaine penicillin in a bougie (Penstix). The ointment levels were after the insertion of 50,000 and 100,000 units of penicillin in a petrolatum base.

Vehicle	Units of penicillin used	<u>Hours after udder insertion</u>								
		6	12	24	36	48	60	72	84	96
		Units of penicillin per ml. of milk								
Mastic	25,000	200	8	1	0.06	-0.06				
"	50,000	400	32	1	0.1	0.03	-0.03	-0.03		
"	100,000	400	64	2	0.5	0.03	0.03	-0.03		
"	200,000	800	320	8	1	0.12	0.06	-0.06		
Penstix	25,000	200	64	0.5	0.06	0.03	-0.03			
"	50,000	400	64	2	0.5	0.06	-0.03			
Ointment	50,000	128	64	2	1.5	1	0.5	0.06	0.06	-0.06
"	100,000	200	64	2	1.3	0.8	0.6	0.12	0.06	-0.06

Terminal levels of 0.5 and 2, 0.06 and 0.03 units per ml. existed at 24 and 48 hours after inserting 25,000 and 50,000 units of bougie containing procaine penicillin. Levels reported by Bolton et al. (4) of 225 units per ml. at six hours after inserting a procaine penicillin bougie of 50,000 units compare to a level of 400 units per ml. in Table III.

Penicillin in ointment form administered at dosages of 50,000 and 100,000 units resulted in a terminal 84 hour level of 0.06 units per ml. for both amounts of penicillin. This was a 24 hour longer persistence of the level of penicillin than where 200,000 units of penicillin in bougie form was administered. After 60 hours the 50,000 units of penicillin ointment maintained a level of 0.5 units per ml. compared to the terminal level at 60 hours of 0.06 units per ml. for the 200,000 units in bougie form.

Foley et al. (11) have presented the only data relative to levels obtained following the administration of penicillin in a water-in-oil emulsion. Their levels following infusion of 100,000 units of an aqueous suspension of penicillin in 20 ml. of water-in-oil showed at 24, 48, and 72 hours 25, 8 and 4 units per ml. of milk respectively. The production record was not reported but it was likely that the above assays were from quarters producing varying volumes of milk.

The penicillin milk levels in cow B following the infusion of 100,000 units in a water-in-oil emulsion, Table V, were 1, 0.25 and 0.12 units per ml. for the same amount at 24, 48 and 72 hourly intervals. This variation may be explained by the lack of production

records in the former data. Increasing the unitage to 200,000 or decreasing it to 50,000 units of penicillin did not vary the level significantly from that obtained after the infusion of 100,000 units of penicillin. This was difficult to explain but was similar to the levels following the infusion of penicillin in sterile water.

The next type of penicillin used was the soluble repository (S-R) type. The data of Table VI present the levels of penicillin in milk following the infusion of 25,000, 100,000, and 400,000 units of S-R penicillin per quarter. At 12, 36 and 60 hours levels of 32, 0.5 and 0.06; 80, 0.5 and 0.06; and 400, 4 and 0.06 units per ml. of milk existed respectively. The level of 0.06 units per ml. of milk was the same for the three different dosages at 60 hours, however, the 400,000 units dosage produced a final level of 0.03 units per ml. at 84 hours while the 100,000 units kept a level of 0.06 units per ml. through 72 hours.

The infusion of 300,000 units of procaine penicillin in 1 ml. of sterile peanut oil suspension maintained a level of 0.06 units per ml. or higher for 192 hours (8 days); Table VII. At 6, 24, 48, 96 (4 days), 144 (6 days), and 192 hours the existing levels were: 3,200, 64, 2, 0.5, 0.12 and 0.06 units of penicillin per ml. of milk. Levels of 100,000 and 200,000 units terminated with 0.03 units per ml. at 96 (4 days) and 120 hours (5 days), after yielding levels at 24 hours of 4 and 32 units per ml. respectively. Procaine penicillin in aqueous solution (300,000 units per ml.) was infused into the udder in 100,000, 200,000 and 300,000 unit amounts. Levels maintained at 24

hours were 16, 8, and 2 while those at 72 hours were 0.2, 0.12 and 0.06 units of penicillin per ml. of milk. A level of 0.06 units per ml. was present at 96 hours after the infusion of 300,000 units in 1 ml. of water. This was 12 hours longer than the terminal levels of the other two dosages in water.

The milk levels obtained after the administration of 25,000 units of penicillin in various vehicles were presented in Table VIII. In descending order the persisting levels of 0.03 or 0.06 units per ml. are as follows: S-R (60 hours), Penstix (48 hours) Mastic and 10 ml. water were equal (36 hours). Fifty and 100 ml. of water produced terminal levels of 0.5 and 0.06 units per ml. at 24 hours.

The vehicles reported in Table IX containing 50,000 units of penicillin maintained milk levels descending in the following order; ointment (84 hours) Penicle (72 hours), Penstix, Mastic, 10 ml., and 50 ml. water (48 hours) and 100 ml. water (36 hours).

Penicillin in 100,000 units amounts per quarter produced the level of 0.03 units per ml. of milk or higher in descending order: 1) procaine penicillin in peanut oil (96 hours), 2) penicle and ointment (84 hours), 3) aqueous procaine and S-R (72 hours), 4) mastic and 10 ml. water (60 hours), 5) 50 and 100 ml. water (48 hours). This data was presented in Table X.

TABLE V. The penicillin milk level in cow B following the infusion of 50,000, 100,000 and 200,000 units per quarter of an aqueous solution of penicillin in a water-in-oil emulsion (Penicle). Cow B was in the middle of her lactation period.

Ml . of vehicle infused	Units of penicillin used	<u>Hours after udder infusion</u>					
		6	12	24	36	48	60 72 84 96
		Units of penicillin per ml. of milk					
20	50,000	320	64	1	0.8	0.5	0.06 0.03 -0.03
20	100,000	320	64	1	0.5	0.25	0.12 0.06 -0.06
20	100,000	400	100	2	1	0.12	0.06 -0.06
20	200,000	400	64	1	0.5	0.12	0.06 -0.06

TABLE VI. The penicillin milk level in cow B (middle of lactation) following the infusion of 25,000, 100,000 and 400,000 units of Soluble Repository (S-R) penicillin per quarter, using sterile distilled water as a vehicle.

