tion it may be seen that a 2,000 unit inhalational dose resulted immediately in an effective level (0.33 units/cc) which dropped to 0.04 units/cc at 1 hour. A 2,000 unit intramuscular dose resulted in a serum level twice as high immediately (0.6 units/cc) but at 1 hour it equaled the serum concentration attained at this period following inhalation. The 5,000 unit intramuscular dose gave the highest serum levels which were maintained at inhibitory concentrations through 2½ hours.

Streptomycin Concentrations. In Fig. 2 are presented figures for streptomycin levels in serum and lungs at various times following administration of the following doses: 2,300 μg by inhalation, 2,500 μg and 5,000 μg by intramuscular injection. Each bar in the diagram represents the average value obtained from determinations made on 2 rats (in 70% of the assays the values were identical for the 2 animals).

Lungs. Immediately following inhalation of 2,300 μg of streptomycin the concentration in the lungs was 41.6 μg/cc of lung extract, whereas intramuscular injections of 2,500 and 5,000 μg resulted in immediate concentrations of only 8.7 and 11.6 μg, respectively. At 1 hour following inhalation the lung concentration was 18.7 μg, while only 2 and 4 μg, respectively, were detected following injection of 2,500 and 5,000 μg. Even at 2 and 3 hours after inhalation the lungs contained 10 μg and 3 μg, respectively, although none was detectable at these times following a similar dose by injection. At 2 and 3 hours following a 5,000 μg injection 1.2 and 0.4 μg, respectively, were found.

Serum. It is to be noted that the concentration of streptomycin in the serum following the 2,300 μg inhalational dose remained at less than 1 μg/cc at all 4 time intervals. This indicates that, in spite of the high lung concentration, this drug is poorly absorbed into the blood from the lungs. A 2,500 μg injection resulted in serum levels ranging from 15 μg/cc immediately to 1 μg at 3 hours. And following a 5,000 μg injection serum levels ranged from 40 μg/cc immediately to 3 μg at 3 hours.

Summary. 1. Penicillin and streptomycin were found in the lungs of rats in far greater concentrations following inhalational administration than after intramuscular injection of similar and even higher doses.

2. Penicillin was retained in the lungs in a high concentration for 1 hour following inhalation. It was still detectable in smaller amounts at 2 hours.

3. Streptomycin was retained in the lungs in an effective concentration for at least 3 hours following inhalation.

4. Penicillin was present in the blood stream in effective concentrations for 2 hours following inhalation.

5. Streptomycin was poorly absorbed from the lungs into the blood following inhalation.

Penicillin and streptomycin used in this study were generously supplied by Chas. Pfizer and Company, Brooklyn, N. Y.

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Effect of Infused Streptomycin in the Mammary Gland.*

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With the establishment of streptomycin as a potent chemo-therapeutic agent in the treatment of many infections resistant to either sulfonamides or penicillin the possibility of its use in the treatment of some forms of infectious bovine mastitis became apparent. This present study was undertaken to deter-

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TABLE I.
Records of Amounts of Streptomycin Infused and Recovered in the Milk with Varying Time Intervals and Quantities of Milk Secreted.

Animal No.	Quarter infused	Streptomycin infused, units × 1000	Time between infusion and milking hr	Total am't of milk obtained at milking ml	Concentration per ml milk units	Amount recovered units ×1000
3	R.R.	500	24	2450	172	421.4
3	L.F.	300	24	1750	100	175
3	R.R.	200	24	2400	50	120
1	R.R.	200	24	2875	49	140.888
3	L.F.	200	24	1100	73	80.3
3	R.F.	200	24	1100	70	77
3	L.F.	200	16	1000	112	112
3	R.F.	200	12	1000	72	72
3	L.R.	200	40	1400	75	105
659	R.F.	150	24	2320	40	92
763	R.F.	150	24	4165	20	82
659	R.R.	150	12	1150	67	77
763	R.R.	150	12	1680	42	70.5
841	R.R.	100	12	970	47-64	—
3	L.R.	100	24	1000	63	63
841	R.F.	100	12	570	80-116	—

TABLE II.
Concentration of Streptomycin in Successive Milkings Following Infusion and Proportion of the Infused Drug Recovered.

Animal No.	Quarter infused	No. of units/ml ×1000	Units per ml of milk at various milking intervals following infusion of quarter					Total recovery in %
			12 hr	24 hr	32 hr	48 hr	72 hr	
763	R.R.	150	42	14	—	—	—	—
3	L.F.	200	—	73	50	18	8	41
3	L.R.	200	—	80	64	30	15	62
3	R.F.	200	72	28	25	7	—	50
3	R.R.	200	50	21	17	—	—	64
3	R.R.	500	—	172	56	30	None	92
3	L.F.	300	—	100	80	30	"	104
3	L.R.	100	—	63	40	17	"	76
3	R.F.	200	—	70	20+	20+	—	—
3	R.R.	200	—	64	20+	20+	—	—

mine the pharmacological properties of the antibiotic when infused into the non-infected gland. Points of major interest included the relationship of size of dose, to concentration in the gland, maintenance of concentration, manner of elimination from the gland, and toxicity.

Materials and Methods. Five grade Holstein cattle and one grade Togenberg goat were employed. All animals were lactating normally and were free of disease during this study. The history and physical examination of the gland of one cow indicated the presence of disease at some previous time.

The cup-plate method of assay slightly modified as reported by Beadle, Mitchell and Bonner[1] and adapted to measurement of antibiotic in milk by Weirether, Jasper and Peterson[2] was used for determining the concentration of streptomycin in the milk samples. Notable differences from the latter were, the agar used, the test organisms, and the procedure of handling the assay plates after their preparation. The agar was the commercial Streptomycin Assay Agar. Originally the test organism was a strain of *Staphylococcus aureus* isolated at the University of Minnesota which later was replaced by a culture of *Staphylococcus aureus, S. M.*

[1] Beadle, G. W., Mitchel, H. K., and Bonner, D., *Bact.*, 1945, **49**, 101.

