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Binding of Antibiotics to Bovine and Ovine Serum

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The degree of binding of 37 antibiotics to bovine and ovine serum, after treatment at therapeutic doses, was determined by equilibrium dialysis and ultrafiltration methods. In general, binding values obtained by the two methods were comparable. The extent of binding varied from 0% for cephaloridine and kanamycin to >95% for novobiocin and fusidic acid. Of the 37 drugs studied, one-fourth were less than 25% bound, one-fourth were more than 75% bound, and the percentage binding of about half of the antibiotics ranged from 25 to 75%. Animal to animal variations in the extent of binding of a particular antibiotic were very small. The capacity of bovine or ovine serum to bind antibiotics was, with a few exceptions, similar to the reported capacity of human serum. At drug concentration ranges usually achieved during therapy, variations in drug levels in serum did not influence the degree of binding except with cephalexin, lincomycin, clindamycin, and chloramphenicol. With these antibiotics, the extent of binding increased two- to sevenfold with the decrease in drug concentration in serum.

Serum protein binding of antibiotics appears to be one of the most important determinants of drug distribution in the body (1, 10, 14, 15, 26, 35, 37, 47). Drugs which are highly bound to serum tend to remain in the intravascular compartment, giving high concentrations in the blood, whereas lightly bound drugs may diffuse more rapidly into the interstitial fluid (6, 41). Binding to serum proteins may delay uptake and metabolism of drugs by the liver and slow down renal excretion (1, 5). The possible therapeutic implications of serum protein binding of antibiotics in relation to bacterial infections have been examined (27-30) in light of the general consensus of opinion that protein-bound antibiotics are antibacterially inactive (15).

Most of the data available on the subject have been gathered from studies conducted on humans, small laboratory animals, dogs, and cats, and considerable interspecies differences have been observed (39, 47). In spite of the common use of bovine serum in binding studies, such experiments have been mostly conducted in vitro, under conditions which may not necessarily represent conditions in vivo.

During our investigations on the pharmacokinetic behavior of antibiotics in lactating cows and ewes, a considerable amount of information was gathered on the relative importance of serum binding with regard to rate and extent of passage of antibiotics from blood into milk. The purpose of the present report is to summarize data on the binding of 37 antibiotics to bovine and ovine serum after treatments at or near therapeutic doses, to compare results obtained by equilibrium dialysis methods with those obtained by ultrafiltration methods, to examine animal to animal variations in the extent of binding, and to study the influence of drug concentration on the binding of selected antibiotics.

MATERIALS AND METHODS

Normal lactating Israeli-Freisian cows and Awassi ewes, which were kept under normal feeding and housing conditions, were used.

Unless otherwise stated, all antibiotics were obtained through regular commerical sources and were used well within the stated expiration dates for their declared potencies. Drugs employed and their sources were as follows: penicillin G and penicillin V, Rafa Laboratory, Jerusalem, Israel; ampicillin, cloxacillin, and carbenicillin, through the courtesy of M. J. Soulal, Beecham Research Laboratories, Betchworth, England; cephaloridine, Glaxo Laboratories; cephalexin, Eli Lilly & Co.; tetracycline, Teva, Jerusalem, Israel; oxytetracycline and chlortetracycline, Assia Chemical Laboratory, Israel; demethylchlortetracycline, Lederle Laboratories; methacycline and doxycycline, Pfizer Co., Inc.; minocycline, Lederle Laboratories (through the courtesy of D. Sompolinsky, Bar-Ilan University, Israel); dihydrostreptomycin, Rafa Laboratory; neomycin, Abic Ltd., Israel; kanamycin, Teva; paromomycin, Parke, Davis & Co.; spectinomycin and erythromycin, Abbott Laboratories; oleandomycin and triacetyloleandomycin, Pfizer & Co.; spiramycin, S.P.E.C.I.A.; tylosin, Elanco; polymyxin B, Pfizer &

Co.; colistin and colistimethate, Rafa Laboratory; lincomycin and clindamycin, The Upjohn Co.; chloramphenicol, Abic Ltd.; novobiocin, Glaxo Laboratories; fusidic acid, Leo; vancomycin, Eli Lilly & Co.; and ristocetin, through the courtesy of E. A. Pritchett, Amdal Co., North Chicago, Ill. Rifamycin SV, rifampin, and rifamide (Lepetit) were obtained with their potencies by the courtesy of R. Haber, Abic Ltd.

