amount of dextrin. Feeding of the 15 mice in each group was continued for 20 days or until death, whichever occurred sooner.

Results. The findings are summarized in Table I. To minimize variations in averages due to early deaths, infection and atypical responses on the part of individual mice, the data were computed on the basis of the top 12 animals in each group. In agreement with earlier findings(2) the mice lost weight and failed to survive when fed a purified ration containing 1 mg of aminopterin per kg of diet. The supplements of whole liver powder, water-soluble liver extract and water-insoluble liver residue were without significant effect. Doubling the B vitamin content of the experimental diet and adding vitamin B_{12} also proved ineffective. Only folic acid of all of the substances tested exerted a protective effect. A diet containing 100 mg of folic acid per kg of diet prolonged slightly the average survival time of the mice. Folic acid at a level of 500 mg per kg of diet, however, completely counteracted the deleterious effects of aminopterin on growth and survival under conditions of the present experiment.

Summary. Immature male mice lost weight and failed to survive when fed a purified ration containing 1 mg of aminopterin per kg of diet. These effects were completely counteracted by folic acid during an experimental period of 20 days when administered at a level of 500 mg per kg of diet.

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Antimicrobial Action of Terramycin *in Vitro* and *in Vivo*. (17727)

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In a recent communication(1), the isolation of a new antibiotic, terramycin, has been reported. Terramycin is a highly active antimicrobial agent which is effective both *in vitro* and *in vivo* against certain of the pathogenic bacteria and certain of the rickettsiae (1,2). The studies reported herein were carried out in an attempt to evaluate more extensively the antimicrobial action of terramycin *in vitro* and its chemotherapeutic action in acute experimental infections in animals.

In vitro activity. 1. Materials and methods. Partially purified and crystalline terramycin,* terramycin hydrochloride, terramycin sulfate, and 2 forms of the sodium salt of terramycin (pH 8.5 and 9.8)† have been used to date in determining the sensitivity of various microorganisms to terramycin. Aureomycin

1. Finlay, A. C., Hobby, G. L., P'an, S. Y., Regna, P. P., Routien, J. B., Seeley, D. B., Shull, G. M., Sobin, B. A., Solomons, I. A., Vinson, J. W., and Kane, J. H., *Science*, 1950, v111, 85.

2. Rose, H. M., 1950, personal communication.

*All of the terramycin used throughout this study was prepared by the Departments of Biochemical and Chemical Research and Development, Chas. Pfizer & Co., Inc. We are indebted especially to Dr. B. A. Sobin and Dr. P. P. Regna for the preparation of large quantities of the antibiotic for this study during the early stages of its development, and to Mr. J. H. Kane and Dr. R. A. Patelski for their advice and assistance throughout the study.

† The pH of the sodium salt of terramycin in dilute aqueous solution is approximately 9.8. It was observed early in the study of terramycin that dilute solutions of its sodium salt may be adjusted to pH 8.5 without precipitation of the antibiotic. It is recognized that such solutions probably consist of mixtures of terramycin and its sodium salt. Although this form cannot be prepared readily as a solid product, its pH and its satisfactory solubility have made it a more desirable preparation for certain preliminary laboratory studies than terramycin hydrochloride (pH 1.5), terramycin sulfate (pH 1.5), or the true sodium salt of terramycin (pH 9.8).

hydrochloride‡ and terramycin hydrochloride were tested simultaneously against a limited series of these microorganisms, in order to obtain a base line for interpretation of results obtained by the method used.

In the interests of convenience, the unit of terramycin has been defined as one microgram of the pure amphoteric compound. The activities of the salts of terramycin are stated in terms of the equivalent weight of pure terramycin(3). The antimicrobial activity of terramycin *in vitro* has been evaluated by standard methods used for other antibiotics. The method used in this laboratory for determination of the sensitivity of microorganisms to terramycin is a modification of that previously described by Lenert and Hobby (4) for streptomycin.

