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Distribution and Blood-to-Milk Transfer of Labeled Antibiotics

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Radioactivity distribution was determined in serum and milk of lactating ewes after parenteral administration of five labeled antibiotics: ^{14}C-benzylpenicillin G, ^{3}H-dihydrostreptomycin, ^{3}H-tetracycline, ^{14}C-chloramphenicol, and ^{14}C-spiramycin. Antibiotic levels were measured simultaneously by microbiological assay. Radiochemical and microbiological assay procedures presented similar kinetic patterns for uptake in serum and penetration into milk, except for tetracycline. Small reductions in milk pH markedly increased the excretion of spiramycin and slightly influenced the milk passage of penicillin, dihydrostreptomycin, and tetracycline but did not alter the transfer of chloramphenicol into milk. Thus, it appears that the five antibiotics penetrate milk in accordance with the nonionic passive diffusion principle, and that good agreement is achieved between the calculated and observed milk/serum ultrafiltrate concentration ratios obtained during equilibrium.

The use of radioactive antibiotics for study of their distribution in laboratory animals, dogs, and cats has been widespread (1, 6, 7, 14, 16-18, 20, 21), and the absorption and excretion of some antibiotics, like tritiated dihydrostreptomycin, was also studied in cattle and swine (19). Although a considerable number of reports was published on the levels of various antibiotics in serum and milk of farm animals, these were determined mainly by microbiological and chemical assay procedures. The present study describes the pharmacokinetic behavior of labeled penicillin G, dihydrostreptomycin, tetracycline, chloramphenicol, and spiramycin in lactating ewes, comparing both radiochemical and microbiological assay methods.

MATERIALS AND METHODS

Animals. The studies were conducted on 17 lactating Awassi ewes, weighing 52 to 68 kg each. The animals were at their second or third lactation, yielding 1.4 to 2.0 kg of milk daily. Their udders were normal as determined by palpation, bacteriological testing, and the California Mastitis Test, the secreted milk having a pH of 6.5 to 6.8.

Antibiotics. The following labeled and unlabeled antibiotics were used: (i) Penicillin G sodium (Teva Ltd., Jerusalem) and ^{14}C-benzylpenicillin potassium (potassium-6-phenyl [acet-*1*-^{14}*C*] amid-penicillanate; Radiochemical Center, Amersham; specific activity, 75 μCi/mg); (ii) dihydrostreptomycin (Teva Ltd., Jerusalem) and ^{3}H-dihydrostreptomycin sesquisulfate (Amersham; 2.1 mCi/mg); (iii) buffered tetracycline-hydrochloride (Teva) and [*7*-^{3}*H*]tetracycline (Amersham; 3 mCi/mg); (iv) chloramphenicol sodium succinate (Abic Ltd., Tel Aviv) and ^{14}C-chloramphenicol (NEN, Boston; 70 μCi/mg); (v) spiramycin adipate (Specia, Paris) and ^{14}C-spiramycin (R. P.; 2.7 μCi/mg). The labeled antibiotics were used within 3 weeks of being tested for radiochemical purity and homogeneity.

Treatment. Animals were injected after the morning milking and were weighed before each experiment. Radioactive penicillin G and dihydrostreptomycin were dissolved in distilled water, tetracycline in 0.01 N HCl, and chloramphenicol and spiramycin in propylene glycol. The labeled drugs were added to solutions of the respective carrier antibiotics before treatment.

For determination of the relative distribution volume and the elimination rate from the serum, drugs were administered by a single intravenous (i.v.) injection in a volume of 100 ml, chloramphenicol at a dosage of 50 mg/kg and the other drugs at 20 mg/kg. Penicillin and dihydrostreptomycin were injected into three ewes (each at 1.0 μCi/kg), and the experiments were conducted as a crossover-design study in which the animals were treated alternately with either drug at 3-week intervals. Labeled tetracycline was given to four additional ewes, each receiving 0.8 μCi/kg, and chloramphenicol and spiramycin were administered to single ewes at a dose of 0.5 μCi/kg each.

To maintain a 3 to 5 h constant drug level in blood, the drugs, except for spiramycin, were each injected

into two ewes by a series of multiple intramuscular (i.m.) injections. Penicillin and dihydrostreptomycin were initially given at 20 mg/kg, followed by 4 × 10 mg/kg at 45-min intervals. After the first i.m. injection of tetracycline (20 mg/kg) and chloramphenicol (50 mg/kg), the drugs were administered twice more, at 90-min intervals, at one-fourth of the initial dose.

