

# Absorption of Antibiotics by the Bovine Udder

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## ABSTRACT

Absorption of 39 antibiotics from the nonlactating bovine udder was compared with absorption of [carbon-14] urea as reference. First order kinetics characterized the absorption of urea and most of the antibiotics during the first 8 to 12 h after intramammary infusion. The absorption of polymyxin B, colistin, neomycin, spiramycin, and several tetracyclines was biexponential. The physicochemical properties of drugs which appeared to govern their absorption from the udder were the degree of lipid-solubility of the nonionized fraction and the dissociation constant. Antibiotic protein binding also influenced absorption. Lipid-solubility was the rate-limiting factor with drugs that are mainly dissociated in milk at pH 6.8. These compounds were absorbed at rates related to their degree of lipid-solubility of nonionized fraction. The concentration of the nonionized molecule in milk was the rate-limiting factor with drugs that were highly lipid-soluble. Results with a number of structurally-related antibiotics, and with others of diverse structures and physical properties, added considerable confidence to the assumption that antibiotics are absorbed from the udder by nonionic (passive) diffusion. The blood-milk barrier behaves as an inert lipid membrane to these drugs.

## INTRODUCTION

Experience in the use of intramammary antibiotic preparations for bovine mastitis therapy has indicated that antibiotics are absorbed from the udder. This was shown for benzylpenicillin, penethamate hydroiodide, dihydro-

streptomycin, chlortetracycline, oxytetracycline, and a number of sulfonamides (12 through 16). Iodide, sodium and potassium chloride, calcium salts, phosphates, and sulfates also were absorbed from the udder (6). Absorption rates of several chemotherapeutical agents differed. Sulfadimidine was detected in blood samples taken from the jugular vein as early as 1 min after intramammary infusion whereas for sulfacetamide the interval was 4 to 5 min. This difference corresponded to variation in appearance time for diffusion of these drugs into milk following intravenous injection (12).

Two physicochemical properties of drugs have been considered to be responsible for the wide differences in the rates at which organic compounds enter from blood into milk: the dissociation constant ( $pK_a$ ) and the lipid-solubility as determined by the ratio of organic solvent/water partition ( $K^o/w$ ) (14). It was suggested (13) that a diffusion equilibrium for drugs in blood and milk implies back-diffusion from milk into blood. This process is also governed by the two principles mentioned above. Since the nonionized fraction of a drug diffuses across the blood-milk barrier, diffusion should proceed at a fast rate when the lipid-solubility of the nonionized fraction is high (10, 17). Our aim was to evaluate the importance of the physicochemical properties of a number of antibiotics in influencing their rate of absorption from the bovine udder. Thus, compounds with closely related structures and compounds with widely diverse structures and physical properties were selected to test the assumption that antibiotics are absorbed from the udder by nonionic (passive) diffusion.

## MATERIALS AND METHODS

### Animals

The trial was on 76 Israeli-Friesian cows from an experimental dairy herd. The udders of all the cows in the herd were free from

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*Staphylococcus aureus* and *Streptococcus agalactiae* infection. Less than 10% of them were infected with non-*agalactiae* streptococci. Daily milk production records were collected. Cows usually were dried off when the daily milk yield declined to 8 liters. These cows immediately were used for the absorption experiments. Milk pH at the last milking of lactation ranged from 6.8 to 7.1.