In all instances, the antimicrobial agent was dissolved in broth in a concentration of 100 μg per ml and the resultant solution passed through a Seitz filter to ensure sterility.§ Two-fold serial dilutions of this solution of terramycin were made in nutrient broth, and, in the case of bacteria, 0.9 ml of each solution was seeded with 0.1 ml of a 10^{-1} or 10^{-4} dilution of a standardized broth or blood broth culture of the organism under test. Except when otherwise specified, 16 to 18 hour broth cultures were used throughout. These cultures were diluted in broth to a constant density immediately prior to use. A density allowing 75 to 85% transmission on a Photovolt Lumetron No. 400 was chosen as standard. In the case of rapidly growing organisms, the 10^{-4} dilution was used. In the case of poorly growing organisms, a 10^{-1} dilution was more satisfactory. Incubation was carried out at 37°C for a period of 48 hours. The sensitivity of an organism was accepted as the least amount of antibiotic capable of causing complete inhibition of growth as evidenced by absence of gross turbidity. In view of the two-fold nature of the serial dilution assay used, however, the sensitivity may have been somewhat greater than that indicated by the accepted end points.

Levinthal's broth was used for studies on *H. influenzae*(5) and the Bordet-Gengou medium for those on *H. pertussis;* an incubation period of 72 hours was used for *H. pertussis*. All studies on *M. tuberculosis* were carried out in the Dubos liquid medium containing Tween 80, as described in a previous communication(6). An incubation period of 7 days at 37°C proved satisfactory for this purpose.

In the case of fungi,Sabaraud's agar was used routinely(7). High concentrations of terramycin were obtained by dilution and Seitz filtration in Sabaraud's broth, followed by subsequent mixing with an equal volume of Sabaraud's agar containing twice the normal concentration of agar. Sabaraud's agar slants (10 ml) containing varying concentrations of terramycin then were inoculated with 0.1 ml of a heavy suspension of a 9-day culture of the organism under test. All tests were incubated at 37°C and the amount of growth read at 3 and 5 days. The sensitivity of an organism in this case was accepted as the least amount of antibiotic necessary to inhibit growth as evidenced by absence of colonial growth on the surface of the agar slant. A comparison of the *in vitro* activity of terramycin and its salts has indicated that there is no significant difference in their

‡ We are indebted to Dr. Stanton M. Hardy of the American Cyanamid Company for the aureomycin hydrochloride used in these studies.

3. Kersey, R. C., *J. Am. Pharm. Assn., Scient. Edition,* 1950, in press.

4. Lenert, T. F., and Hobby, G. L., PROC. SOC. EXP. BIOL. AND MED., 1947, v65, 235.

§ Studies on the degree of adsorption of terramycin onto Seitz filter pads have indicated that little or no terramycin is removed from solution during filtration of small volumes (20 to 50 ml) of broth or serum broth solutions of terramycin (50 to 100 μg per ml). Fifty to 75% of terramycin present in aqueous solutions, in contrast, may be removed from solution during filtration. Throughout the studies reported herein, all terramycin, therefore, was dissolved in plain or 25% serum broth prior to filtration.

5. Alexander, H., *et al., J. Exp. Med.,* 1947, v85, 329, 607.

6. Hobby, G. L., Lenert, T. F., and Dougherty, N., *Annals of the N. Y. Acad. of Sci.,* 1949, v52, 775.

7. Hobby, G. L., Regna, P. P., Dougherty, N., and Steig, W. E., *J. Clin. Invest.,* 1949, v28, 927.

TABLE I.
Antimicrobial Action of Terramycin *in vitro*.