Sampling and treatment of samples. Blood samples were taken from the jugular vein before treatment and at 1- to 2-h intervals until the 12th or 14th h post-treatment. Blood sampling was continued twice daily for 5 days. Each udder was hand-stripped, and milk pH and volume of each sample were determined. Blood was allowed to clot at room temperature for about 2 h, blood and milk samples were centrifuged, and samples of serum and skim milk were removed and kept frozen at −18 C until assayed.

Ultrafiltrates of fresh serum and skim milk were prepared (23) from the samples collected during the period when the levels of serum antibiotics were constant. Samples of serum and skim milk, and their ultrafiltrates, were kept deep frozen pending assay.

Assay procedures. Microbiological assays of penicillin, dihydrostreptomycin, tetracycline, and spiramycin were performed by the cylinder cup method (2), and chloramphenicol was assayed by the turbidimetric method (2). Standards for the antibiotics were prepared in serum and milk before treatment and were assayed in the ultrafiltrates by preparing standards in protein-free dialysates of antibiotic-free serum and skim milk.

Radioactivity was measured in an automatic Tri-Carb liquid scintillation spectrometer (Packard) attached to an "On Line" electronic computation of net count with percent standard deviation. To 20 ml of scintillation fluid, 0.5 ml of sample was added, and activity was measured for 10 min three times. The efficiency of the counting procedure was monitored by internal standardization.

RESULTS

Semilogarithmic plots of drug concentrations and radioactivities in serum after intravenous administration yielded biphasic time curves (Fig. 1-5). In presenting the data graphically, mean values obtained by the microbiological and radiochemical assay methods at the end of the initial rapid fall in drug activities were made to overlap. A good agreement was generally observed between the slopes obtained in serum by the two analytical procedures carried out during the first 6 to 8 h after treatment. Curves of this type generally fit the equation $C_P = A_e^{-t} + B_e^{-t}$ derived from the two-compartment open-system model (12); however, in treatment of the serum data, the initial "distributional" phase was ignored, and the one-compartment open-system model was used as a basis for assessment. By extrapolating the terminal linear portion of the log C_P versus time curve to zero time, the apparent initial drug concentration and radioactivity in serum (C_0) was obtained (4, 13). The half-life in serum ($T_{½}$ beta) was determined graphically from the same segment of the curve. The apparent first-order disappearance rate constant (K_d beta), expressed as percent per hour, was determined, and the relative distribution volume per 100 kg of body weight (V'_d) was calculated by dividing the dose, in micrograms per kilogram, by C_0 (4, 13).

When calculations were based on total (bound and unbound) drug concentration in serum from 2 to 10 or 12 h after treatment, and by applying the single-compartment model to

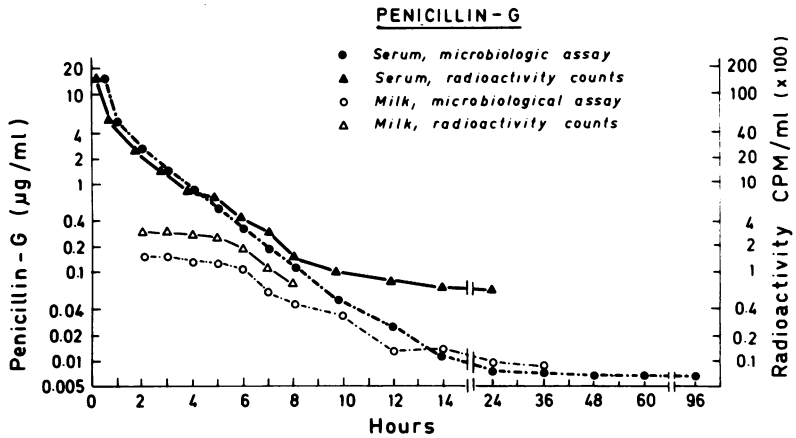

FIG. 1. *Mean concentration of penicillin G in serum and milk of three lactating ewes injected i.v. with* ^{14}C-*benzylpenicillin, 20 mg/kg (1.0 µCi/kg). Values determined by radioactivity counts and microbiological assay.*

analyze the data, highest C_0 values were observed with dihydrostreptomycin (Table 1). This drug also showed the shortest $T_{1/2}$ beta and the smallest V'_d. Compared to the other drugs, its elimination from serum was the fastest, whereas chloramphenicol and spiramycin had the slowest elimination rate, and the former was calculated to occupy space equivalent to more than 150% of wet total body weight on account of its low C_0.