#### Drugs and Drug Administration

The following antibiotics were obtained from regular commercial sources: methicillin sodium<sup>1</sup>, benzylpenicillin sodium<sup>2</sup>, phenoxymethylpenicillin potassium<sup>2</sup>, phenethicillin potassium<sup>1</sup>, oxacillin sodium<sup>1</sup>, cloxacillin sodium<sup>1</sup>, dicloxacillin sodium<sup>1</sup>, ampicillin sodium<sup>1</sup>, penethamate hydroiodide<sup>3</sup>, cephaloridine sodium<sup>4</sup>, cephaloglycin monohydrate<sup>4</sup>, cephalixin monohydrate<sup>4</sup>, sodium fucidate<sup>3</sup>, rifamycin SV<sup>5</sup>, rifampicin<sup>5</sup>, dihydrostreptomycin sulfate<sup>2</sup>, neomycin sulfate<sup>6</sup>, kanamycin sulfate<sup>7</sup>, paromomycin sulfate<sup>8</sup>, spectinomycin HCl<sup>9</sup>, polymyxin B sulfate<sup>10</sup>, colistin sulfate<sup>2</sup>, erythromycin base<sup>2</sup>, erythromycin estolate<sup>2</sup>, oleandomycin base<sup>10</sup>, triacetyloleandomycin<sup>10</sup>, tylosin tartrate<sup>11</sup>, spiramycin adipate<sup>12</sup>, lincomycin HCl<sup>13</sup>, chloramphenicol base<sup>6</sup>, tetracycline HCl<sup>7</sup>, oxytetracycline HCl<sup>2</sup>, chlorotetracycline HCl<sup>2</sup>, demethylchlortetracycline<sup>14</sup>, methacycline HCl<sup>14</sup>, doxycycline HCl<sup>10</sup>, minocycline HCl<sup>14</sup>, pyrrolidinomethyl-tetracycline<sup>15</sup>, and tetracycline-L-methyl lysine<sup>16</sup>.

Aqueous solutions or suspensions of the

antibiotics were prepared in 15 ml of sterile distilled water to which were added 2  $\mu\text{Ci}$  of [<sup>14</sup>C] urea<sup>17</sup>, specific activity 937  $\mu\text{Ci}/\text{mg}$ . Within 2 h after the last milking before drying-off, different antibiotics and [<sup>14</sup>C] urea were infused into the four quarters of each cow. Only quarters secreting milk at pH 6.8 were used. The dose of each antibiotic varied according to volume of milk produced at the last milking of lactation and sensitivity of the assay method. The dose was calculated so that a 2000 times dilution of it would result in an antibiotic concentration which would be equal to the highest concentration employed in constructing the standard curve for the microbiological assay of the drug in milk. For example, the dose of rifamycin SV was 100  $\mu\text{g}/\text{quarter}$ , whereas chloramphenicol was infused at a dose of 100,000  $\mu\text{g}/\text{quarter}$ . The standard dose of [<sup>14</sup>C] urea was 2  $\mu\text{Ci}/\text{quarter}$ .

Udder quarters of several cows were infused with considerably higher doses of a number of antibiotics. Solutions containing mixtures of two or three antibiotics were also infused into a number of quarters. Following infusion, the udders were massaged thoroughly for 30 s to promote distribution of the drugs.

#### Sampling Procedures

At intervals of 30 min, the udders were gently massaged, teat ends were wiped dry with separate pieces of cotton wool soaked in 70% ethanol, and a 2 ml milk sample was removed by hand after discarding the first stream of milk. According to previous experience with the antibiotic, samples were taken for 8 or 12 h. Milk samples were kept at -18 C pending their assay.

#### Assay Procedures

Antibiotics were assayed microbiologically within 4 days of sampling by the cylinder-cup method (2). Radioactivity in the samples was measured as previously described (19).

#### Stability Studies

The stability of antimicrobial activity of each antibiotic in milk was determined. Antibiotic solutions in milk at concentrations corresponding to the midpoint concentrations used in the construction of the standard curve were incubated for 12 h at 37 C. The percentage loss of antibiotic activity after incubation was then calculated.

<sup>1</sup> Beecham Research Laboratories, Brentford, England.

<sup>2</sup> Rafa Laboratories, Jerusalem, Israel.

<sup>3</sup> Leo Pharmaceutical Products, Ltd., Bullerup, Denmark.

<sup>4</sup> Glaxo, Grenford, England.

<sup>5</sup> Lepetit, Milan, Italy.

<sup>6</sup> Abic, Ltd., Ramat Gan, Israel.

<sup>7</sup> Teva, Ltd., Jerusalem, Israel.

<sup>8</sup> Parke Davis & Company, Detroit, MI.

<sup>9</sup> Amdal Division of Abbott Laboratories, Ltd., England.

<sup>10</sup> Pfizer, Inc., Brussels, Belgium.

<sup>11</sup> Elanco Products, Ltd., London, England.

<sup>12</sup> SPECIA, Paris, France.

<sup>13</sup> Upjohn Company, Kalamazoo, MI.