I. Antibacterial Activity of terramycin

Microörganisms	Sensitivity: μg of terramycin per cc—37°C 24 hr	48 hr	Microörganisms	Sensitivity: μg of terramycin per cc—37°C 24 hr	48 hr
Strep. hemolyticus, Group A			*Bruc. bronchiseptica*	<.3	1.5
C203Mv	<.3	1.5	*H. influenzae* (St.)	2.0	4.0
Type 18	.6	1.5	(DeR.)	2.5	2.5
Strain Br-Mn.	1.5	1.5	*H. pertussis*		5.0†
Strep. hemolyticus, Group C			*K. pneumoniae* (Kin.)	1.5	2.5
Griffith 20	2.5	21.	*K. pneumoniae* (Nol.)	.9	1.5
" 21	.6	1.5	Streptomycin-resistant mutant	21.	21.0*
" 7	.6	.6	*A. aerogenes*	1.5	2.5
H 76	<.3	<.3	Streptomycin-resistant mutant	2.5	2.5*
H 81	.6	.6	Unidentified Gram Neg. Rod (SMR)	5.0	5.0*
D. pneumoniae			*E. coli* (BW 41)	2.5	5.0
I/230, type I	<.02	.02	(W 87)	2.5	5.0
D/39, " II	<.3	<.3	(Bristol)	5.0	5.0
A66, " III	<.3	<.3	*Ps. pyocyaneus* (St.)	5.0	21.
M_3R	<.3	2.5	(ATCC 9027)	21.	21.
BR.	<.3	.5	*Proteus vulgaris*	>167.	>167.
Sch.	<.4	<.4	*S. typhosa* (St.)	1.5	2.5
Strep. viridans (Hawth.)	<.3	2.5	Streptomycin-resistant mutant (NAII)	<.3	1.5*
Enterococcus	.6	.6	Streptomycin-resistant mutant (NAIV)	.6	1.5*
Staph. aureus (H)	.7	1.5	*S. paratyphosa* (Fr.)	2.5	5.0
(Br.)	1.5	1.5	*S. dysenteriae* (Duf.)	2.5	5.0
Staph. albus (Bell)	168.	>168.	*S. typhi murium* (L.I.)	2.5	5.0
C. diphtheriae (No. 210)	.6	.6	*S. typhi murium* (B.I.)	3.0	5.5
Bc. subtilis (W)	.4	.7	*S. newport* (B.I.)	5.5	5.5
(PCI-224)	42.	42.	*S. enteritidis* (B.I.)	3.0	5.5
(No. 6633)	.6	.6	*S. cholera suis* (B.I.)	3.0	5.5
Cl. welchii	<.3	11.			
N. catarrhalis (ATCC)	2.5	2.5			
N. meningitidis	<.3	.5			

* *K. pneumoniae* strain requires 1400 μg streptomycin per cc for inhibition; *A. aerogenes* strain requires 2800 μg; both strains of *S. typhosa* require >5000 μg; and the unidentified gram negative rod requires >100,000 μg of streptomycin for inhibition.

† Readings in this instance were made at 72 hr (37°C).

II. Tuberculostatic Action of Terramycin Hydrochloride:

Microörganisms	Sensitivity: μg of terramycin per cc 5 days—37°C	12 days—37°C	Sensitivity to streptomycin, μg per cc (12 days)
$H_{37}Rv$ (No. 2678)	3.0	13.0	<.3
Freshly isolated strains			
Ni.	3.0	16.0	6.0
Mu.	1.5	26.0	>1000.
Mo.	3.0	13.0	.5
Be.	3.0	26.0	25.0
St.	1.5	26.0	5000.

antimicrobial action. Terramycin, in concentrations as high as 298 μg per ml of medium, was incapable of inhibiting the growth of nine strains of fungi (*Nocardia asteroides, Sporotrichum schenkii, Microsporum canis, Trichophyton rubrum, Cryptococcus neoformans, Trichophyton mentagrophytes, Monilia albicans, Epidermophyton floccosum,* and *Microsporum audouini*).

Preliminary observations on the nature of the *in vitro* activity of terramycin were made by means of growth curves(8). The rate of growth of *Streptococcus hemolyticus* was

8. Hobby, G. L., Meyer, K., and Chaffee, E., PROC. SOC. EXP. BIOL. AND MED., 1942, v50, 281.

TABLE II.
Antimicrobial Action of Terramycin Hydrochloride: Comparison with Aureomycin Hydrochloride.

