TABLE I.
Ulcer Formation in Intestines by Enzyme Solutions under 90 cm H_2O Pressure.

Dog No.	0.1% solution of	pH	Time exposed (min.)	Remarks	Severity
1-12	rennin* in N/10 HCl	1.2	60-180	perforated ulcer in all dogs	4.0 in all dogs
13	trypsin† in N/10 $NaHCO_3$	8.1	580	necrosis through ½ thickness of mucosa entire area	2.0
14	" " " "	"	698	necrosis through ⅓ thickness of mucosa over entire area, 10 small spots into submucosa	2.0
15-17	" " " "	"	326-384	normal mucosa	0
18	" " " "	"	696	necrosis through ⅓ thickness of mucosa entire area, 5 small spots into submucosa	2.0
19	" " " "	"	694	surface necrosis over ¾ area of loop	1.0
20-21	" " " "	"	690-692	normal mucosa	0
22	erepsin† in N/10 $NaHCO_3$	"	640	surface necrosis	1.0
23-24	" " " "	"	636-638	normal mucosa	0
25	" " " "	"	634	necrosis entire mucosa, some patches into muscle	3.0
26-30	" " " "	"	284-632	normal mucosa	0
31-41	steapsin† in N/10 $NaHCO_3$	"	458-810	" "	0
42-53	Amylopsin† in N/10 $NaHCO_3$	"	290-745	" "	0
54-65	N/10 HCl	1.0	91-320	perforated ulcer in all dogs	4.0 in all dogs

* Armour, Rennet, N.F.
† Pfanstiehl.

alkaline solution alone.

Summary. 1. The proteolytic enzymes, rennin, trypsin, and erepsin, under hydrostatic pressure of 90 cm H_2O produced necrosis of the intestines of dogs, rennin being the most active of the three. 2. The average time required to produce a perforation with N/10 HCl alone was about 50% more than with rennin in N/10 HCl. 3. Neither steapsin nor amylopsin caused necrosis under the above condition.

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Effect of Infused Penicillin in the Bovine Mammary Gland.*

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Reports[1-3] have been published dealing with the therapy of bovine mastitis by infusion of penicillin but none has been found that deals with the physiologic behavior of this antibiotic when introduced into the mammary gland. This report deals primarily with studies on the rate of decline and irritating effects of infused penicillin. A knowledge of both of these aspects is essential to a rational approach to the use of the drug in therapy.

For the majority of experiments the sodium salt of penicillin was made up with sterile distilled water so that 1 ml of solution contained 5,000 Oxford Units. Immediately after

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1 Kakavas, J. C., *North Am. Vet.*, 1944, **25**, 408.

2 Klein, L. A., Crissman, D. W., and Moor, J. W., *Am. J. Vet. Research*, 1945, **6**, 3.

3 Bryan, C. S., Horwood, R. E., and Huffman, C. F., *Vet. Med.*, 1945, **40**, 87.

milking infusions were made via the teat canal, in 5, 10, 30, 40, 50, 100, and 200 thousand unit doses in normal glands and in 10, 30, 50, and 100 thousand unit doses in pathologic glands. All glands were carefully palpated before and after treatment. Milk samples were taken before and for varying periods after treatment and examined grossly and for bacterial, chloride and cell content. Yields of milk before and after treatment were also ascertained. Blood and urine samples were taken periodically for penicillin determination.

Sampling of milk for penicillin determination varied. In some trials small samples were drawn at hourly intervals, in others the intervals were longer. Complete milking varied from 10 to 72 hours after the infusions were made.

In one series the penicillin was infused in quantities of water equal to the volume of milk withdrawn.

The method used for assaying the amount of penicillin in milk was a slight modification of the cylinder-plate procedures described by Schmidt and Moyer[4] and Beadle, Mitchell, and Bonner.[5] It was noted early in the work that the zones of inhibition due to penicillin in milk stimulated the growth of the test organism (*Staphylococcus aureus* No. 209) at the periphery of the zone. In order to standardize the appearance of the zones of inhibition of the unknown and the standard solutions the following procedure was adopted. Sterile skim milk was used instead of 1% phosphate buffer for diluting the 50 Oxford Units per ml penicillin standard to 0.25, 0.5, 1.0, 2.0 and 3.0 Oxford Units per ml. These skim milk standards were run, in duplicate, on each day of assay for the standard curve[4] and all dilutions of milk samples for assay were made in sterile skim milk.

Culture dishes[5] were covered with a piece of celotex instead of a glass plate during the incubation period in an incubator humidified so that the relative humidity was between 40 and 50%.