<sup>14</sup> Lederle Laboratories, U. S. A.

<sup>15</sup> Hoechst, Germany.

<sup>16</sup> Carlo Erba, Milan, Italy.

<sup>17</sup> The Radiochemical Center, Amersham, England.

## RESULTS

Semilogarithmic plots of drug concentrations and radioactivity counts over time, as measured in the periodically collected milk samples, resulted in monoexponential rates of decline of all drugs during the first 8 h. A second slower rate of decline was observed 8 to 12 h following the infusion of polymyxin B, colistin, neomycin, spiramycin, and several tetracyclines. First order kinetics were determined by a least squares procedure for fitting an exponential curve, and the  $t_{1/2}$  values were determined. The results of the tests with 276 infused quarters showed the  $t_{1/2}$  values for radioactivity counts ranged from 1.0 to 3.4 h. Differences were much greater among the  $t_{1/2}$  values of the different antibiotics. These values ranged from .5 h for chloramphenicol and cephalexin to more than 8 h for polymyxin B.

Analysis of the concentration-time curves and  $t_{1/2}$  values showed considerable variation for different cows. Variation was greatest between front and rear quarters. However, for a given antibiotic, the ratios of its  $t_{1/2}$  to the  $t_{1/2}$  of radioactivity counts were almost the same in all the infused quarters. This ratio, i.e.,  $t_{1/2}$  antibiotic/ $t_{1/2}$  radioactivity counts, will be a basis for comparing absorption rates of antibiotics from the udder.

Among the penicillins (Table 1), the ratios ranged from 1.85 for methicillin to .48 for penethamate. Penethamate was absorbed from the udder almost twice as rapidly as [ $^{14}$ C]urea and almost four times as rapidly as methicillin. Ratios for other weak acids are in Table 2. Rifamycin SV was absorbed most slowly from the udder while cephalexin was absorbed almost five times as fast as [ $^{14}$ C]urea. The poorly lipid-soluble basic aminoglycoside antibiotics and the basic polypeptides were absorbed much slower than the highly lipid-soluble basic macrolides (Table 3). Differences were great in the rate of absorption of the nine tetracycline analogs (Fig. 1). Ratios of  $t_{1/2}$  tetracycline to  $t_{1/2}$  [ $^{14}$ C]urea were .8, 2.4, and 3.2 for minocycline, doxycycline, and methacycline. These were the most lipid soluble tetracycline analogs. The ratios for tetracycline, pyrrolidinomethyltetracycline, and tetracycline-L-methyl lysine, which were the least lipid soluble tetracyclines in the series, were equal to or higher than 8. The latter tetracyclines exhibited a biexponential rate of decline in

TABLE 1. Absorption rate of several penicillin derivatives from the bovine mammary gland in comparison to the absorption rate of [ $^{14}$ C] urea.

Type of penicillin	pK <sub>a</sub> <sup>a</sup>	Percentage nonionized at pH 6.8 <sup>b</sup>	Coefficient of lipid-solubility <sup>a</sup>	Percentage nonionized X coefficient of lipid-solubility	No. of quarters infused	Ratio of $t_{1/2}$ antibiotic to $t_{1/2}$ [ $^{14}$ C] urea	
						Mean	SD
Methicillin sodium	2.7	.01	13	.13	4	1.85	.24
Benzylpenicillin sodium	2.7	.01	58	.58	12	1.60	.28
Phenoxyethylpenicillin potassium	2.7	.01	102	1.02	6	1.25	.48
Phenethicillin potassium	2.7	.01	155	1.55	5	1.05	.22
Oxacillin sodium	2.7	.01	219	2.19	6	.85	.20
Cloxacillin sodium	2.7	.01	275	2.75	8	.84	.32
Dicloxacillin sodium	2.7	.01	672	6.72	4	.80	.20
Ampicillin sodium	2.7, 7.2	.63	150	94.50	8	.70	.18
Penethamate hydroiodide	8.5	4.80	58	278.40	6	.48	.14

<sup>a</sup>Data are from Hou and Poole (5).

<sup>b</sup>Percentage nonionized =  $100 - [100/(1 + \text{antilog}(pK_a - \text{pH}))]$  Albert (1).