	Sensitivity: μg (total wt) per cc	
	Terramycin HCl	Aureomycin HCl (A377)
Strep. hemolyticus (C203Mv)	.9	.9
D. pneumoniae (I/230)	.9	.9
Staph. aureus (H)	.9	.9
K. pneumoniae	2.0	3.5
A. aerogenes	2.0	7.0
E. coli (BW 41)	7.0	14.0
" (W 87)	7.0	14.0
S. typhosa	7.0	7.0
S. dysenteriae	7.0	14.0
S. paratyphosa	7.0	14.0
Proteus vulgaris	450.	>450.
Pseud. pyocyaneus	28.	57.
Brucella bronchiseptica	.9	.9

Tests carried out simultaneously under identical conditions.
Incubation period 48 hr at 37°C.

measured in the presence and absence of varying concentrations of terramycin, by determination of the number of organisms per ml at varying times during the incubation period.

2. *Results.* The results of these studies are illustrated in Tables I, II, and III, and in Fig. 1 and 2. From the data available, it appears that that terramycin is more like penicillin than streptomycin in that its action is influenced only slightly by environmental factors. As is the case with other antibiotics, however, the concentration of terramycin present influences markedly the rate and extent of inhibition of growth of a given organism. Terramycin is a highly bacteriostatic agent although, under certain conditions, bactericidal action may be apparent. It is effective *in vitro* in low concentrations against a wide variety of microorganisms belonging to both the gram-positive and gram-negative groups.

In vivo activity. 1. Materials and methods. The preparations of terramycin used in these experiments were comparable with those which have been described above. Procedures employed in the evaluation of other antimicrobial agents have proved highly satisfactory for study of the chemotherapeutic action of terramycin, and the methods used in this laboratory were ones which have been described in detail in a communication concerned with penicillin(9). With the exception of those experiments pertaining to the chemotherapeutic action of terramycin against infections due to *Borrelia recurrentis,* all tests were carried out in male white mice (Rockland Regular Strain) weighing 15 to 20 g. In the case of *S. typhosa, K. pneumoniae,* and *H. influenzae,* cultures were diluted in mucin prior to injection; in the case of *H. pertussis,* cultures were diluted in Casamino acid, while in other instances dilutions were made in nutrient broth. Fourteen to 16 hour broth or blood broth cultures of all organisms, except *H. influenzae* and *H. pertussis,* were used. In the case of *H. influenzae,* a suspension of organisms, washed from a 5½-hour Levinthal's agar plate and having a density equivalent to 75% transmission (Photovolt Lumetron No. 400) was used; in the case of *H. pertussis,* a suspension of organisms in 1% Casamino acid, made from a 48-hour Bordet-Gengou plate, and having a density equivalent to 35% transmission, was used. Mice were infected with *H. influenzae* by the intraperitoneal injection of 1 ml of a 10^{-5} dilution of the standardized suspension of culture, diluted in mucin. Mice were infected with *H. pertussis* by the intracerebral injection of 0.01 ml of the undiluted suspension of culture described above. Animals were infected with all other organisms by the intraperitoneal injection of 1.0 ml quantities of varying dilutions of the culture under test.

All mice were treated with terramycin either in a single dose administered one-half hour after infection, or in divided dosage, twice daily beginning one hour after infection and continuing for a period of 2½ days. Both the subcutaneous and oral routes of administration were used.

Rats belonging to the Sherman strain and weighing approximately 90 to 110 g were infected intraperitoneally with 0.6 ml of citrated hearts' blood from previously infected rats in which the presence of *Borrelia*

9. Hobby, G. L., *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1946, v63, 296; *J. Bact.*, 1947, v54, 305.

TABLE III.
Antimicrobial Action of Terramycin: Comparison of the Action of Terramycin and Its Salts.