The following radioactive products were purchased from the Radiochemical Centre, Amersham, England: ^{14}C-benzylpenicillin, potassium (specific activity, 75 μCi/mg), tetracycline-7-^{3}H (3 mCi/mg), and dihydrostreptomycin-^{3}H, sesquisulfate (2.1 mCi/mg). Spiramycin-1-^{14}C (2.7 μCi/mg) was the product of Rhone-Poulenc, France, and ^{14}C-chloramphenicol (70 μCi/mg) was obtained from New England Nuclear Corp., Boston, Mass.

Antibiotics were administered by single or multiple intravenous or intramuscular injections. Dosages for the polymyxins were either 3.5 or 7.5 mg/kg, and chloramphenicol was injected at 50 mg/kg. All other drugs were given at 20 mg/kg. The radioactively labeled drugs were administered at about 100 and 250 μCi per ewe or cow, respectively, mixed in the respective carrier antibiotics, at dosages stated earlier.

Blood specimens were obtained before and at intervals after treatments. Serum was separated as soon as possible and stored at 4 C for 24 to 48 hr.

Binding studies by the equilibrium dialysis technique were made with 0.25-inch (6.35-mm) diameter Visking cellulose casing (21). Casings were stored refrigerated in buffer. Amounts of 2 ml of serum were dialyzed against 4.0 ml of 0.1 M phosphate buffer, *p*H 7.4, in screw-cap vials or bottles. Vials or bottles were gently rotated at 4 C for 48 hr for some antibiotics and for 60 hr for others. Samples of serum and buffer were then removed and stored at -20 C until the time of assays. Dialysis controls, for verification of free passage and for the determination of inactivation during dialysis, were prepared by adding known amounts of each antibiotic to buffer and dialyzing against buffer. Lack of protein in serum dialysates was confirmed by the addition of 50% trichloroacetic acid to samples of the dialysates. Ultrafiltration was conducted according to the procedure described by Rolinson and Sutherland (39). About 5 min after starting the application of positive pressure, filtration was stopped and the filtrate obtained (about 0.2 ml) was pipetted off and discarded. Filtration was then resumed until the volume of ultrafiltrate obtained reached about 10% of serum volume in the bag. Suitable ultrafiltration controls were prepared in buffer. Lack of protein was ascertained by testing with 50% trichloroacetic acid.

Assays for antibiotic activity were conducted by the cup-plate method with recommended media and test organisms (2). Carbenicillin, however, was assayed by use of a local *Pseudomonas aeruginosa* strain highly susceptible to the drug and resistant to benzylpenicillin. Spectinomycin and the polymyxins were sometimes assayed with local *Escherichia coli* strains.

For estimation of antibiotic content in serum of cows and ewes, standards were prepared in pooled antibiotic-free bovine and ovine serum, respectively. Concentrations of unbound drugs in the buffer samples and ultrafiltrates were determined by preparing antibiotic standards in buffer which had been previously dialyzed against antibiotic-free serum of cows and ewes and had been shown to be free from protein.

Radioactivity of sera, buffer dialysates, and ultrafiltrates was determined with a liquid scintillation spectrometer (Packard Tri-Carb). To 0.5 ml of samples in glass vials were added 1.0 ml of Bio-solv (Beckman Instruments, Inc.) and 20.0 ml of toluene containing 2,3-diphenyloxazolyl at a concentration of 5.0 g/liter and dimethyl 5-phenyloxazolyl at a concentration of 0.3 g/liter. The efficiency of the counting procedure was monitored by internal standardization, with the appropriately labeled toluene.

For the purpose of this report, the concentrations of antibiotics were subject to the extent of binding of each drug and the sensitivity of the assay, but, in general, these concentrations were in the ranges obtained at half-life values and below. Determinations were also made at more than one drug concentration, and the extent of binding of some compounds as affected by drug concentration is also reported.

Serum protein binding, expressed in percent, was determined by the formula: percent bound = 100 $\times$ (concentration in serum $-$ concentration in buffer or ultrafiltrate)/concentration in serum.