TABLE 2. Absorption rate of several weakly acidic antibiotics from the bovine mammary gland in comparison to the absorption rate of [<sup>14</sup>C] urea.

Antibiotics	pK <sub>a</sub> <sup>a</sup>	Percentage nonionized at pH 6.8	Degree of lipid-solubility	No. of quarters infused	Ratio of t <sub>1/2</sub> antibiotic to t <sub>1/2</sub> [ <sup>14</sup> C] urea	
					Mean	SD
Cephaloridine	3.4	.04	Low	4	1.38	.46
Cephaloglycin	4.9, 7.6	26.00	High	6	.78	.20
Cephalexin	5.3, 7.8	36.00	High	5	.22	.08
Sodium fusidate	5.4	3.80	High	4	.36	.10
Rifamycin SV	2.8, 6.7	.22	High	6	4.35	1.05
Rifampicin	7.9	92.6	High	5	1.05	.25

<sup>a</sup>Data are from Hou and Poole (5) and Umezawa (18).

drug concentration. The slower rate of decline started 8 and 9 h after infusion.

For several antibiotics, the rate of absorption from the udder relative to the absorption of [<sup>14</sup>C] urea was independent of dose (Table 4) and the presence of other antibiotics (Table 5).

For most antibiotics, the same diameters of inhibition zones were measured by a given concentration of an antibiotic before and after incubation for 12 h at 37 C. Loss of activity during incubation was 3.5% to 8.0% for methicillin, erythromycin, and tetracycline. The extent of degradation in microbiological activity of these antibiotics was too small to interfere with analysis of the data.

DISCUSSION

The examination of several classes of antibiotics indicated that the extent of inactivation in milk during the observation period was minimal. This also has been reported by others (11). Absorption from the udder can explain the reduction in antibiotic concentrations in the samples removed periodically.

In studies aimed at elucidating the mechanism of absorption from the udder of sulfonamides, antipyrine, and urea, the slope of the absorption curve for each drug differed on repeated infusions of the test substance to the same quarter (12 to 14). It is reasonable to

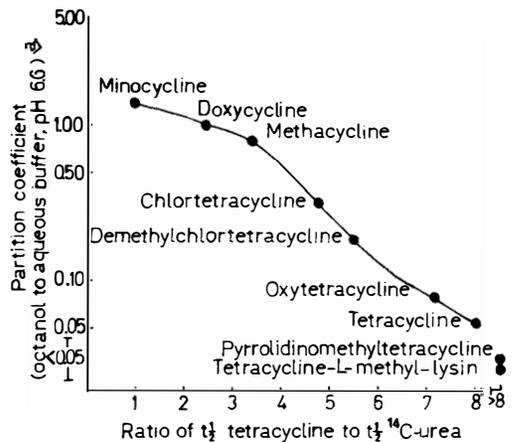


FIG. 1. Absorption rate of nine tetracycline analogs from the bovine mammary gland in comparison to the absorption of [<sup>14</sup>C] urea. Each tetracycline was infused to the 8 quarters of 2 cows.

<sup>a</sup>Data are from Colaizzi and Klink (3).

TABLE 3. Absorption rate of several poorly lipid-soluble and highly lipophilic basic antibiotics from the bovine mammary gland in comparison to the absorption of [<sup>14</sup>C] urea.

Antibiotics	pK <sub>a</sub> <sup>a</sup>	Percentage nonionized at pH 6.8	Extent of binding to udder-tissue homogenates <sup>c</sup>	No. of quarters infused	Ratio of t <sub>1/2</sub> antibiotic to t <sub>1/2</sub> [ <sup>14</sup> C] urea	
					Mean	SD
<b>Poorly lipid-soluble</b>						
Dihydrostreptomycin	7.7 <sup>b</sup>	11.2	Great	8	> 8.0	
Neomycin	7.7 <sup>b</sup>	11.2	Great	5	> 8.0	
Kanamycin	7.7 <sup>b</sup>	11.2	Great	4	> 8.0	
Paromomycin	6.3, 8.3	24.0	Moderate	4	4.5	1.20
Spectinomycin	6.9, 8.7	9.1	Moderate	4	4.4	1.65
Polymyxin B	10.0 <sup>b</sup>	.06	Very great	6	> 8.0	
Colistin	10.0 <sup>b</sup>	.06	Very great	4	> 8.0	
<b>Highly lipid-soluble</b>						
Erythromycin base	8.8	1.0	Moderate	6	2.2	1.0
Erythromycin estolate	7.0	38.5	Moderate	4	1.0	.3
Cleandomycin base	8.5	2.0	Moderate	6	2.3	.8
Triacetyloleandomycin	6.6	61.0	Moderate	6	.8	.2
Tylosin base	7.1	34.0	Moderate	6	3.4	.4
Spiramycin base	7.6	13.7	Great	8	6.8	.4
Lincomycin base	7.6	13.7	Weak	10	.6	.2