Organism	Sensitivity: μg of terramycin per cc			
	Terramycin	Terramycin hydrochloride	Terramycin sulfate	Terramycin sodium salt*
Strep. hemolyticus (C203Mv)	5.0	3.0	3.0	3.5
D. pneumoniae (I/230)	<1.5	<1.5	<1.5	<1.5
Staph. aureus (H)	<1.5	<1.5	<1.5	<1.5
Bc. subtilis (W)	<1.5	<1.5	<1.5	<1.5
K. pneumoniae	5.0	6.0	3.0	<1.5
A. aerogenes	5.0	6.0	5.5	3.5
E. coli (BW 41)	11.0	6.0	5.5	3.5
E. coli (W 87)	11.0	6.0	5.5	6.5
Ps. pyocyaneus	42.0	23.0	11.0	26.0
S. dysenteriae	21.0	12.0	5.5	6.5
S. typhosa	5.0	6.0	3.0	3.5
S. paratyphosa	11.0	12.0	5.5	26.0
Proteus vulgaris	>600	>742	>716	>837
Bruc. bronchiseptica	2.5	3.0	<1.5	<1.5
M. tuberculosis				
$H_{37}Rv$ Isolate No. 2678		6.5	6.0	7.5
Freshly isolated strains				
St.		6.5	6.0	4.5
Be.		6.5	6.0	7.5
Mo.		6.5	6.0	
Mu.		4.0	6.0	3.5
Ni.		6.5	6.0	7.5

Except in the case of *M. tuberculosis*, incubation was carried out for 72 hours at 37°C. Cultures of *M. tuberculosis* were incubated for 7 days at 37°C. Longer incubation was not used in view of the fact that the stability of terramycin at 37°C (pH 7.0-7.8) does not permit accurate readings after longer periods of time.

* pH 8.5 sodium salt of terramycin used.

recurrentis‖ was apparent by examination of Giemsa-stained blood smears. A portion of the infected rats were held as controls. Terramycin was administered to the remaining animals by the intramuscular route, once or twice daily, beginning 1 hour after infection and continuing for 4 days.

2. *Results.* The results of extensive studies, carried out by the methods described, to determine the chemotherapeutic action of terramycin against a variety of experimental infections in animals are illustrated in Tables IV and V. From these data, it is apparent that terramycin is a highly active antimicrobial agent capable of protecting mice against infections due to *Streptococcus hemolyticus, D. pneumoniae, K. pneumoniae, H. influenzae, H. pertussis, S. typhosa, S. newport, S. enteritidis* and *S. cholerae suis.*¶ It is capable, furthermore, of suppressing temporarily experimental infections in mice due to *S. typhimurium.* It is effective when administered by the oral as well as the parenteral routes. Terramycin is ineffective in the control of experimental mouse infections due to *Ps. pyocyaneus* or *Proteus vulgaris.* Preliminary studies in rats infected with *Borrelia recurrentis* suggest that 0.82 mg of terramycin per 100 g of body weight, administered intramuscularly once daily for 4 days, is sufficient to sterilize the blood.

Data on the chemotherapeutic action of terramycin against experimental mouse infections due to *M. tuberculosis* will be reported in detail elsewhere(10). Preliminary studies indicate, however, that 7.1 mg of terramycin per day administered subcutane-

‖ We are indebted to Dr. Fordyce Heilman of the Mayo Clinic, Rochester, Minn., for the strain of *Borrelia recurrentis* used.

¶ Studies on the chemotherapeutic action of terramycin against experimental infections due to the Clostridia are in progress and will be reported at a later date. The observations made so far would suggest that this antimicrobial agent is effective against this group of organisms.