RESULTS

Table 1 compares the binding of 15 antibiotics in serum of both cows and ewes and the binding of another 22 antibiotics in serum of ewes, as determined by equilibrium dialysis and ultrafiltration methods. Among the compounds tested, binding to serum of either species varied from 0% for cephaloridine and kanamycin to >95% for novobiocin and fusidic acid, and the extent of binding of most antibiotics ranged between 26 and 75% (Table 2). Marked differences were also observed in the extent of serum binding within groups of closely related antibiotics. Thus, among the penicillins, average binding ranged from 13.2% for ampicillin to 78% for cloxacillin; among the tetracyclines, binding ranged from 18.8% for oxytetracycline to 91.8% for doxycycline; and among the aminoglycosides, from 0% for kanamycin to 56.3% for neomycin. The degree of serum binding of the macrolide antibiotics ranged between 18 and 45.4%, whereas the polypeptide antibiotics were 32.8 to 72.6% bound. Ristocetin was undialyzable in either serum or buffer control, and therefore the determination of its serum binding capacity could not be made.

The extent of binding to either bovine or ovine serum of the 15 antibiotics tested in both species was in general very similar. This was found to be true for the extensively bound drugs, i.e., cloxacillin, and for the moderately bound antibiotics,

TABLE 1. *Binding of antibiotics to bovine and ovine sera as determined by equilibrium dialysis and ultrafiltration methods*

Antibiotic	Type of animal	No. of animals	Concn tested (μg/ml)	Equilibrium dialysis method		Ultrafiltration method	
				No. of assays	Percentage bound (mean ± SD)	No. of assays	Percentage bound (mean ± SD)
Penicillins							
Penicillin G	Cows	4	2.0–4.0[a]	16	28.5 ± 3.8	32	32.6 ± 2.5
	Ewes	5		18	30.4 ± 2.5	30	36.8 ± 4.7
Phenoxymethyl-ampicillin	Ewes	3	2.0–4.0	8	78.0 ± 7.6	15	81.0 ± 3.4
	Cows	2	0.5–2.0	6	18.0 ± 2.5	10	18.0 ± 2.0
	Ewes	4		10	13.8 ± 3.2	16	15.6 ± 3.8
Cloxacillin	Cows	4	10.0–20.0	12	71.3 ± 6.8	20	78.0 ± 5.4
	Ewes	4		12	72.6 ± 3.5	20	74.0 ± 4.0
Carbenicillin	Ewes	4	15.0–30.0	12	25.2 ± 3.8	10	34.2 ± 3.2
Cephalosporins							
Cephaloridine	Ewes	4	1.5–3.0	12	0.0	12	6.2 ± 1.4
Cephalexin	Ewes	3	15.0–25.0	9	10.6 ± 1.2	9	12.6 ± 2.7
Tetracyclines							
Tetracycline	Cows	4	2.5–5.0	12	31.6 ± 3.3	30	41.4 ± 2.1
	Ewes	6		16	27.5 ± 4.5	30	32.0 ± 4.0
Oxytetracycline	Cows	6	2.5–5.0	18	18.6 ± 2.2	30	22.4 ± 3.8
	Ewes	6		18	21.2 ± 3.2	30	24.6 ± 4.2
Chlortetracycline	Cows	6	1.0–4.0	18	46.8 ± 2.6	30	51.2 ± 5.4
	Ewes	6		18	45.6 ± 3.0	30	49.6 ± 3.8
Demethylchlortetra-cycline	Ewes	3	1.0–4.0	9	68.8 ± 2.6	12	70.2 ± 5.8
Methacycline	Ewes	3	2.0–4.0	8	79.2 ± 2.8	12	88.8 ± 3.5
Doxycyline	Ewes	4	0.5–1.5	12	84.0 ± 3.8	16	90.2 ± 2.4
Minocycline	Ewes	3	1.0–2.5	8	72.8 ± 2.0	12	80.2 ± 4.5
Aminoglycosides							
Dihydrostreptomycin	Cows	4	2.5–5.0	16	8.0 ± 2.5	30	10.2 ± 2.0
	Ewes	6		24	11.6 ± 3.2	30	15.0 ± 3.8
Neomycin	Cows	2	5.0–10.0	6	44.8 ± 2.6	10	50.0 ± 3.4
	Ewes	4		8	49.4 ± 3.6	16	54.2 ± 4.4
Kanamycin	Ewes	3	2.5–5.0	12	0.0	12	4.0 ± 1.0
Paromomycin	Ewes	3	2.5–5.0	12	36.6 ± 2.8	12	38.0 ± 5.2
Spectinomycin	Cows	6	12.5–25.0	24	6.0 ± 2.0	24	8.4 ± 1.8
Macrolides							
Erythromycin	Cows	4	1.0–4.0	12	18.0 ± 1.6	18	20.4 ± 2.8
	Ewes	6		12	23.0 ± 3.4	18	24.8 ± 3.6
Oleandomycin	Ewes	3	5.0–7.5	6	20.0 ± 1.2	12	23.6 ± 3.4
Triacetyloleandomy-cin	Ewes	2	10.0	6	32.4 ± 1.4	8	34.6 ± 2.2
Spiramycin	Cows	6	2.5–5.0	18	37.6 ± 4.2	30	38.0 ± 3.8
	Ewes	6		18	29.8 ± 3.4	30	32.6 ± 4.6
Tylosin	Cows	2	2.5–5.0	6	33.5 ± 2.2	20	44.0 ± 2.0
	Ewes	6		18	38.0 ± 5.0	30	45.4 ± 3.8
Polypeptides							
Polymyxin B	Cows	2	6.2–12.5	6	54.0 ± 3.0	8	74.6 ± 6.2
	Ewes	4		12	32.8 ± 4.4	12	42.2 ± 3.8
Colistin sulfate	Cows	2	6.2–12.5	6	56.0 ± 2.8	8	69.8 ± 5.6
	Ewes	4		12	60.4 ± 2.6	12	71.6 ± 4.2
Colistimethate	Ewes	4	6.2–12.5	12	34.0 ± 4.2	12	42.8 ± 3.8
Others							
Lincomycin	Ewes	5	2.0–4.0	ND[b]	—	15	34.2 ± 3.2
Clindamycin	Ewes	5	1.2–2.5	ND	—	15	46.0 ± 3.0
Chloramphenicol	Cows	4	20.0–40.0	16	36.6 ± 3.2	18	38.2 ± 2.6
	Ewes	4		8	32.2 ± 2.8	19	33.8 ± 1.8
Novobiocin	Ewes	3	2.5–5.0	12	96.4 ± 3.8	12	97.8 ± 2.0
Fusidic acid	Ewes	4	15.0–30.0	16	95.8 ± 3.8	24	98.0 ± 4.2
Vancomycin	Ewes	2	10.0–20.0	ND	—	8	54.8 ± 3.0
Ristocetin	Ewes	3	10.0–20.0	9	Undialyzable	ND	—
Rifamycin SV	Ewes	2	1.0–4.0	ND	—	4	72.6 ± 4.6
Rifampin	Ewes	2	0.1–0.4	ND	—	4	84.2 ± 5.6
Rifamide	Ewes	2	2.0–4.0	ND	—	4	70.2 ± 4.6