Penicillin assay of urine samples was carried out in the same manner as for milk except 1% phosphate buffer was the diluent instead of skim milk.

Several methods were utilized at various times to assay the penicillin content in blood. The method of choice was the serial dilution assay employing *Bacillus subtilis* described by Hickey.[6] This method, in our experience, usually detects 0.031 Oxford Units per ml in blood plasma.

To determine the length of time a therapeutic level of penicillin would remain in the milk after its infusion into normal glands, small samples of milk were taken at varying intervals for penicillin determination. All of the milk was removed at intervals from 10 to 72 hours following infusion and penicillin therein determined. Twelve normal quarters were infused with solutions containing 5,000 Oxford Units per ml water and 7 normal quarters with solutions containing 100 and 200 Oxford Units per ml.

The rate of decline in concentration of penicillin per ml of milk for infusions of 5,000 and 40,000 Oxford Units in 1 and 8 ml of water respectively, is presented in Fig. 1. The rate of decrease is the greatest during the first 10 hours after which the disappearance of penicillin is at a slower rate. The rate of decrease is greater for each increment of the dose administered over the range studied which was up to 200,000 Oxford Units per quarter. In all cases where 30,000 Oxford Units or more were infused the milk contained 0.5 or more units of penicillin per ml 24 hours after the infusion was made. At no time was there sufficient penicillin in the blood to be detected by the assay methods used, but that some of the drug found its way into the blood is evidenced by its presence in the urine. The urine levels are presented in Fig. 1 for a period of 24 hours when a total of 100,000 Oxford Units were infused in all 4 quarters. The highest level of 11 Oxford

[4] Schmidt, W. H., and Moyer, A. J., *J. Bact.*, 1944, **47**, 199.

[5] Beadle, G. W., Mitchell, H. K., and Bonner, D., *J. Bact.*, 1945, **49**, 101.

[6] Hickey, R. J., Commercial Solvents Corporation, according to information furnished in communication March 14, 1945.