10. Hobby, G. L., Lenert, T. F., Donikian, M., and Pikula, D., unpublished data.

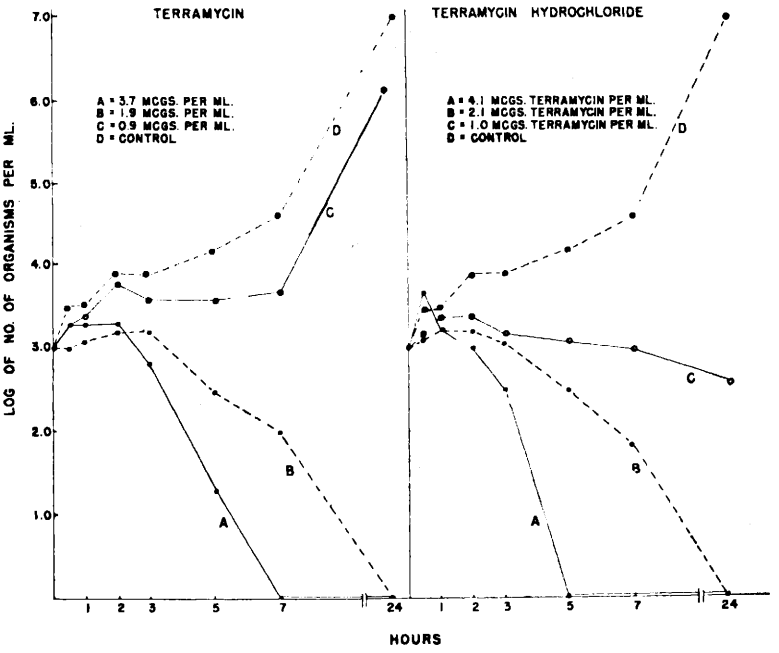

FIG. 1.

Comparison of the rate of action of varying concentrations of terramycin and terramycin hydrochloride against *Streptococcus hemolyticus* ($C_{203}Mv$) *in vitro*.

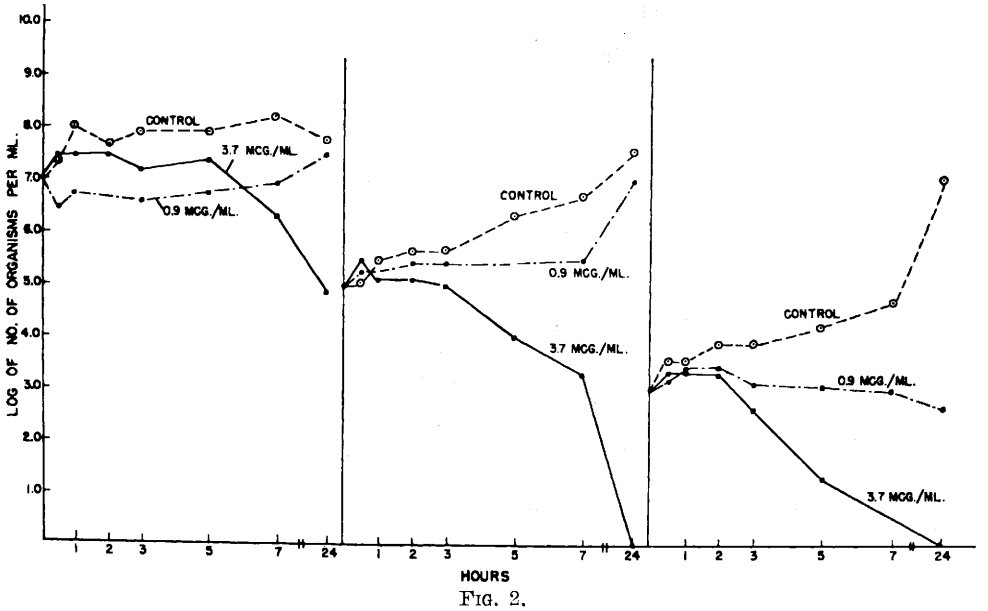

FIG. 2.

The rate action of terramycin against varying numbers of *Streptococcus hemolyticus* (C203Mv) *in vitro*.

ously is sufficient to exert a suppressive effect on the tuberculous infection in mice. Terramycin is absorbed rapidly into the circulatory system following oral or parenteral administration.

Significant concentrations may be detected in the urine of injected animals in a biologically active form(11). Terramycin is a substance of low toxicity. Preliminary studies in mice indicate that at

TABLE IV.
Chemotherapeutic Action of Terramycin and Its Salts Against Experimental Infections in Mice.