Mls. of water used	Units of penicillin infused	<u>Hours after udder infusion</u>									
		6	12	24	36	48	60	72	84	96	
Units of penicillin per ml. of milk											
5*	25,000	320	32	2	0.5	0.25	0.06	-0.06			
0.25	100,000	800	80	1	0.5	0.5	0.06	0.06	-0.06		
1.0	400,000	3200	800	16	4	1	0.12	0.1	0.03	-0.03	
1.0	400,000	3200	400	16	4	1	0.06	0.06	0.03	-0.03	

5* 0.25 ml. containing 100,000 units was diluted to 20 ml. so 5 ml contained 25,000 units.

TABLE VII. The penicillin milk level in cow B (middle of lactation) following the infusion of 100,000, 200,000 and 300,000 units of procaine penicillin in peanut oil and in sterile water as a vehicle; these amounts were suspended in 0.25, 0.5 and 1 ml. of each vehicle respectively.

Units of penicillin infused	<u>Hours after udder infusion</u>									
	6	12	24	36	48	72	96	120	144	192
	Units of penicillin per ml. of milk									
In oil:										
100,000	200	32	4	0.5	0.5	0.06	0.03	-0.03		
200,000	640	320	32	2	1	0.12	0.06	0.03	-0.03	
300,000	1600	400	40	4	3	1.5	1	0.4	0.4	0.1*
300,000	3200	320	64	16	2	1	0.5	0.25	0.12	0.06*
300,000	3200	320	64	16	2	1	0.5	0.25	0.12	0.06*
In water:										
100,000	800	200	2	2	0.25	0.06	-0.06			
200,000	1600	800	8	1	0.6	0.12	-0.06			
300,000	6400	1600	16	1	0.8	0.2	0.06	-0.06		

* less than 0.06 units per ml in next 24 hours.

TABLE VIII. A comparison of the penicillin milk levels in cow B(Middle of lactation) following the infusion of 25,000 units of penicillin in various forms and vehicles.
(Na for sodium and Pr. for procaine).

Type of penicillin	Vehicle (volume in mls.)	<u>Hours after udder infusion</u>				
		6	12	24	36	48
					60	72
<u>Units of penicillin per ml. of milk</u>						
Sodium	Water (10)	200	8	1	0.06	-0.06
Sodium	Water (50)	100	4	0.5	-0.06	
Sodium	Water (100)	64	2	0.06	-0.06	
Sodium	Mastic	200	8	1	0.06	-0.06
Procaine	Penstix	200	64	0.5	0.06	0.03
					-0.03	
Na. and Pr.	S-R	320	32	2	0.5	0.25
					0.06	-0.06

TABLE IX. A comparison of the penicillin milk levels in Cow B (middle of lactation) following the infusion of 50,000 units per quarter of penicillin in various forms and vehicles.

Type of penicillin	Vehicle (ml. volume)	Hours after udder infusion								
		6	12	24	36	48	60	72	84	96
Units of penicillin per ml. of milk										
Sodium	Water (10)	400	16	1	0.25	0.06	-0.06			
Sodium	Water (50)	400	8	0.5	0.1	0.03	-0.03			
Sodium	Water (100)	200	4	0.12	0.06	-0.06				
Sodium	Penicile (20)	320	64	1	0.8	0.5	0.06	0.03	-0.03	
Ointment (Sodium)	Petrolatum	128	64	2	1.5	1	0.5	0.06	0.06	-0.06
Procaine	Penstix	400	64	2	0.5	0.06	-0.06			
Sodium	Mastic	400	32	1	0.1	0.03	-0.03			

Determinations in Cow D: Cow D, a Holstein giving an average of 45 pounds of milk daily, was used because she was at the beginning of the lactation period. The penicillin levels of Table XI were somewhat unusual; 25,000 units in 5, 10, 50 and 100 ml. of sterile water all give the same level (0.06) at 24 hours. The levels produced by both 50,000 and 100,000 units terminated at 36 hours (0.03) in 10, 50 and 100 ml. regardless of the unitage or the volume of the vehicle used. Packer (25) gives the summaries of 107 separate trials using 44 different quarters, with striking similarity of the penicillin levels at either 12 or 24 hours after the injection of various doses. Average readings obtained on milk taken 12 hours after injection of either 50,000 or 100,000 units were identical. He reported no marked differences in the quantity of penicillin 24 hours after the infusion of 25,000 or 200,000 units, and that these observations would suggest that there may be "threshold levels" maintained in the udder which may be somewhat independent of dosage. Other data previously reported show that much more penicillin actually leaves the udder in a low producing cow than in a high producing one, resulting in about the same level of penicillin in each case at 24 hours after the injection. This observation does not agree with the recommendation of Schalm of larger doses (33) for cows in high production in order to maintain a high concentration of the drug in the milk. Murnane (21) presented data showing that 95 to 97 per cent of a 15,000 unit injection was absorbed at the end of 12 hours in a cow in the middle stage of lactation, whereas, a cow in the "drying off" stage demonstrated only 68-70 per cent absorption of the total infusion. That the increased concentration of penicillin following its infusion with large quantities of water suggested that

the infused water is rapidly absorbed from the gland leaving most of the penicillin behind was an observation of Weirether et al. (46). The preceding study also revealed that the rate of disappearance of penicillin from pathologic glands presented the same picture as for the normal except in coliform infections (decline greater). Only when the total of 455,000 units was infused could there be any penicillin (0.06 units per ml.) detected in the blood.

Although somewhat lower, the data in Table XI compare favorably with that of Packer (25) and Little et al. (19); also Schofield (34). Little et al. (19) reported levels of 3.75 and 16.4 units per ml. at the end of 12 hours following the infusion of 25,000 and 50,000 units in 50 ml. water, respectively. This compares favorably with levels in Table XI of 4 and 2 units in similar order.