[2] Weirether, V. J., Jasper, D. E., and Petersen, W. E., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 282.

After preparation the assay plates were handled in the usual manner for streptomycin cup-plate procedures. Herrel and Heilman.[3]

Procedure. Milk samples were obtained by milking out the quarter completely and retaining a small portion of the whole for assay. The interval between infusion of a quarter with streptomycin and evacuation of the milk for assay varied from 12 to 40 hours. In some instances a quarter was milked out completely several times; a sample being retained each time for assay. The interval between successive milkings varied from 3 to 24 hours and between the first and last milking from 12 to 72 hours. Samples were either assayed immediately or refrigerated until assay could be performed.

Assay of urine samples was made in some cases. Urine samples from the cow were obtained by artificial stimulation of the micturation reflex. Samples from the goat were secured by catheterization. Following an infusion, 5 to 11 samples were obtained over a period of 24 to 32 hours.

Blood samples following infusion of the mammary gland in the goat were obtained for assay in two instances. Over a 24-hour period 10 samples were drawn in each case. The blood was permitted to clot and the serum saved for assay.

Gross appearance of the milk, its quantity, pH, and cell and chloride contents were determined as a basis for evaluating the irritating and toxic effects of the drug on the mammary glands.

The amount of drug infused varied from 100,000 units to 500,000 units per quarter in the cow. In the goat either 140,000 or 200,000 units were infused into one-half of the udder. The streptomycin was dissolved in sterile distilled water. The concentration in the solution to be infused was uniformly maintained at 20,000 units per ml. The volume of material infused thus varied with the total number of units infused.

Table I illustrates the general relationship between concentration per ml in the sample and (1) size of dose (2) time, *i.e.*,

[3] Herrel, Wallace E., and Heilman, Fordyce R., *Am. J. Med.*, 1947, **2**, 421.

TABLE III.

Elimination of Streptomycin in the Urine at Varying Time Intervals Following Intramammary Infusions.

Animal	Amount infused ×1000	Quarter (cow) of half (goat) of udder infused	Units/ml of urine in sample drawn at various intervals (hr) following infusion															
			1	2	4	5	6	7	8	9	10	11	13	14	17	24	26	27	32
Goat	140	left	9	9	15+	—	15+	—	15+	15+	—	15+	—	15+	—	15+	—	15+	—
Goat	200	,,	—	—	—	—	—	12	—	15	13	—	13	—	—	—	—	13	—
Cow	1,100	all 4	—	—	—	2	—	125	—	14	15	—	135	14	—	11.3	8	8.5	9.5

interval between infusion and sampling and (3) dilution or production of the quarter. It should be expected that these three factors would be among the more important ones in determining concentration at any given time. An interesting feature presented here is the seemingly small amount of influence a 24-hour variation of factor No. 2 had. Another notable feature is that on the dosage schedules used the concentration did not fall below 20 units / ml for periods up to 40 hours. Assays of the first and last drawn milk gave equivalent values.

The above observations promoted speculation concerning the elimination of streptomycin from the gland. In Table II are presented total recovery percentages and figures identifying the presence of streptomycin at various intervals following infusion. The data accumulated would seem to indicate that whatever the mechanism for elimination be, it must represent a relatively slow process. Also, since each milking was a complete one, in some instances aided by the intravenous injection of 10 units of oxytocin, and since successive milkings revealed considerable amounts of streptomycin, it was thought that a certain percentage might temporarily be fixed locally in the tissues. The amount so fixed, it was postulated, diminished as accumulating secretions in the gland became equilibrated with the tissues.

Among the more obvious ways by which the streptomycin unaccounted for might disappear from the normal gland are (1) destruction in the tissues and (2) absorption into the blood and elimination by the kidneys in the urine. The first named possibility was not investigated. For exploring the second the goat was first employed. In this animal the volume of blood and urine are relatively small thereby increasing the chance for detection of the antibiotic. After two such experiments on the goat a third was performed on the cow. The data are presented in Table III. The levels reached in the urine are not exceptionally high but are maintained for a considerable length of time at a fairly consistent value. Assays were not continued over a long enough time to determine the end point. However, if the normal average urine production of these animals be considered it would appear that the major portion of the antibiotic not accounted for in the milk might be excreted in the urine. Further experiments are planned to study the rate of renal excretion following infusion.

At no time was there sufficient streptomycin present in the blood to be detected by the assay methods used.

In only 3 experiments (those for cows 763, 841, and 659) were the pH, chlorides and cell counts completed for the milk before and after the infusions. No changes in the pH or chloride values were observed and only a slight increase in cell numbers which lasted for only a few milkings. No irritating effects could be detected by clinical inspection of the udders and the milk appeared normal except for a slight discoloration occasionally observed in the first milking following the infusion.

Summary. (1) Streptomycin infused into the normal bovine mammary gland in amounts ranging from 100,000 to 500,000 units per quarter could be detected in milk samples as long as 48 hours following infusion. As determined by the assay procedure used, the concentration did not fall below 20 units / ml in any of the samples after a 24-hour interval. (2) The concentration per ml in the sample was found to vary with (1) the size of the dose (2) time interval between infusion and sampling and (3) milk production of individual quarters. (3) At no time was there sufficient streptomycin present in the blood to be detected by the assay procedure used. However, in both the cow and goat significant amounts were found in urine samples as long as 27 hours following infusion. (4) Under the conditions of these experiments streptomycin was found to be relatively non-toxic when infused into the normal bovine mammary gland.

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