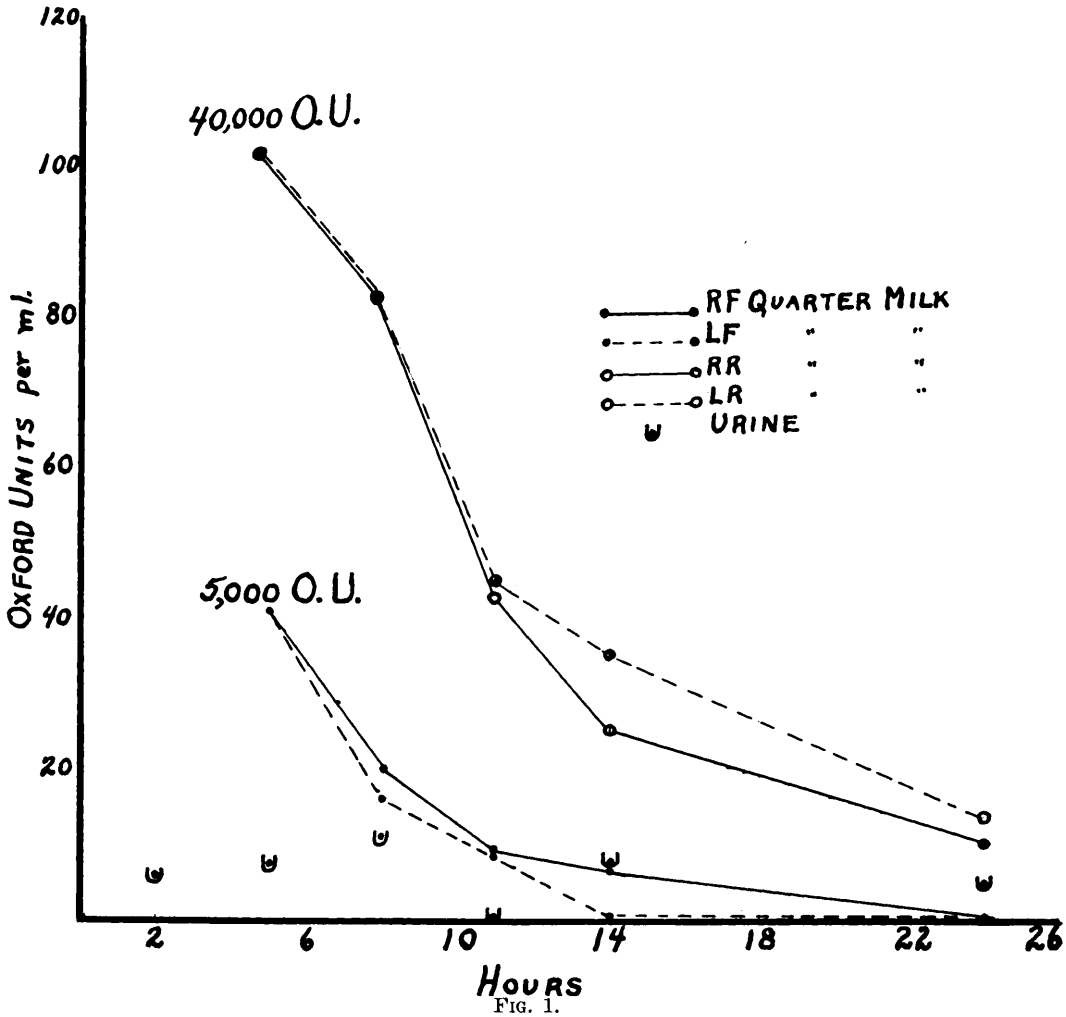

FIG. 1.

Penicillin content in milk and urine following intramammary infusions of 5,000 and 40,000 Oxford Units in 1 and 8 ml of sterile distilled water.

Units per ml urine was obtained at 8 hours and 5 Oxford Units per ml at the end of 24 hours after infusion.

The rate of disappearance of penicillin from pathologic glands presented the same picture as for the normal except in coliform infections in which the rate of decline was much greater. In the second and third infusions in consecutive 24 hour periods however, the rate of decline was progressively lower.

In order to determine whether the rate of penicillin migration out of the gland would be increased with water migration, the penicillin was infused in quantities of water equal to the quantities of milk withdrawn in 7 quarters. The results for 4 quarters are presented in Fig. 2. It will be noted that up to more than a 4-fold concentration was reached in one case 3 hours after the infusion was made. After the maximum concentration is reached the rate of decline presents much the same picture as for the experiments where the smaller amounts of water were used. The concentrations of penicillin in the urine, also presented in Fig. 2 are of the order of magnitude expected and indicate no greater rate of absorption from the gland than when small amounts of water were used.

The increased concentration of penicillin following its infusion with large quantities of water suggests that the infused water is