Radioactivity was detected in serum for up to 24 h after the injection of penicillin G, but the drug was determined by the more sensitive microbiological assay method for up to 60 h after treatment (Fig. 1). Radioactivity was found in serum up to 96 h after dihydrostreptomycin, tetracycline, and chloramphenicol administration. Spiramycin was detected microbiologically for 84 h and measured radiochemically for up to 120 h after injection.

Chloramphenicol and spiramycin were first detected in milk, by both methods, as early as 30 min after i.v. administration. Tetracycline was found in the milk 1 h after its injection, whereas penicillin and dihydrostreptomycin could not be recovered from milk until the 2nd h after treatment. When peak concentrations and radioactivities of penicillin G and dihydrostreptomycin were observed in milk, these values were close to the concentrations of activities prevailing at the same time in serum (Fig. 1 and 2). Microbiological assay of tetracycline in milk showed levels higher than in serum, whereas radioactivity levels in milk were consistently below those found in serum (Fig. 3). Radioactivity and microbiological assay of milk yielded concentrations of chloramphenicol about one-half that of serum (Fig. 4), the drug being eliminated by a similar pattern from both milk and serum. At 1 h after spiramycin treatment, concentrations and radioactivity in serum and milk were equilibrated (Fig. 5). Thereafter, the drug was observed by both analytical methods, at higher concentrations in milk than in serum. Milk at pH 6.5 contained significantly higher concentrations of the drug than milk at pH 6.8.

Table 2 presents the ranges of serum levels obtained for each of five antibiotics during 3 to 5 h, and the means of drug concentrations and radioactivity in milk during the corresponding period. No changes in milk pH were noted during that time interval. For each antibiotic,

TABLE 1. *Pharmacokinetic constants for the disappearance of labeled and unlabeled penicillin G, dihydrostreptomycin, tetracycline, chloramphenicol, and spiramycin from the serum of ewes after a single i.v. injection*

Antibiotic	Ewe no.	Amt of C_0[a]		$T_{1/2}$ (h)[b]		K_d (%/h)[c]		V'_d[d] microbiological assay
		Counts per min per ml	μg/ml	Radiological assay	Microbiological assay	Radiological assay	Microbiological assay	
Penicillin G	1	6,550	6.2	1.2	1.6	57.7	43.3	32.4
	2	5,400	5.0	1.6	1.8	43.3	38.6	40.0
	3	6,150	5.8	1.4	1.5	50.0	46.3	34.3
	Mean	6,030	5.7	1.4	1.6	50.3	42.1	35.5
Dihydrostreptomycin	1	2,000	15.0	1.1	1.2	63.0	57.7	13.0
	2	1,950	16.0	1.2	1.0	57.7	69.3	12.5
	3	2,100	14.0	1.0	1.1	69.3	93.0	14.3
	Mean	2,015	15.0	1.1	1.1	63.3	63.3	13.3
Tetracycline	4	2,000	3.8	4.5	3.0	15.4	23.1	52.6
	5	1,850	3.0	5.0	3.5	13.9	19.8	66.6
	6	1,600	1.4	6.0	6.0	11.6	11.6	142.8
	7	1,800	1.8	5.1	4.5	13.9	15.4	111.0
	Mean	1,810	2.5	5.1	4.2	13.4	17.5	93.2
Chloramphenicol	8	300	3.2	14.0	14.0	5.0	5.0	156.2
Spiramycin	9	320	3.4	14.0	14.0	5.0	5.0	58.8

[a] C_0, Drug concentrations extrapolated to zero time.
[b] $T_{1/2}$, Half-life concentrations.
[c] K_d, Apparent first-order disappearance rate constant.
[d] V'_d, Percent relative distribution volume per kilogram of body weight.