[a] International units.
[b] Not done.

i.e., penicillin G, tetracycline, neomycin, tylosin, and chloramphenicol, as well as for the slightly bound compounds, i.e., ampicillin, oxytetracycline, streptomycin, erythromycin, and spectonomycin. Polymyxin B was a notable exception in this respect, as it was found to be less bound to ovine than to bovine serum.

Values of serum binding determined by the ultrafiltration method were in general slightly higher than those obtained by the equilibrium dialysis method, again with the exception of polymyxin B. This antibiotic was found by the ultrafiltration method to be on the average 13.4 and 12.7% more bound to bovine and ovine serum, respectively, compared with the binding data obtained by the equilibrium dialysis method. It is also noteworthy that cephaloridine and kanamycin were found to be about 5% bound by the ultrafiltration technique but entirely unbound by the equilibrium dialysis method.

TABLE 2. *Grouping of antibiotics according to their degree of binding to sera of cows and ewes as determined by ultrafiltration*

Classification	Antibiotics	
Slightly bound (<25%)	Ampicillin	Dihydrostreptomycin
	Cephaloridine	Kanamycin
	Cephalexin	Spectinomycin
	Oxytetracycline	Erythromycin
Moderately bound (25–75%)	Penicillin G	Tylosin
	Carbenicillin	Polymyxin B
	Tetracycline	Colistin
	Chlortetracycline	Colistimethate
	Demethylchlortetracycline	Lincomycin
	Neomycin	Clindamycin
	Paromomycin	Chloramphenicol
	Oleandomycin	Vancomycin
	Triacetyloleandomycin	Rifamycin SV
	Spiramycin	Rifamide
Extensively bound (>75%)	Penicillin V	Minocycline
	Cloxacillin	Novobiocin
	Doxycycline	Fusidic acid
	Methacycline	Rifampin

Results presented in Table 3 indicate that, at least as far as the five radioactively labeled antibiotics tested were concerned, the binding data obtained correlated well with the data obtained by the microbiological assay method.