Available in Table XII are data on levels in milk following the insertion of penicillin in solid and semi-solid forms. Ointment containing 100,000 units penicillin again maintained a level twice as long (72 hours) as did either Mastic or Penstix (36 hours). With the exception of 25,000 units in Penstix (24 hours) all bougies produced a level that terminated after 36 hours. Bougies containing 200,000 units of penicillin gave a high level of 4 units per ml. after 24 hours and 0.03 units per ml. after 60 hours as compared to 0.12 and 0.0 for 100,000 units. Trussell and Stevenson (43) reported an average level of 5.4 units, compared to 8 units per ml. in Table XII following the insertion of 25,000 units of penicillin in bougie form in a large number of quarters. Barnum (3) found 1.2 units per ml. upon the assay of samples of milk from a cow producing 40 pounds of milk following the use of the bougie containing 25,000 units. Six hours after insertion of a 25,000

TABLE XI. The penicillin milk level in cow D (following the infusion of 25,000, 50,000 and 100,000 units per quarter of penicillin in varying amounts of sterile distilled water as vehicle. Cow D was at the beginning of her third lactation.

Ml. of water infused	Units of penicillin used	Hours after udder infusion				
		6	12	24	36	48 60
Units of penicillin per ml. of milk						
50	25,000	32	2	0.06	-0.06	
10	"	64	4	0.06	-0.06	
50	"	64	4	0.06	-0.06	
100	"	32	1	0.06	-0.06	
10	50,000	100	4	0.12	0.03	-0.03
50	"	200	2	0.06	0.03	-0.03
100	"	32	1	0.12	0.03	-0.03
10	100,000	128	8	0.5	0.06	-0.06
50	"	50	1	0.06	0.03	-0.03
100	"	64	1	0.1	0.03	-0.03

unit procaine penicillin bougie Bolton et al. (4) were able to demonstrate 275 units per ml. which compared to 200 units per ml. for the same period in Table XII.

It is difficult to compare the data of Table XIII with those published by Foley et al. (11) due to critical variations in the levels obtained. It is likely that the 8 quarters they infused were not in high production. The level reported in a dry cow was not much, if any, higher than the other levels. In a water-in-oil emulsion (20 ml.) 200,000 units of penicillin did not produce a level any appreciably higher than did 50,000 and 100,000 unit infusions. The reported object of this water-in-oil emulsion was to transport the penicillin high into the alveoli of the udder by negative geotropism. It was evident in a report by Murmane (21) that just sterile oil did not produce any higher levels than did plain water.

It is difficult to explain the higher levels obtained by rediluting, to a volume of 5 and 10 ml., quantities of S-R penicillin. These data, presented in Table XIV, were collected following the infusion of diluted and undiluted S-R penicillin into the udder of cow D. Schalm et al. (32) found a considerable variation in the penicillin concentration per ml. of foremilk between quarters of the same udder. The secretory activity of the various quarters was not impaired in any way; therefore, the variations in penicillin content of the foremilk among quarters of the same udder cannot be attributed to difference in milk volume. Although the undiluted infusion (0.25 ml.) maintained a much higher level at 24 hours (2 units per ml.) than the same amount diluted in 10 ml. of sterile water (0.5) it terminated 12 hours sooner (36 hours).

The highest levels produced in cow D, recorded in Table XV, resulted from the infusion of procaine penicillin in sterile peanut oil. Each ml. contained 300,000 units of procaine penicillin and was divided into 0.33 and 0.66 ml. to obtain 100,000 units and 200,000 units. One ml. of the above maintained a very high level, terminating at 72 hours, one-third of this amount maintained a lower level for 60 hours. The data presented in Table XV indicated that a water suspension was equally as efficient in producing a long level as was oil. This was not true in cow B where the level was maintained eight days or twice as long after the injection of the oil suspension.

The data presented in Table XVI were the only results obtained after penicillin infusion in cow C. The ~~left~~ front quarter on cow C was partially blind. There were tight sphincters at the opening of each teat and much difficulty was experienced inserting some of the bougies used. The cow was highly nervous even when drawing samples so it was decided not to use her as she produced exactly the same volume of milk each day as did cow B. The results in Table XVI are comparable to those obtained in cow B (Table IV).

The data of Tables XVII, XVIII and XIX agree with Packer's (25) report that 24 hours after the infusion of 25,000, 50,000 and 100,000 units of penicillin in aqueous solution it would be difficult to predict which quarter had received either of the doses as an original injection. For example, 24 hours after the injection of the three above amounts in 50 ml. of water the same level (0.06) was present for all three. Thorp et al. (42) found that there was less variation in the assays for penicillin when 100 ml. of diluent was employed, while the most variations were encountered when 20 ml. of

diluent was used. This could have been due to the varying dilution factors of the milk secretion.

Both the procaine and penicillin bougies containing 25,000 units gave levels at 36 hours or 12 hours longer than was present after any of the three aqueous infusions. This was not true for the 50,000 and 100,000 unit bougies, their final level for these two amounts were the same (36 hours) as the levels of the water solutions.

It was shown in the data in Tables XVII, XVIII and XIX that 25,000 and 100,000 units of penicillin in water solution and suspended in a water-in-oil emulsion gave levels (0.06 units per ml.) in each vehicle 36 and 48 hours after infusion, respectively. These levels are twelve hours longer than those obtained when the penicillin was given in water solution or either bougies. Whereas, at 36 hours after instillation of 50,000 units in a water-in-oil emulsion the final levels for all of the water dilutions, bougies and emulsions were the same. In this case, however, the emulsion maintained a much higher level through 24 hours (4 units per ml.) compared to the water and bougies (0.12 and 0.06 respectively).

Data on 50,000 and 100,000 unit levels revealed that penicillin ointment produced the higher levels up to 36 hours after insertion, 0.1 and 0.5 respectively, when compared to procaine penicillin in oil or S-R penicillin. Thirty-six hours after injection of 100,000 units of the latter two there was a level of 0.12 and 0.06 respectively, although oil maintained a level for the same length of time (60 hours) as the ointment did.