Microörganism	Terramycin: Injection schedule: Frequency	Total No.	Route	CD_{50} (19/20 Confidence Limits): Mg per 20 g mouse: Amphoteric	HCl	SO_4	Sodium salt
Strep. hemolyticus (C203Mv)	b.i.d.	5	Subcu.	.03 (.01- .07)	.01 (.01- .07)	.04 (.02- .07)	.03 (.01- .06)
	q.d.	1	"	.10 (.06- .16)	.35 (.23- .54)	.35 (.20- .63)	.25 (.17- .36)
	b.i.d.	5	Oral	.36 (.08- 1.62)	.74 (.49- 1.11)	.69 (.33- 1.45)	.50 (.19- 1.30)
	q.d.	1	"	2.5 (1.84- 3.40)	1.20 (.67- 2.16)	3.10 (1.55- 6.20)	2.50 (1.32- 4.75)
D. pneumoniae (I/230)	b.i.d.	5	Subcu.	.26 (.16- .42)	.27 (.20- .36)	.24 (.18- .32)	.30 (.19- .47)
	q.d.	1	"	1.3 (.72- 2.34)	2.6 (1.62- 4.16)	2.3 (1.05- 5.06)	2.20 (1.58- 3.06)
	b.i.d.	5	Oral	17 (12.69-22.78)	6.40 (4.92- 8.30)	8.10 (6.43-10.20)	6.80 (5.20- 8.84)
	q.d.	1	"	>25	25 (16.45-38)	>25	>25
K. pneumoniae (Nol.)	b.i.d.	5	Subcu.	.18 (.11- .30)	.098(.04- .22)	.19 (.11- .33)	.10 (.06- .16)
	q.d.	1	"	.68 (.40- 1.16)	.13 (.07- .23)	.31 (.18- .54)	.50 (.35- .72)
	b.i.d.	5	Oral	.84 (.21- 3.36)	2.3 (1.53- 3.45)	2.50 (1.75- 3.58)	1.1 (.69- 1.76)
	q.d.	1	"	2.40 (1.32- 4.37)	1.60 (.50- 5.14)	4.50 (3.00- 6.75)	3.20 (2.37- 4.30)
S. typhos	b.i.d.	5	Subcu.	.08 (.06- .11)	.24 (.12- .48)	.16 (.08- .31)	.34 (.16- .71)
	q.d.	1	"	.56 (.37- .84)	.60 (.16- 2.88)	1.00 (.55- 1.82)	.96 (.41- 2.26)
	b.i.d.	5	Oral	12 (8.89-16.2)	10.5 (6.18-17.85)	Approx. 33	
	q.d.	1	"	25 (13.51-46.25)	7.0 (3.18-15.40)	21 (7.5 -58.8)	13 (9.29-18.2)
H. influenzae	b.i.d.	5	Subcu.	.12 (.02- .66)			
	q.d.	1	"	1.7 (.36- 7.99)			
H. pertussis	b.i.d.	5	"	Approx. .7			
	q.d.	1	"	4.1 (2.28- 7.38)			

In all instances, a minimum of 10 mice per dosage level and 4 to 8 dosages were used to determine each of values above. The CD_{50} (19/20 Confidence Limits) values were calculated according to the method of Litchfield & Wilcoxon(13).

TABLE V.
Chemotherapeutic Action of Terramycin Against Experimental Infections in Mice.

	Terramycin (Amphoteric)				
	Injection schedule			CD_{50} (19/20 Confidence Limits): mg per 20 g mouse Observation period	
Microörganisms	Frequency	Total No.	Route	96 hrs	10 days
S. typhi murium	b.i.d.	5	Subcu.	0.13 (.08- .22)	>1.00
	b.i.d.	5	Oral	3.0 (1.76-5.10)	44 (18.33-105.6)
S. cholerae suis	b.i.d.	5	Subcu.	0.10 (.04- .26)	0.22 (0.09- 0.53)
	b.i.d.	5	Oral	2.0 (1.18-3.4)	>20
S. enteritidis	b.i.d.	5	Subcu.	0.09 (.05- .17)	3.8 (1.09- 13.3)
	b.i.d.	5	Oral	6.2 (3.9 -9.9)	>20
S. newport	b.i.d.	5	Subcu.	0.05 (.02- .06)	0.18 (0.08- 0.4)
	b.i.d.	5	Oral	2.0 (.57-7.)	7.2 (5.14- 10.08)

In all instances, min. of 10 mice per dosage, 4 to 8 dosages were used to determine each value above. The CD_{50} (19/20 Confidence Limits) values were calculated according to the method of Litchfield & Wilcoxon(13).