Some animal to animal variations in the extent of binding were observed, as indicated in Table 4. These variations, however, were slight and might well be less than the experimental errors inherent in the dialysis, filtration, and microbiological assay methods.

The effect of variations of antibiotic concentration on the extent of binding of three extensively bound drugs, i.e., cloxacillin, doxycycline, and novobiocin, and three slightly bound drugs, i.e., ampicillin, cephalexin, and streptomycin, are given in Table 5. For each antibiotic tested, a concentration range was selected which produced, in the undiluted serum with the respective assay system, diameters of inhibition zones between 35.0 and 15.0 mm. The percentage binding of cloxacillin, doxycycline, and novobiocin increased slightly with the decrease in concentrations, whereas with ampicillin and streptomycin the percentage binding appeared to be essentially unchanged at the concentration ranges studied. The extent of binding of cephalexin, on the other hand, was significantly influenced by the concentration

TABLE 3. *Binding of radioactively labeled antibiotics to sera of cows and ewes treated with each drug, as determined by equilibrium dialysis and ultrafiltration methods*

Antibiotic	Type of animal	No. of animals	Concn tested[a]	Equilibrium dialysis		Ultrafiltration	
				No. of assays	Binding (%)	No. of assays	Binding (%)
^{14}C-penicillin G-	Cows	1	1.5	4	31.2 ± 3.2	10	39.6 ± 3.2
	Ewes	2	2.5, 3.6	4	34.8 ± 4.8	8	36.0 ± 2.1
Tetracycline-7-^{3}H	Cows	2	5.0, 6.2	8	30.0 ± 4.1	12	34.4 ± 4.6
	Ewes	2	4.0, 5.5	8	25.3 ± 1.8	12	28.2 ± 3.0
^{3}H-streptomycin	Ewes	2	6.8, 7.5	8	9.6 ± 2.2	10	13.6 ± 3.5
^{14}C-spiramycin	Ewes	1	7.2	4	34.8 ± 1.5	8	38.2 ± 2.6
^{14}C-chloramphenicol	Ewes	1	27.5	4	32.8 ± 2.0	8	32.5 ± 3.7

[a] Concentrations of unlabeled and labeled antibiotic are given, expressed in units per milliliter for penicillin G and in micrograms per milliliter for the other drugs.

TABLE 4. *Variations in the extent of binding of five antibiotics with sera of individual cows, as determined by equilibrium dialysis*[a]

Antibiotic	Cow no.	Percent bound
Penicillin G	1	29.6
	2	24.3
	3	28.5
	4	32.0
Tetracycline	5	27.3
	6	32.6
	7	30.8
	8	34.8
Streptomycin	1	5.8
	2	6.2
	3	11.0
	4	8.3
Erythromycin	5	21.6
	6	17.4
	7	18.3
	8	19.0
Chloramphenicol	9	29.2
	10	32.8
	11	25.0
	12	24.2

[a] For penicillin G, streptomycin, and chloramphenicol, each value represents the mean of four separate dialysis experiments; for tetracycline and erythromycin, each value represents the mean of three separate experiments.

of the drug. About a sevenfold increase in percentage binding was found as the concentration of cephalexin in serum decreased from 16.2 to 0.5 μg/ml. A twofold increase in the proportion of unbound to bound drug in serum was observed with lincomycin and clindamycin over a concentration range of 15.0 to 1.0 μg/ml. The percentage of chloramphenicol binding to serum was also increased about twofold, from 22% to about 45%, as the concentration of the drug in serum decreased from 65 to 10.0 μg/ml.

DISCUSSION

In the present study, serum binding was determined directly in the blood of animals receiving each drug and by two independent methods. Equilibrium dialysis and ultrafiltration assay methods were also controlled by addition of tracer amounts of ^{14}C- or tritium-labeled antibiotics, when available. Results obtained with these methods were found to be remarkably consistent with each other, and variations among individuals of the same species were very small.