TABLE XIII. The penicillin milk levels in cow D (beginning of lactation) following the infusion of 25,000, 50,000, 100,000 and 200,000 units per quarter of an aqueous solution of penicillin in 20 ml. of a water-in-oil emulsion(Penicile).

Units of penicillin infused (20 ml. Penicile) (as the vehicle)	Hours aft er udder infusion					
	6	12	24	36	48	60 72
	Units of penicillin per ml. of milk					
25,000	4	0.5	0.06	0.06	-0.03	
50,000	32	16	4	0.03	0.03	-0.03
100,000	50	32	4	0.06	0.06	-0.06
100,000	50	8	1	0.06	0.06	0.03 -0.03
200,000	64	16	1	0.1	0.06	0.03 -0.03

TABLE XIV. The penicillin milk levels in cow D (beginning of lactation) following the infusion of 25,000, 50,000, 100,000, 200,000 and 400,000 units per quarter of S-R penicillin (400,000 units per ml.). One ml. was diluted with sterile water to obtain the higher dilutions.

Mls of water	Units of penicillin	<u>Hours after udder infusion</u>					
		6	12	24	36	48	60
infused	used	Units of penicillin per ml. of milk					
5	25,000	4	1	0.25	0.06	0.03	-0.03
10	50,000	8	2	0.25	0.06	0.03	-0.03
10	100,000	32	4	0.5	0.06	0.06	-0.06
0.25	100,000	320	4	0.25	0.06	-0.06	
0.25	100,000	200	8	2	0.06	-0.06	
1	400,000	1600	32	2	0.25	0.03	-0.03
1	400,000	800	16	2	0.25	0.06	-0.06

TABLE XV. The penicillin milk levels in cow D (beginning of lactation) following infusion of 100,000, 200,000 and 300,000 units per quarter of procaine penicillin. The procaine penicillin was suspended in sterile peanut oil and also in sterile water; each ml. contained 300,000 units and was injected in 0.33, 0.66 and 1 ml. amounts.

Units of penicillin	Hours after udder infusion						
	6	12	24	36	48	60	84
In oil:	Units of penicillin per ml. of milk						
100,000	200	10	1	1	0.06	0.03	-0.03
100,000	100	10	2	0.12	0.06	0.06	-0.06
200,000	320	80	8	1	1	0.03	-0.03
300,000	3200	400	32	8	1	0.12	0.06
300,000	3200	200	40	1	0.5	0.5	-0.06
In water:							
100,000	400	50	1	0.06	0.03	-0.03	
200,000	800	100	1	0.12	0.1	0.06	0.03
300,000	1600	200	3	0.25	0.12	0.1	-0.06

TABLE XVI. The penicillin milk level in cow C (middle of lactation) following the insertion of 25,000, 50,000 and 100,000 units per quarter of crystalline sodium penicillin in bougie form(Mastic).

Units of penicillin used	<u>Hours after insertion in udder</u>							Units of penicillin per ml. of milk
	6	12	24	36	48	60	72	
25,000	200	32	1	0.25	0.06	-0.06		
50,000	800	32	1	0.12	0.06	-0.06		
100,000	300	64	1	0.25	0.12	0.06	-0.06	
100,000	1600	64	2	0.12	0.12	0.03	-0.03	

TABLE XVII. A comparison of the penicillin milk levels in cow D (beginning of lactation) following the infusion of 25,000 units per quarter of crystalline sodium penicillin G in various forms and vehicles. Penstix and S-R contain procaine penicillin.

Type of penicillin used	Type of vehicle	<u>Hours after udder infusion</u>						
		6	12	24	36	48	60	72
inrused (ml. volume)		Units of penicillin per ml. of milk						
Sodium	Water (10)	64	4	0.06	-0.06			
Sodium	Water (50)	64	4	0.06	-0.06			
Sodium	Water (100)	32	1	0.06	-0.06			
Procaine	Penstix	200	4	0.06	-0.06			
Sodium	Penicle (20)	4	0.5	0.06	0.03	-0.03		
Sodium	Mastic	50	8	0.06	0.03	-0.03		
Sodium and Procaine	S-R (5)	4	1	0.25	0.06	0.03	-0.03	

TABLE XVIII. A comparison of the penicillin milk levels in cow D (beginning of lactation) following the infusion of 50,000 units per quarter of penicillin in various forms and vehicles.

Type of penicillin	Type of vehicle	Hours after udder infusion						
		6	12	24	36	48	60	72
Units of penicillin per ml. of milk								
Sodium	Water (10)	100	4	0.12	0.03	-0.03		
Sodium	Water (50)	200	2	0.06	0.03	-0.03		
Sodium	Water (100)	32	1	0.12	0.03	-0.03		
Sodium	Mastic	100	8	0.06	0.03	-0.03		
Procaine	Penstic	200	8	0.06	0.03	-0.03		
Procaine & Sodium	Water (10)	8	2	0.25	0.06	0.06	-0.06	
Sodium	Penicle (20)	32	16	4	0.03	0.03	-0.03	
Sodium	Ointment	64	8	1	0.1	0.06	-0.06	

TABLE XIX. A comparison of the penicillin milk levels in cow D (beginning of lactation) following the infusion of 100,000 units per quarter of crystalline sodium penicillin G in various forms and vehicles.

Type of penicillin	Type of vehicle (volume ml.)	6	<u>Hours after udder infusion</u>							
			12	24	36	48	60	72		
		Units of penicillin per ml. of milk								
Sodium	Water (10)	128	8	0.5	0.06	-0.06				
Sodium	Water (50)	50	1	0.06	0.03	-0.03				
Sodium	Water (100)	64	1	0.1	0.03	-0.03				
Sodium	Mastic	64	4	0.06	0.06	-0.06				
Procaine	Penstix	400	8	0.25	0.06	-0.06				
Sodium and Procaine	S-R (0.25)	320	4	0.25	0.06	-0.06				
Procaine	Water (0.33)	400	50	1	0.06	0.03	-0.03			
Sodium	Penicle	50	32	4	0.06	0.06	-0.06			
Sodium and Procaine	Water(10)	32	4	0.5	0.06	0.06	-0.06			
Procaine	O il (1)	100	10	2	0.12	0.06	0.06	-0.06		
Sodium	Ointment	200	32	2	0.5	0.12	0.03	-0.03		

Comparison of the Stages in Lactation: Problem number two in this work was to compare the effects of the stages of lactation on the levels obtained after udder infusion of various amounts of penicillin in different vehicles.