<sup>a</sup>Data are from Umezawa (18).

<sup>b</sup>Estimated values.

<sup>c</sup>Data are from Ziv et al. (20).

TABLE 4. Effect of dose on the absorption rate of selected antibiotics from the bovine mammary gland in comparison to the absorption rate of [ $^{14}\text{C}$ ] urea.

Antibiotic	Dose/quarter i.u. or $\mu\text{g}$ .	No. of quarters infused	Ratio of $t_{1/2}$ antibiotic to $t_{1/2}$ [ $^{14}\text{C}$ ] urea	
			Mean	SD
Benzylpenicillin sodium	500	12	1.60	.28
Benzylpenicillin sodium	10000	4	1.58	.34
Ampicillin sodium	250	8	.70	.18
Ampicillin sodium	10000	3	.82	.18
Rifamycin SV	100	6	4.35	1.05
Rifamycin SV	200000	3	4.70	1.18
Sodium fusidate	10000	4	.36	.10
Sodium fusidate	500000	2	.43	.16
Chloramphenicol	100000	6	.25	.06
Chloramphenicol	500000	4	.22	.08
Chloramphenicol	1000000	4	.28	.10

believe that these differences, and those observed by us, were caused by variations in the distribution of the test substance in the mammary gland and by the volume of residual milk. The influence of these factors can be overcome by using, as a reference, 200 mg of the fully diffusible urea, which is entirely nonionized, and infusing it together with the drug (13). While the technique seemed to be satisfactory on preliminary trials, we considered that more extensive investigations should be made by substituting 2  $\mu\text{g}$  of a radioisotope for the nonlabeled urea to speed up the analytical processing and to minimize the risk of udder irritation.

The rates at which the drugs were absorbed from the udder were governed by their physical

characteristics rather than by their molecular configuration. Two important physical properties appear to influence absorption from the udder, the lipid-solubility of the undissociated drug and the dissociation constant ( $\text{pK}_a$ ) which determines the concentration of the undissociated form in milk. Among the 39 antibiotics, those with a higher lipid-to-water partition coefficient ( $K^o/w$ ) were absorbed faster than those with a lower  $K^o/w$ . This can be explained by the assumption (10, 14, 17) that the blood-milk barrier behaves as an inert lipid layer.

The importance of the degree of lipid-solubility is indicated by the comparison of the rate of absorption of drugs which are structurally-related and are highly ionized in milk. The

TABLE 5. Absorption rate of antibiotic mixtures from the bovine mammary gland in comparison to the absorption rate of [ $^{14}\text{C}$ ] urea.

Antibiotic mixture	No. of quarters infused	Ratio of $t_{1/2}$ antibiotic to $t_{1/2}$ [ $^{14}\text{C}$ ] urea
Benzylpenicillin sodium and dihydrostreptomycin	2	Benzylpenicillin - 1.55, Dihydrostrep. - > 8.0 Benzylpenicillin - 1.55, Dihydrostrep. - > 8.0
Penethamate hydroiodide and neomycin	2	Penethamate - .44, Neomycin - > 8.0 Penethamate - .38, Neomycin - > 8.0
Tylosin and spectinomycin	2	Tylosin - 1.4, Spectimomycin - 3.2 Tylosin - 1.2, Spectimomycin - 3.0
Lincomycin, benzylpenicillin sodium, and colistin	2	Lincomycin - .7, Benzylpen. - 1.58, Colistin - > 8 Lincomycin - .6, Benzylpen. - 1.46, Colistin - > 8