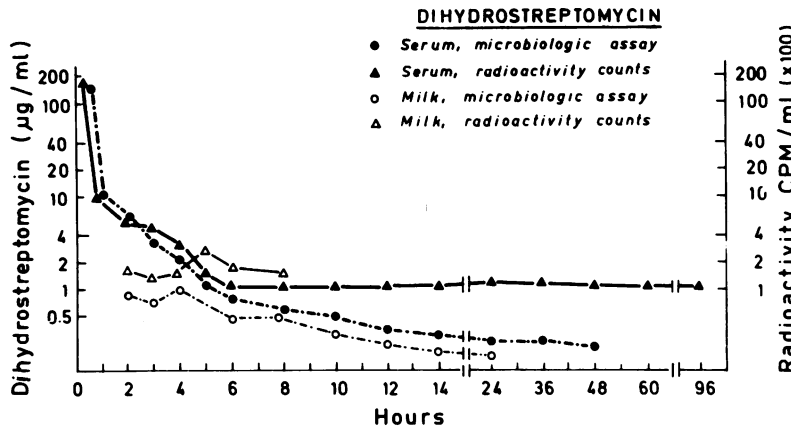

FIG. 2. *Mean concentration of dihydrostreptomycin in serum and milk of three lactating ewes injected with ³H-dihydrostreptomycin, 20 mg/kg (1.0 µCi/kg). Values determined by radioactivity counts and microbiological assay.*

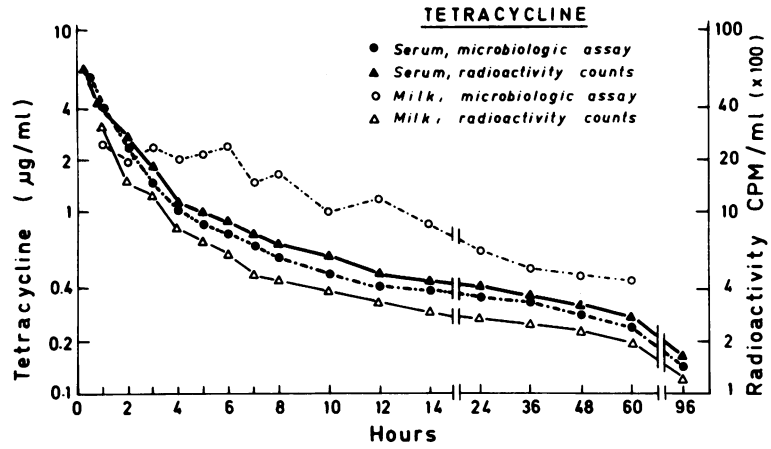

FIG. 3. *Mean concentration of tetracycline in serum and milk of four lactating ewes injected i.v. with ³H-tetracycline, 20 mg/kg (0.8 µCi/kg). Values determined by radioactivity counts and microbiological assay.*

the observed ratios of radioactivity in milk/serum ultrafiltrate were close to the calculated ones. The microbiologically observed and the calculated ratios for chloramphenicol and spiramycin were in good agreement; however, the ratios observed by microbiological assay of penicillin G and tetracycline were higher than the respective calculated ratios, whereas those observed for dihydrostreptomycin were markedly below the calculated ones. Variations in kinetic constants among animals treated with penicillin G and dihydrostreptomycin were minimal, but values varied to a greater extent among the four ewes treated with tetracycline (Fig. 3, Table 1).

Less than 0.001% of the doses of penicillin G and dihydrostreptomycin were recovered microbiologically from milk after intravenous injection. The recoveries of tetracycline, chloramphenicol, and spiramycin were 0.001%, 0.004%, and 4.6%, respectively.

DISCUSSION

Several reports have been published in the last 15 years on the pharmacokinetic behavior of drugs in lactating animals (10, 15). Antibacterial agents, like other drugs, are weak organic acids or bases, and a few of them were shown to penetrate from blood into milk according to the pH-pK passive-diffusion concept (8, 9). It has been pointed out that the kinetics of most exogenous substances may best be described with the use of a two-compartment open-system model (12). For the optimal analysis of the data according to the two-compartment model, blood should have been sampled at very fre-

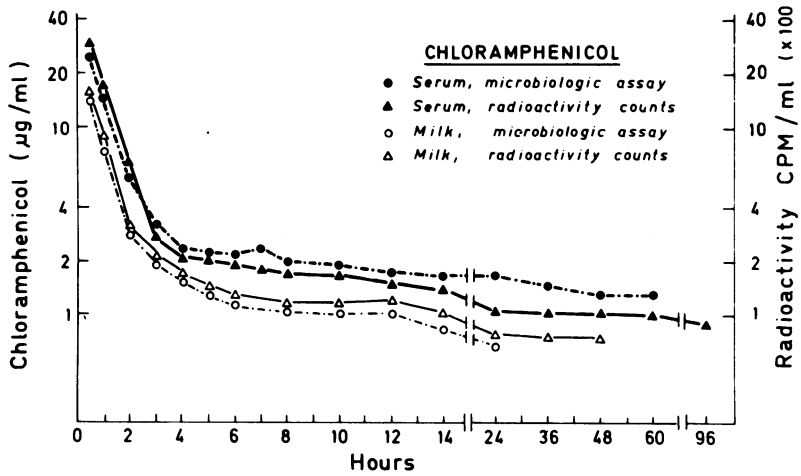

FIG. 4. *Concentration of chloramphenicol in serum and milk of a lactating ewe injected i.v. with ^{14}C-chloramphenicol, 50 mg/kg (0.5 µCi/kg).* Values determined by radioactivity counts and microbiological assay.