The data presented in Tables I, III and XI compared the levels obtained after udder infusion of 25,000, 50,000 and 100,000 units of penicillin in 10, 50, and 100 ml. of sterile water. Practically all the data showed the definite trend of the quarters producing the smaller amounts of secretion maintained penicillin levels longer and higher than did the quarters in higher production. For example, 50,000 units in 50 ml. of water, cow D (45 pounds of milk daily) maintained a level for 36 hours, cow B (15 pounds of milk daily) showed a level for 48 hours and cow A (2 pounds of milk daily) had a final level at 60 hours. At 24 hours the levels were 4, 0.5 and 0.06 units of penicillin per ml. respectively. If the level was not present for a longer period it was usually maintained at a higher level during the major part of that period as in 25,000 and 50,000 units in 100 ml. infused in cows B and D. At 12 hours the levels were 4 and 1, and 2 and 1 respectively, although the final level disappeared at the same periods (24 and 36 hours in respect to the initial dosage). Murnane (21) stated that the penicillin concentration within the udder at any time after administration was inversely related to milk volume, and the rate of absorption. This would explain the trend of the penicillin levels in Tables I, III and XI. Schalm (31) points out that the rate of absorption varied with individual quarters of the same udder, and Weirether (46) found the rate equal in infections, except for coliform, to that of the normal. The suggestion of "threshold levels" by Packer (25) would explain why

higher unitage does not produce longer levels in individuals and, therefore, suggested that frequency of administration was much more important than the quantity (within limits) of penicillin injected in maintaining substantial levels in the udder.

Data on the levels after insertion of solid forms of penicillin vehicle in the mammary gland was presented in Tables II, IV, XII and XVI. The same trend was evident with bougie administration as was with water vehicles. After being in the quarters 24 hours a 25,000 unit bougie containing procaine penicillin gave levels of 8, 0.5 and 0.06 units per ml. in cows A, B and D respectively and the final assays were at 72, 48 and 24 hours in similar order. But, a penicillin bougie containing the same amount produced a terminal level of 0.06 and 0.03 units per ml. in both cows B and D at 36 hours. The same reasons for the trend of the levels in water vehicles was evident with bougies. Presumably, the bougies quickly released the penicillin contained and it went into solution with the water in the milk, which is the same situation as occurs with water solutions.

There was no indication of a change in the trend of higher levels in lower producers in the data of levels obtained after intramammary administration of penicillin ointment, aqueous solutions in a water-in-oil emulsion, procaine penicillin in oil and in water, or S-R penicillin. These data are compiled in Tables II, V, VI, VII, XII, XIII, XIV and IV.

A level in cow B was still evident 84 hours after infusion of 100,000 units of procaine penicillin in 0.33 ml. of peanut oil, whereas, the same amounts instilled in cow D produced a level for only 48 hours. An increase to 200,000 units did not increase

the level enough to warrant use of that amount; the levels were of the same duration as those just discussed in both cows. This was the same conclusion made by Thorp et al. (42) for the same number of units in water vehicle.

It is questionable as to the value of the lower levels of penicillin maintained in the udder. These low levels may inhibit the infectious organisms in a test tube, but Stevenson (4) stated that 0.5 units per ml. was necessary in the udder for therapeutic value.

The data compiled in Tables XX and XXI attempts to compare all penicillin levels of the various vehicles.

Cow	Penicillin(units)	Vehicle		Hours:	12	24	36	48	60	72	84	96
		(vol.ml)	(vol.ml)									
B	25,000	Water	(10)	200	8	1	0.06	-0.08				
D	"	"	"	64	4	0.06	-0.06					
A	50,000	"	"	200	64	16	2	0.5	0.12	0.06	-0.06	
B	"	"	"	400	16	1	0.25	0.06	-0.06			
D	"	"	"	100	4	0.12	0.03	-0.03				
A	100,000	"	"	1600	400	32	8	1	0.5	0.25	0.06	-0.06
B	"	"	"	400	32	4	1	0.25	0.12	-0.06		
D	"	"	"	100	4	0.06	0.03	-0.03				
A	25,000	Water	(50)	256	64	1	0.12	-0.06				
B	"	"	"	100	8	1	0.03	-0.03				
D	"	"	"	64	4	0.06	-0.06					
A	50,000	"	"	128	32	4	0.5	0.1	0.06	-0.06		
B	"	"	"	400	8	0.6	0.1	0.03	-0.03			
D	"	"	"	200	2	0.06	0.03	-0.03				
A	100,000	"	"	800	200	16	2	0.5	0.25	0.06	-0.06	
B	"	"	"	400	8	2	1	0.06	-0.06			
D	"	"	"	50	1	0.06	0.03	-0.03				
A	25,000	Water	(100)	128	64	1	0.1	-0.06				
B	"	"	"	64	2	0.06	-0.06					
D	"	"	"	32	1	0.06	-0.06					
B	50,000	"	"	200	4	0.12	0.06	-0.06				
D	"	"	"	32	1	0.12	0.03	-0.03				
A	100,000	"	"	800	128	8	1	0.5	0.06	0.03	-0.03	
B	"	"	"	200	3	1	0.12	0.03	-0.03			
D	"	"	"	64	1	0.1	0.03	-0.03				
B	100,000(S-R)	Water	(0.25)	800	80	8	1	0.5	0.06	0.06	-0.06	
D	100,000(S-R)	"	"	200	8	2	0.06	-0.06				
B	400,000(S-R)	Water	(1)	3200	800	16	4	1	0.12	0.1	0.03	-0.03
D	400,000(S-R)	"	"	800	16	2	0.25	0.06	-0.06			

TABLE XX. Penicillin milk levels following intra-mammary infusion in water vehicles.