penicillins (Table 1) and the tetracyclines (Fig. 1) were absorbed at rates corresponding to their  $K^o/w$ , indicating that the rate-limiting factor for their absorption from the udder is the milk concentration of the lipid-soluble moiety of the molecule. However, ampicillin and penethamate (an ester of benzylpenicillin) being considerably less ionized in milk than the other penicillins, were absorbed at the fastest rates in spite of their lower lipid-solubility characteristics. The importance of the  $pK_a$  in determining the rate of passage of drugs from milk into blood is further illustrated by antibiotics possessing high lipid-solubility coefficients. In the case of these drugs, the  $pK_a$  clearly becomes the rate-limiting factor for absorption from the udder. This is illustrated by comparing the absorption rates of cephaloglycin with cephalixin, rigamycin SV with rifampicin (Table 2), erythromycin base with its estolate-ester, and oleandomycin base with its triacetyl-ester (Table 3).

Although both lipid-solubility and the  $pK_a$  are pertinent in governing the rate of drug absorption from the udder, it appears that with some antibiotics the extent of protein binding can influence the rate of absorption. The interaction of drug molecules with milk is probably of little significance in slowing down the absorption of antibiotics from the udder. Several studies (11, 13, 14, 19) have shown that most antibiotics are bound to milk to a limited extent. Polymyxin B, colistin, and neomycin were extensively bound to udder tissue homogenates (20) and to other organs in the body (7, 8). Among the macrolide antibiotics, only spiramycin was bound to udder tissue homogenates to the same extent (20). Udder tissue binding is probably an additional factor, possibly of lesser importance than the poor lipid-solubility, which contributed towards the limited absorption of the former antibiotics from the udder. On the other hand, tissue binding must have assumed a greater role in slowing down the absorption of spiramycin, as is suggested when the physical properties of this antibiotic and lincomycin are compared (Table 3).

The biexponential shape of the concentration-time curve for these antibiotics could have resulted from udder tissue binding which limited their absorption. Extensively-bound drugs cross biological membranes with difficulty (9), and the extent of binding, which follows the

Langmuir adsorption isotherm (4), will tend to increase with the progressive decrease in drug concentration.

Table 4 may be accepted as additional supporting evidence for the concept of nonionic passive diffusion of antibiotics across the blood-milk barrier. If enzyme-dependent, energy-requiring active transport mechanisms have been involved in the absorption of drugs like rifamycin SV from the udder, then a 2000 times increase in substrate concentration should have caused a relative decrease in the rate of absorption.

The choice of antibiotic mixtures for absorption studies was limited by the lack of availability of quantitative methods for assaying each drug separately. Nevertheless, the limited data in Table 5 indicate that absorption of each antibiotic from the udder was unaffected by another antibiotic, thus strengthening the validity of the passive diffusion concept.

Results of pharmacokinetic studies, besides their academic interest, are also of great practical importance. It is important to know to what extent and at which rate an antibiotic is absorbed from the udder. The practical importance of these factors is greatest when the drug has been incorporated into pharmaceutical forms since in therapeutics the effects of dosage forms are related to the rate and/or the extent of drug absorption. With intramammary treatment of bovine mastitis during lactation, it is desirable that the period of antibiotic residues in milk be short. On the other hand, in dry-period intramammary therapy, high and persistent concentrations of antibiotics in the udder are advantageous. It is of importance, therefore, to predict *a priori* the kinetic behavior in the udder of new antimastitis antibiotics prior to their evaluation in the field. To make meaningful progress towards this objective, an understanding of the theoretical aspects of the problem is necessary. With such knowledge, it is possible in principle to choose judiciously the specific antibiotic, or through molecular modification to "design" a derivative with the suitable optimum physicochemical properties to effect the desired rate of absorption from the udder.

Cows on the first day of their dry-period are convenient experimental models for basic comparative studies on the persistence of antibiotics in the udder because little milk is being

produced, the problem of milk being contaminated with antibiotics is avoided, and no traces of radioactivity are usually in milk and urine 48 h after infusion of 2  $\mu$ Ci of [ $^{14}$ C] urea (Ziv, unpublished data).

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