It has been pointed out (10) that, whereas the equilibrium dialysis method permits measurements of true dynamic equilibrium at a variety of concentrations, the ultrafiltration method should be criticized for not being thermodynamically sound, since equilibrium conditions are altered in the course of filtration by the local accumulation of protein on the membrane. In our studies, the effect of protein accumulation was minimized by limiting the volume of the ultrafiltrate obtained to about 10% of that of serum. The slightly higher binding values found by the ultrafiltration technique may indicate that some protein accumulation did occur on the membrane but to a minor extent. The ultrafiltration method is, however, a very simple one which permits a large number of samples to be studied repeatedly in a relatively short time. Furthermore, drug deterioration, which may occur with some antibiotics during dialysis, is negligible during ultrafiltration.

The unsuitability of these two methods for evaluating the extent of binding of ristocetin to human serum was already noted (22) and was confirmed by us with respect to ovine serum. It

TABLE 5. *Effect of variations in drug concentrations on the extent of binding to ovine serum, as determined by equilibrium dialysis*

Antibiotic	Concn (μg/ml)	Percent bound
Cloxacillin	17.5	70.7
	11.2	72.0
	4.6	76.8
	3.2	78.0
Doxycycline	5.6	82.7
	1.8	85.0
	0.8	85.0
	0.1	88.2
Novobiocin	16.0	93.6
	5.8	95.3
	2.4	95.8
	1.0	96.8
Ampicillin	5.2	14.5
	2.0	13.0
	0.8	14.2
	0.1	13.0
Cephalexin	16.2	3.0
	7.5	5.8
	2.3	11.0
	0.5	22.4
Streptomycin	6.0	13.2
	2.4	12.5
	1.8	11.0
	1.0	14.6

was, however, shown (17) that the presence of 10% rabbit serum or 20% horse blood did not interfere with the antibacterial activity of this drug. This suggests the absence of any marked degree of binding of ristocetin with serum protein.

The data presented in Tables 1 and 2 support the earlier conclusions (39) that the nature of the drug is perhaps the most important factor which affects the equilibrium between protein and free drug on the one hand and protein-bound antibiotic on the other. The capacity of bovine or ovine serum to bind different antibiotics could not, however, be related to either the acidic or alkaline nature of the drug, and hence to the degree of ionization in serum. For example, great differences were found in the extent of binding among the acidic drugs, such as the penicillins, cephalosporins, novibiocin, fusidic acid, and the rifamycins, and among the basic antibiotics, i.e., the aminoglycosides and polymyxins, as well as among the amphoteric tetracyclines.

Several studies have shown that among homologous series of drugs, such as fatty acids (45), penicillins (4, 19), and tetracyclines (40), as well as others (12), an increase in lipid solubility of the compound in the series is accompanied by an increase in serum protein binding. In Table 6, the partition coefficients for several penicillins and tetracyclines are compared with our binding data.

TABLE 6. *Bovine and ovine serum protein binding and apparent distribution coefficients between octanol or chloroform and aqueous buffers*

Antibiotic	Avg percent binding	Partition coefficient	Reference
Penicillin G	31.5	58×10^{3}[a]	4
Penicillin V	80.0	102×10^{3}[a]	4
Cloxacillin	77.0	275×10^{3}[a]	4
Oxytetracycline	23.5	25×10^{3}[b]	8
		7.2×10^{3}[c]	40
Tetracycline	34.0	36×10^{3}[b]	8
		95×10^{3}[c]	40
Chlortetracycline	49.5	130×10^{3}[b]	8
Demethylchlortetracycline	69.5	50×10^{3}[b]	8
		72×10^{3}[c]	40
Minocycline	76.5	$1,480 \times 10^{3}$[d]	8
Methacycline	84.0	430×10^{3}[b]	8
		72×10^{3}[c]	40
Doxycycline	88.0	430×10^{3}[b]	8
		475×10^{3}[c]	40

[a] Octanol-water, *p*H 7.0.
[b] Octanol-water, *p*H 7.5.
[c] Chloroform-water, *p*H 7.4.
[d] Octanol-water, *p*H 6.6.