Units	Vol.(mL) Level	12	24	36	48	60	72	84	96
Cow	Penicillin Vehicle	6							
B	25,000 Mastic	200	1	0.06	-0.06				
D	"	50	0.06	0.03	-0.03				
B	50,000	400	1	0.1	0.03	-0.03			
D	"	100	0.06	0.03	-0.03				
A	100,000	1600	16	1	0.5	0.25	0.03	0.03	-0.03
B	"	400	2	0.5	0.03	0.03	-0.03		
D	"	64	0.12	0.06	-0.06				
A	25,000 Penstix	512	8	0.5	0.5	0.06	0.03	-0.03	
B	"	200	0.5	0.06	0.03	-0.03			
D	"	200	0.06-0.06						
B	50,000	400	2	0.5	0.06	-0.06			
D	"	200	0.06	0.03	-0.03				
D	100,000	400	0.25	0.06	-0.06				
B	50,000 Ointment	128	2	1.5	1	0.5	0.06	0.06	-0.06
D	"	64	1	0.1	0.06	-0.06			
B	100,000	200	2	1.3	0.8	0.6	0.12	0.06	-0.06
D	"	200	2	0.5	0.12	0.03	-0.03		
B	50,000 Penicle(20)	320	1	0.8	0.5	0.06	0.03	-0.03	
D	"	32	4	0.03	0.03	-0.03			
B	100,000	400	2	1	0.12	0.06	0.06	-0.06	
D	"	50	4	0.06	0.06	-0.06			
B	200,000	400	1	0.5	0.12	0.06	0.06	-0.06	
D	"	64	1	0.1	0.06	0.03	-0.03		
Procaine:									
B	100,000 Peanut oil	200	8	0.25	0.12	0.06	0.06	-0.06	
D	"	100	2	0.12	0.06	0.06	-0.06		
B	Water(0.33)	800	2	0.06	0.25	0.06	0.06	0.06	-0.06
D	"	400	1	0.06	0.03	-0.03			
B	200,000 (0.66)	1600	8	1	0.6	0.12	0.12	0.06	-0.06
D	"	800	1	0.12	0.1	0.06	0.03	-0.03	
B	300,000 (1)	6400	16	1	0.8	0.25	0.2	0.1	0.06 -0.06
D	"	1600	3	0.25	0.12	0.1	0.06	-0.06	24 hrs.
B	200,000 Peanut oil	640	32	1	1	0.25	0.12	0.06	0.06 longer
D	"	320	8	1	1	0.03	-0.03		

TABLE XXI. Penicillin milk levels following intramammary in various vehicles.

SUMMARY AND CONCLUSIONS

The highest and most persistent levels were produced following the instillation of 300,000 units of procaine penicillin in 1 ml. of sterile peanut oil. In cow B (middle of lactation) a level of greater than 0.06 units per ml. of milk was assayed 8 days after udder infusion. In cow D (beginning of lactation) the level after the same infusion was 0.06 units of penicillin per ml. at the 72 hour period. Data on procaine penicillin in sterile water suspension in cow D showed the same level as did oil but it was not maintained at the same height. After 36 hours the level of procaine penicillin in oil was 4 units per ml. compared to that of procaine penicillin in water of 0.25 units per ml.

There were no appreciable variations in the levels of penicillin per ml. of milk 24 hours after udder infusion of 25,000, 50,000 and 100,000 units of penicillin in similar amounts of water vehicle. The levels following the instillation of penicillin in 10 ml. of water were maintained at a higher level for the first 24 hours than after infusion of 50,000 or 100,000 ml. in the same amount of vehicle, although the final period maintaining an assayable level was the same in cow D.

Penicillin in an ointment base produced a level in cow B of 0.06 units per ml. 84 hours after udder insertion of both 50,000 and 100,000 units, whereas, in cow D the level 0.03 units per ml. of milk was present at 60 hours.

An increase to 200,000 units as compared to 100,000 units of penicillin in a water-in-oil emulsion did not produce a higher or

longer level of penicillin per ml. of milk in cow B. After 24 hours the levels were 2 and 1 units respectively per ml. and the final levels for both were 0.06 units per ml. at the 60 hour period.

Levels of 3,200 and 800 units per ml. of milk were assayed 6 hours after the administration of 400,000 units of S-2 penicillin in 1.0 ml. of sterile water, in cows B and D respectively. The terminal level in cow B was 0.03 units per ml. at 84 hours, whereas, 200,000 and 300,000 units of procaine penicillin alone, in water, gave a level of 0.06 and 0.1 units per ml. respectively at the same period.

There were no clinical signs of irritation observed after any of the vehicles containing penicillin, except in cow B when the 100 ml. quantity of sterile water was used. Similar quantities of penicillin in 10 and 50 ml. of the water yielded no reaction.

In this experiment it was proven that the concentration of penicillin per ml. of milk in the udder was inversely proportional to the volume of milk produced. There was not an appreciable advantage 24 hours after administration of 100,000 units over 50,000 and 25,000 units in the same vehicle.