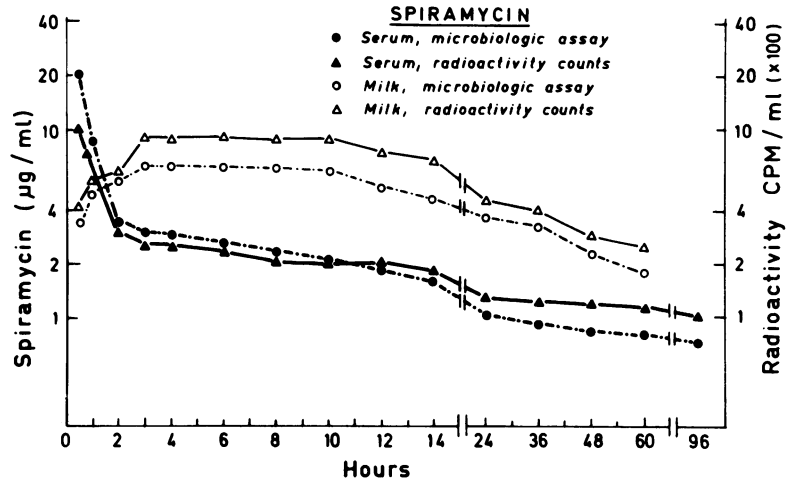

FIG. 5. *Concentration of spiramycin in serum and milk of a lactating ewe injected i.v. with ^{14}C-spiramycin, 20 mg/kg (0.5 µCi/kg). Values determined by radioactivity counts and microbiological assay.*

quent intervals during the early distributional phase (12). This, however, was not a practical possibility in the present study. The single-compartment model was therefore chosen for the kinetic analysis of serum antibiotic levels with the full realization of its limitation (12). Data presented in this study suggest that milk, almost free of serum proteins, may be kinetically considered as an extravascular compartment, and useful information on drug distribution throughout the body may be obtained by studying drug levels in serum and milk provided that pH differences which exist between blood and milk and that intrinsic differences in drug activity in the two media are taken into consideration. Some interesting relationships were found between the observed milk/serum ultrafiltrate ratios obtained during equilibrium on the one hand, and the C_0, K_d, and V'_d values as well as the earliest time of detection in milk and percentage recovery in milk after intravenous injection, on the other.

Chloramphenicol, a completely un-ionized and highly lipid-soluble drug, as well as spiramycin, a lipophilic weak base, penetrated milk more readily and in greater amounts than penicillin G and dihydrostreptomycin, being more ionized and poorly lipid soluble at physiological pH. The better penetration of the former drugs into milk may be related to their better

TABLE 2. *Concentrations of labeled and unlabeled penicillin G, dihydrostreptomycin, tetracycline, chloramphenicol, and spiramycin in serum, milk, and their ultrafiltrates*

Antibiotic	Ewe no.	Milk pH	Radiochemical assay				Microbiological assay			
			Serum level (counts per min per ml)	Activity in milk (counts per min per ml)	Milk/serum ultrafiltrate		Serum level (μg/ml)	Concn in milk (μg/ml)	Milk/serum ultrafiltrate	
					Observed	Calculated[b]			Observed	Calculated[b]
Penicillin G (pK 2.7)	10	6.5	2,300-3,100	370	0.14	0.12	2.4-3.3	0.62	0.22	0.12
	11	6.5		410	0.16	0.12		0.55	0.24	0.12
	11[a]	6.8		720	0.28	0.25		0.78	0.32	0.25
Dihydrostreptomycin (pK_a 7.6)	12	6.5	460-520	1,950	4.0	5.2	4.2-5.0	6.5	1.4	5.2
	13	6.8		1,050	2.0	2.8		3.7	0.8	2.8
Tetracycline (pK 3.3, 7.7, 9.7)	14	6.5	2,150-2,700	1,920	0.80	0.71	2.2-3.0	4.55	1.82	0.71
	15	6.8		1,650	0.66	0.71		4.10	1.54	0.71
Chloramphenicol	16	6.5	1,200-2,050	2,050	1.20	1.00	12.5-21.0	19.5	1.30	1.00
	16[a]	6.8		2,180	1.10	1.00		18.0	1.15	1.00
	17	6.5		1,850	1.00	1.00		16.5	0.90	1.00
	17[a]			1,900	1.05	1.00		20.0	1.15	1.00
Spiramycin (pK 8.0)	9	6.5	210-310	1,660	7.4	7.6	2.0-2.9	16.2	6.6	7.6
	9[a]	6.8		890	3.5	3.8		7.4	3.2	3.8