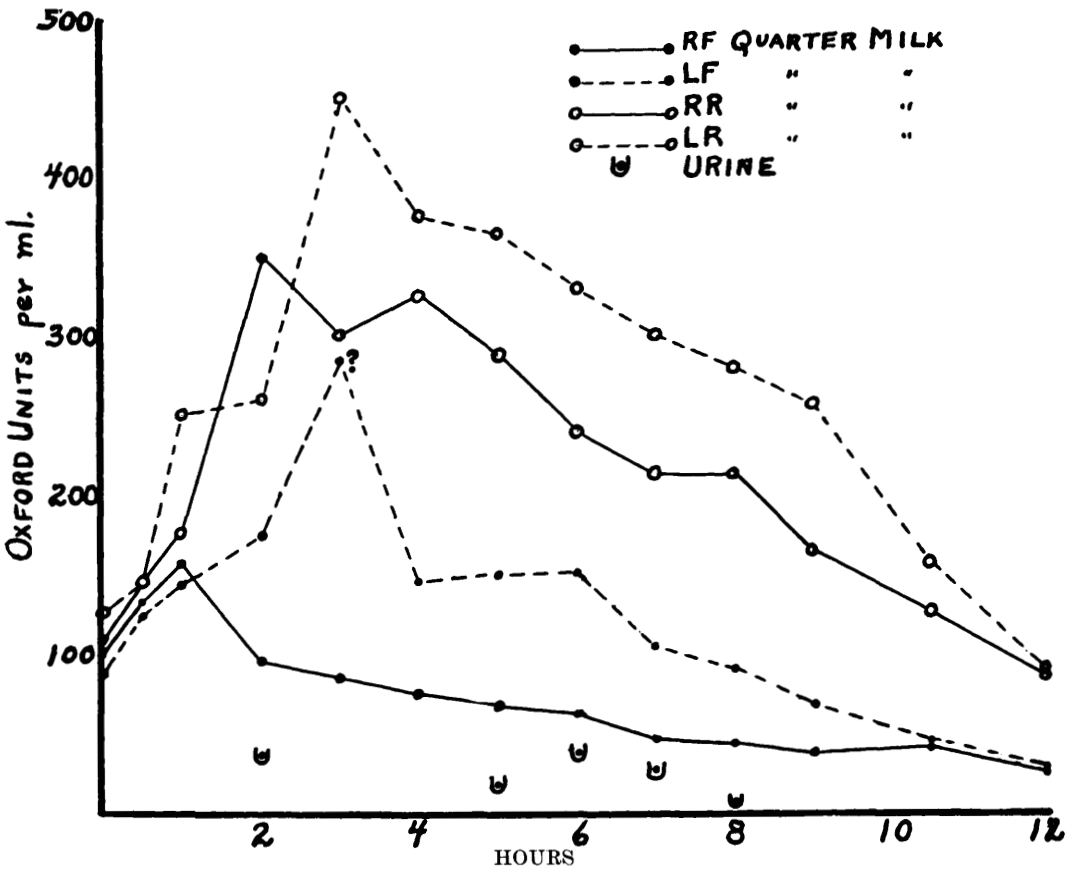

FIG. 2.

Penicillin content in milk and urine following intramammary infusions of sterile distilled water containing 100 and 200 Oxford Units per ml. The quantity of penicillin solution infused was equal to the quantity of milk withdrawn prior to the infusion.*

* RF quarter—750 ml containing 100 Oxford Units per ml. Total, 75,000 Oxford Units.
LF quarter—600 " " 100 " " " " " 60,000 " "
RR quarter—950 " " 200 " " " " " 190,000 " "
LR quarter—650 " " 200 " " " " " 130,000 " "

rapidly absorbed from the gland leaving most of the penicillin behind. Subsequent decreases in penicillin content can be accounted for chiefly by dilution with milk which is being secreted and by absorption from the gland. The absorption from the gland, however, takes place very slowly. Only when the total amount infused was 455,000 Oxford Units could any penicillin (0.06 O. U. per ml) be detected in the blood. While no evidence was obtained that penicillin is destroyed in the normal gland this possibility must be considered as another probable cause for its disappearance.

As measured by physical examination† of the gland, body temperature effects and changes in the character and quantity of the milk, infusion into the normal quarter of 5,000 to 200,000 units of penicillin in small quantities of distilled water had but a slight irritating effect. In some cases, no swelling could be detected. In others a slight swelling occurred in 3 to 4 hours after the infusions were made which completely subsided in 10 hours. In no case was any temperature rise noted.

In the experiments where the cows were milked at regular intervals after infusions, no demonstrable decrease in the quantity of milk was observed. In cases where the intervals between milkings were lengthened,

† Palpation and visual aspect.

there were decreases in the amounts of milk that could be accounted for by the intraglandular pressure resulting from the delay in withdrawing the milk.

In all cases, there were observed changes in the character of the milk which varied in general with the quantity of penicillin infused. A few small flakes, with increases in chloride, and leucocytes were noted, as well as a slightly yellow color. The milk returned to normal in 3 to 4 days.

When the penicillin was infused in quantities of distilled water to equal the quantity of milk withdrawn just before the infusion was made it was followed by a significant rise in temperature with tremors and greater changes in the character of the milk. These more severe effects may be accounted for by pyrogenic properties in the water used. More experiments are in progress to clarify this point.

The majority of pathologic quarters treated were infected with coliform organisms against which penicillin is not very effective. These quarters were treated in an effort to determine what effect a very high local concentration of the drug would have upon the coliform organisms in the mammary tissue. A marked decline in temperature and an improvement in the general condition of the animal resulted following the initial infusion of penicillin in two cases of acute mastitis. However, in the majority of cases, there was no clear evidence that penicillin exerted either a favorable or an unfavorable influence upon the course of coliform mastitis. The presence of penicillin in the pathologic gland does not seem to augment the inflammatory processes already present, even though the etiologic agent is not susceptible to the drug.

Summary. Aqueous solutions of penicillin (sodium salt) containing 5,000 Oxford Units per ml had slight irritating effects on the normal bovine mammary gland as measured by physical examination of the gland, body temperature and changes in the character and quantity of milk. The largest dose infused was 40 ml (200,000 O.U.). Increase in body temperature, tremors and greater changes in the character of the milk were observed after penicillin was introduced in large volumes of water. Largest volume infused was 950 ml (190,000 O. U.). These effects may have been due to the pyrogenic properties in the water used.

In glands infused with small volumes of water containing 5,000 O. U. per ml., the rate of decline of penicillin per ml of milk is greatest during the first 10 hours after which the decline is at a slower rate. The milk contained 0.5 or more units of penicillin per ml 24 hours after the infusion of 30,000 or more units. The disappearance rate from pathologic glands was of the same order as for the normal gland except in coliform infections. When penicillin was introduced in large volumes of water the infused water was rapidly absorbed leaving most of the penicillin in the gland.

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