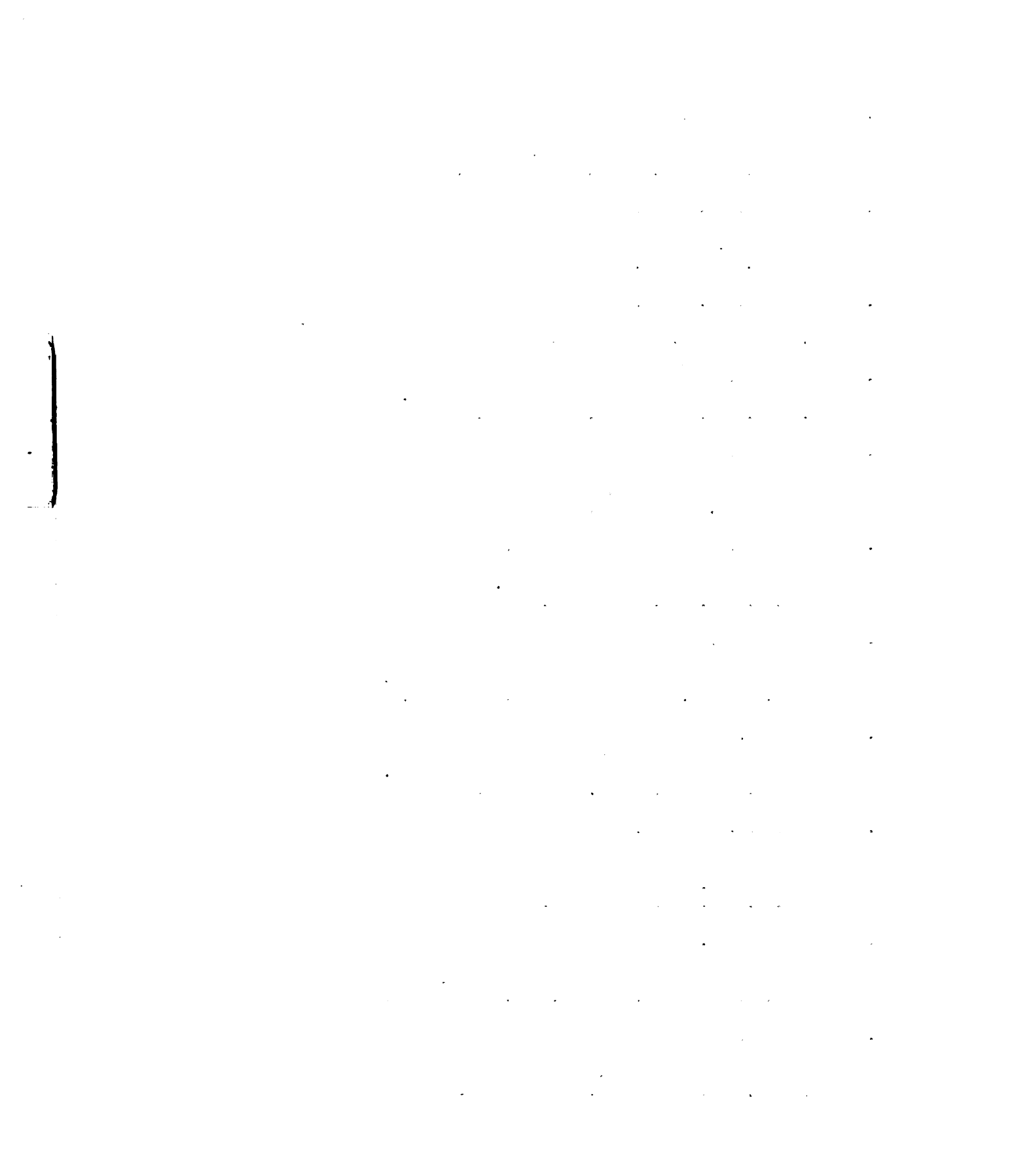
BIBLIOGRAPHY

1. Barker, C. A. V. (1945)
Observations on the use of penicillin in the treatment of bovine mastitis.
Canad. J. of Comp. Med. and Vet. Sci. 9:235-243.
2. Barker, C. A. V., and H. P. Dussault (1945)
Observations on the permeability of the lactating bovine mammary gland to penicillin.
Canad. J. of Comp. Med. and Vet. Sci. 9:332-335.
3. Barnum, D. A. (1947)
Preliminary observations on the use of the penicillin bougie in the bovine udder.
Canad. J. of Comp. Med. and Vet. Sci. 11:65-67.
4. Bolton, W. D., J. M. Frayer, J. H. Cady and E. F. Waller (1949)
Procaine penicillin G levels in the udder during treatment of chronic mastitis.
Vet. Med. 44:244-245.
5. Bryan, C. S. (1946)
Penicillin nasties in the treatment of chronic streptococcic mastitis.
Vet. Med. 41:429-432.
6. Bryan, C. S., J. W. Cunkleman, F. W. Young, and E. E. Visger (1945)
The treatment of acute infectious bovine mastitis with penicillin.
Vet. Med. 41:94-98.
7. Byrne, J. L., F. J. Pullin and H. Konisk (1946)
Studies in infectious mastitis: I. Penicillin as a therapeutic agent.
Canad. J. of Comp. Med. and Vet. Sci. 10:140-142.
8. Farrag, H. H. (1948)
The action of penicillin in vitro on organisms found in bovine mastitis.
J. of Amer. Vet. Med. Assoc. 112:371-374.
9. Fleming, Alexander (1946)
Penicillin, Its Practical Application, chapter: Pharmacy of penicillin, 46-58.
Chapel River Press, Andover, Great Britain.
10. Foley, E. J., and S. W. Lee (1948)
Factors concerned in streptococcal growth in the bovine udder and their relation to pathogenesis and treatment of bovine mastitis by penicillin.
Cornell Vet. 38:367-379.

11. Foley, E.J., A. W. Stults, S. W. Lee, and D. J. Bryne (1949)
Studies of vehicles for sustaining penicillin levels in the
bovine mammary gland.
Amer. J. of Vet. Res. 10:66-70.
12. Foster, J. W., and H. B. Woodruff (1945)
Microbiological aspects of penicillin. I. Methods of assay.
J. Bact. 46:187-202.
13. Cerber, I. E., G. Schwartzma, and G. Balhr (1946)
Penetration of penicillin in foci of infection.
J. of Amer. Med. Assoc. 8:257-259.
14. Heishman, J. D. (1947)
Sensitivity to penicillin of microorganisms associated with
bovine mastitis.
Amer. J. of Vet. Res. 8:257-259.
15. Kakavas, J. C. (1944)
Penicillin in the treatment of bovine mastitis.
North Amer. Vet. 25:408-412.
16. Klein, L. A., D. W. Crisman, and J. W. Moor (1945)
Effect of local injections of penicillin on staphylococci in
the cows udder.
Amer. J. of Vet. Res. 6:3-8.
17. Langer, P. H., R. L. Burkhart, C. R. Schroeder, and M. Welsh (1948)
Blood levels following intramammary infusion of penicillin.
J. Dairy Sci. 31:103-107.
18. Lee, S. W., E. J. Foley, and E. R. Caley (1945)
Fissibactericidal nature of penicillin action.
Nature. 136:49-50.
19. Little, R. B., and W. H. Plastringe (1946)
Bovine mastitis.
McGraw-Hill, N. Y. First edition. Chapt. XII. Treatment of
mastitis. 363-416.
20. Murnane, D. (1945)
A preliminary report on the treatment of clinical and subclinical
streptococcal and staphylococcal infections of the bovine udder
with penicillin.
21. Murnane, D. (1946)
Second report on the treatment of clinical streptococcal and
staphylococcal infections in the bovine udder with penicillin.
Aust. Vet. J. 22:35-40.
22. Murnane, D. (1946)
Clinical bovine mastitis, its treatment and control.
Aust. Vet. J. 22:156-168.