[a] Milk pH different in the two half-udders of the same ewe.

[b] Theoretical distribution of weak acids and bases at equilibrium between serum and milk can be calculated (10, 15): *for acids* as the ratio milk ultrafiltrate/serum ultrafiltrate = $[1 + 10(\text{pH milk} - pK_a)]/[1 + 10(\text{pH serum} - pK_a)]$ and *for bases* as the ratio milk ultrafiltrate/serum ultrafiltrate = $[1 + 10(pK_a - \text{pH milk})]/[1 + 10(pK_a - \text{pH serum})]$.

distribution throughout other compartments across tissue barriers (3, 5) and to their slower elimination from serum. It has been experimentally documented in cows that, during equilibrium, weak acids like penicillin G present milk/serum ratios lower than 1.0, whereas weak bases like erythromycin present ratios higher than 1.0, as expected from the non-ionic diffusion concept (8-11). On that basis it was postulated (10) that a ration of 1.0, independent of milk pH, should be observed with respect to chloramphenicol, and this was experimentally confirmed in our earlier (23) and present studies. Although conclusion concerning spiramycin excretion in milk is based on a single animal, results were in agreement with our previous report (22) where a milk-to-serum ratio of 2.8 was found in alkaline milk and a ratio of 8.0 in the more acidic milk. With tetracycline, however, a ratio twice as high as the expected one was observed in cows (15). This discrepancy was partly explained by the fact that tetracycline has three pK_a values, i.e., 3.3, 7.7, and 9.7. Differences of a similar nature were found in the present study when the drug was assayed microbiologically (Table 2). The theoretically calculated ratios for tetracycline were based on the surmise that the three titratable groups of the tetracycline molecule play an equal role in determining the degree of ionization. Supporting evidence for this assumption is the good agreement between the calculated and the radiochemically observed ratios.

Differences between the observed and calculated ratios obtained when dihydrostreptomycin was assayed microbiologically were much greater than those obtained by the radiochemical assay of the drug. The superiority of the radiochemical over the microbiological assay methods in kinetic studies was elaborated by Snell (16-18), who indicated that, although the multiplicity of factors which interfere with the bioassay of antibiotics was realized, no satisfactory method has yet been found for completely eliminating or neutralizing them. Interference with the test, due to potentiation or antagonistic effects, chelation, serum binding, absorption by cellular elements, or pH differences in the various body fluids, is inherent in the bioassay of many antibiotics, particularly tetracyclines. The magnitude of the difficulties involved in interpreting serum and milk levels of tetracycline is evident from Fig. 3.

Use of radioactive antibiotics can offer better quantitative data which resolve many of the problems encountered in bioassay. The known findings of wide individual differences with regard to serum levels of tetracycline, determined biologically, and the narrower margin found radiochemically can be cited as an addi-

tional example of the higher precision obtained by employing the latter method. There are, however, problems with regard to the relationship between bioactivity and radioactivity at various time periods. When penicillin G and dihydrostreptomycin were radiochemically assayed in serum, elimination rates from about the 7th h post-treatment onwards were slower, compared to the rates determined microbiologically. Serum protein binding, which was about 35% for penicillin G and less than 10% for dihydrostreptomycin, can provide only a partial explanation for these differences in rates. Both biologically active drugs and inactive metabolites were probably measured radiochemically during that period, and it is important to note that these differences in rate were found with the centrally labeled ^{14}C-penicillin but not with the peripherally labeled $C_7{}^3H$-tetracycline. It was stated (18) that a compound which is labeled in its nucleus with ^{14}C, as is the case with penicillin, is not subject to losing its label by peripheral attack, as might be accomplished by dehydrogenation of a tritium-labeled compound.

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