A good correlation can be seen between the degree of lipid solubility and the capacity of bovine and ovine serum to bind these drugs. Several anomalous results can be seen with respect to cloxacillin, demethylchlortetracycline, and minocycline. Such results have been explained (4) by interaction between functional groups in the side chains of the molecules. The relevance of the correlation to the mechanism of binding of penicillins to serum albumin has been discussed (4). It should, however, be recalled that, although several of the extensively bound drugs, such as fusidic acid, novobiocin, and the rifamycins, are highly lipophilic (9, 20, 44), and some of the slightly bound drugs, such as streptomycin and kanamycin, are extremely polar, water-soluble compounds (11, 46), the macrolides chloramphenicol and lincomycin were only moderately bound to serum proteins and yet are highly lipophilic compounds. Additional studies are therefore indicated for elucidating the relationship between serum protein binding and the degree to which antibiotics are lipophilic.

That some interspecies differences exist in the extent of serum protein binding of penicillins and tetracyclines has been shown by several workers, but no general conclusions have been drawn regarding the nature of these differences. It was reported (47) that penicillin V was 80 and 64% bound by human and canine serum, respectively, and oxacillin was 93% bound in human serum compared with 65% binding in the dog. Penicillin G was 30 to 51% bound in human, horse, sheep, rabbit, and calf serum, and differences in the binding capacities for cloxacillin ranged from 93.5% in human serum to 70% in horse serum (39). On the other hand, very few differences in the binding of several tetracyclines by canine and human serum were noted (40); the least bound analogue, oxytetracycline, was 20 and 25% bound and the most heavily bound drug in the series, methacycline, was 95 and 93% bound to canine and human serum, respectively.

Our studies seem to indicate that the binding values of most antibiotics studied in bovine and ovine serum approximated the values reported for human serum (3, 13, 30, 32, 42, 43, 48). There were, however, some exceptions. Cloxacillin and methacycline were less bound in bovine and ovine serum compared with their reported binding values in man (3, 29), whereas demethylchlortetracycline, which was about 70% bound to bovine serum, was shown (3) to be more than 90% bound in human serum. Polymyxin B was shown (13, 36) to be about 50% bound in human serum and was found in our study to be bound to a similar extent by bovine serum. In ovine serum,

however, that drug was on the average only 36.5% bound.

The difference between 50 and 90% binding of a drug in two species may not seem very large, but, since it is only the unbound residue which is active, the difference between 50 and 10% active fraction means that a given dose of the drug produces initially only one-fifth of the active concentration in blood. Variations in drug binding to serum proteins among species may also play a role in determining differences in tissue levels of drugs (7, 14, 24), in drug toxicity (33), and in overall drug kinetics, particularly for highly bound drugs (23, 24). These differences are of special importance when a number of substances are evaluated experimentally in one animal species and assumptions are made that the relative extent of binding of the compounds is of the same order in other species.

For some antibiotics, marked changes were observed in the proportion of bound to unbound drug at different concentrations. For example, the percentage binding of cephalexin was significantly altered at drug concentration ranges achieved during treatment at accepted dosages (Table 5). Similar changes were observed (16) in human serum. Some changes were also noted in the serum of cows and ewes treated with chloramphenicol, lincomycin, and clindamycin. A two- to threefold increase in the percentage of serum binding of lincomycin in cows was reported (18, 38) when the drug concentration in serum decreased from about 7.0 to 1.0 μg/ml.

That serum binding can influence drug distribution in the body and that the magnitude of the effect will depend on the strength of association and on the dose of the drug was shown by Martin (34). His model showed that a strongly bound drug at low drug concentrations is concentrated primarily in the plasma compartment. However, at higher dosages, the fraction of the bound drug in serum will be markedly reduced. Another characteristic attributable to serum binding of drugs with high affinities for proteins is that there is a dose range within which small increases in dose result in relatively large increases in the amount of unbound drug in the body. It was stated by Martin that this behavior may have some interesting manifestations on dose-response characteristics and pharmacokinetic properties of drugs, provided that the extent of binding is quite large. Our results with cephalexin and other antibiotics suggest that these relationships could also apply to drugs which are bound to serum to a moderate degree. In reviewing the subject, Meyer and Guttman (35) remarked that Martin's concepts are only simplified representations of what might be a highly complex distributional pattern involving binding to tissue proteins, partitioning into fatty compartments, and the unavailability of certain aqueous compartments separated from blood by biological membranes acting as barriers. Nevertheless, the works of Kunin (25–31) clearly demonstrated that serum protein binding can be a most important factor in the distribution of some antibiotics, but that for others it might not be of significance in spite of in vitro demonstrations that interaction may produce a seemingly high extent of binding.

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