23. Murphy, J. M., and K. O. Pfau (1945)
The value of local injections of penicillin in the treatment of streptococci agalactiae infections and mastitis in cows.
Cornell Vet. 35:87-102.
24. Packer, R. Allen (1948)
Penicillin therapy in chronic bovine mastitis.
I. Sensitivity of bovine mastitis organisms to the action of penicillin.
Amer. J. Vet. Res. 9:140-143.
25. Packer, R. Allen (1948)
Penicillin therapy in chronic bovine mastitis.
II. Penicillin levels in the udder during treatment.
Amer. J. Vet. Res. 9:259-263.
26. Packer, R. Allen (1948)
Penicillin therapy in chronic bovine mastitis.
III. Treatment of mastitis.
Amer. J. Vet. Res. 9:264-269.
27. Petersen, W. E. (1932)
Osmotic pressure and milk secretion.
Proc. Soc. Exp. Bio. and Med. 30:259-264.
28. Porter, J. J., H. M. Campbell, H. F. Weber, and T. F. Reutner (1946)
Dosages of penicillin for streptococci agalactiae mastitis.
J. Amer. Vet. Med. Assoc. 109:60-64.
29. Porter, J. J., and A. C. Kelman (1947)
Effect of volume of distilled water as vehicle for penicillin in treating chronic streptococcal mastitis.
J. Amer. Vet. Med. Assoc. 110:246-248.
30. Randall, W. A., C. W. Price, and H. Welch (1945)
The estimation of penicillin in body fluids.
Science. 101:365-366.
31. Schalm, O. W. (1945)
Recommendations for the use of penicillin in the treatment of bovine mastitis.
Vet. Student. 8:99-101.
32. Schalm, O. W. and N. H. Casselberry (1946)
Treatment of streptococcal agalactiae infection with intramammary infusions of penicillin.
J. Amer. Vet. Med. Assoc. 109:470-477.
33. Schofield, Frank W. (1946)
Penicillin in the treatment of bovine mastitis.
Canad. J. Comp. Med. and Vet. Sci. 10:63-70.

34. Schofield, Frank W., and D. A. Barnum (1946)
Limitations in the use of penicillin in the treatment and eradication of bovine mastitis.
J. Amer. Vet. Med. Assoc. 108:413-421.
35. Seeley, H. W. Jr., E. O. Anderson, and W. N. Plastring (1945)
Non-permeability of the lactating bovine mammary gland to penicillin.
Science. 102:44-45.
36. Seeley, H. W. Jr., E. O. Anderson, and W. N. Plastring (1945)
Action of penicillin against mastitis organisms in milk.
J. of Dairy Sci. 28:887-891.
37. Slanetz, L. W. and F. E. Allen (1945)
Treatment of bovine mastitis with penicillin.
J. Amer. Vet. Med. Assoc. 107:18-21.
38. Spencer, G. R., and M. E. Kraft (1948)
Penicillins, G, K, and F in the treatment of chronic bovine streptococcic mastitis.
Cornell Vet. 38:358-363.
39. Spencer, G. R., M. E. Kraft, and G. K. L. Underbjerg (1947)
Efficiency of intramammary infusions with penicillin and diluents on streptococcic mastitis.
Amer. J. Vet. Res. 8:325-328.
40. Stevenson, W. G. (1946)
The concentration of penicillin in foremilk following intramammary infusion for the treatment of mastitis.
Canad. J. Comp. Med. and Vet. Sci. 10:82-83.
41. Stults, A. W., and E. J. Foley (1948)
Treatment of streptococcus agalactiae mastitis by single injections of penicillin in retaining vehicles.
J. Amer. Vet. Med. Assoc. 113:68-72.
42. Thorp, W. T.S., Irene J. Uhrig, and E. J. Straley (1947)
Concentrations of penicillin in the bovine mammary gland following infusion and penicillin tolerance of certain streptococci.
Amer. J. Vet. Res. 8:157-165.
43. Trussell, Paul C., and W. G. Stevenson (1949)
The penicillin concentration in milk following insertion of penicillin bougies into the bovine udder.
Canad. J. Comp. Med. and Vet. Sci. 13:127-130.
44. Walker, J. W. (1946)
Penicillin in peanut oil with 3% beeswax, local use in bovine staphylococcic mastitis.
J. Amer. Vet. Med. Assoc. 108:413-421.



45. Watts, P. S., and D. H. McLeod (1946)
The estimation of penicillin in blood serum and milk of bovines
after intramuscular injections.
J. Comp. Path. and Therap. 56:170-179.
46. Weirether, F. J., D. E. Jasper, and W. E. Petersen (1945)
Effect of infused penicillin in the bovine mammary gland.
Proc. Soc. Exp. Biol. and Med. 59:282-286.
47. Welsh, M., P. H. Langer, R. L. Burkhart, and C. R. Schroeder (1948)
Penicillin blood and milk concentrations in the normal cow
following parenteral administration.
Science. 108:185-187.

ROOM USE ONLY

MAY 26 1962

ROOM USE ONLY

MICHIGAN STATE UNIVERSITY LIBRARIES



3 1293 03083 2293