TABLE VI.
Subacute Toxicity of Terramycin in Mice.

Dosage (mg/20 g mouse)	No. doses per day	No. of days	Route of administration	No. of mice	% survival
7.45	2	5	Oral	10	100
3.72	2	5	"	10	100
1.86	2	5	"	10	100
11.18	2	5	Subcutaneous	10	60
7.45	2	5	"	10	90
3.72	2	5	"	10	80
11.18	1	5	"	10	60
7.45	1	5	"	10	80
3.72	1	5	"	10	100

least 375 mg may be administered orally twice daily for at least 5 days with no untoward symptoms. By the subcutaneous route, 185 mg per kg body weight may be administered once daily without toxicity. (Table VI). Extension studies by P'an and his associates(12) in mice and other species of animals further illustrate the low toxicity of this compound.

Discussion and summary. The data reported in this and in a previous communication by Finlay *et al.*(1) emphasize the strong antimicrobial activity of terramycin and its salts against a wide variety of aerobic and anaerobic gram-positive and gram-negative bacteria, and against certain of the rickettsiae. In addition, attention is called to the possible tuberculostatic activity of this compound. Terramycin is absorbed readily following oral or parenteral administration and is a highly effective chemotherapeutic agent, active against a variety of experimental infections in mice. A comparative study of the chemotherapeutic activities of terramycin and its salts indicates that the highly insoluble amphoteric form of terramycin as well as its more soluble salts are all chemotherapeutically effective. In most instances, the CD_{50} of the hydrochloride has been less than that of the other forms of terramycin, especially when administered by the oral route. More satisfactory results, as indicated by lower CD_{50} values, have been obtained in some in-

11. Hobby, G. L., Reed, W., Rinne, D., Powers, M., and D'Ambrosia, A., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 511.

12. P'an, S. Y., *et al., J. Pharm. and Exp. Therap.*, 1950, in press.

13. Litchfield, J. T., Jr., and Wilcoxon, F., *J. Pharm. and Exp. Therap.*, 1949, v95, 99.

fections, however, with terramycin (amphoteric) when therapy was carried out by means of a single injection. It is possible that this enhanced chemotherapeutic effect, observed in these infections when treatment is carried out by a single injection only, may be a reflection of the low solubility and slow absorption of terramycin in the amphoteric form.

The toxicity of terramycin has been studied extensively by P'an and his associates(12). It is possible that the main virtue of terramycin resides in its remarkably low toxicity and low chemotherapeutic index. Terramycin, however, is a highly effective chemotherapeutic agent against experimental animal infections and it is believed that terramycin may prove of value in the treatment of certain human infections due to terramycin-sensitive organisms. A therapeutic evaluation of terramycin in human infections, therefore, has been initiated.

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Absorption and Excretion of Terramycin in Animals. (17728)

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The antimicrobial activity of terramycin has been described in detail(1). The present study was undertaken in order to determine the rate of absorption of terramycin in experimental animals and to evaluate the differences, if any, in the rates of absorption of the various salts of terramycin.

Materials and Methods. Throughout this study, partially purified and crystalline terramycin, terramycin hydrochloride, terramycin sulfate, and 2 forms of the sodium salt of terramycin* prepared from crystalline terramycin have been used. Absorption and excretion studies have been carried out in rabbits and dogs. Terramycin has been administered in aqueous solution or suspension by the intravenous, intramuscular, or oral route. In all instances specimens of sera were separated from the blood clot on the day of bleeding and all serum and urine samples frozen on the day of collection. Samples were thawed once only at the time of test. The concentrations of terramycin in serum and urine may be determined by the standard methods used for penicillin. In this laboratory a modification of the method of Tompsett and his associates(2) has been used throughout. The procedure differs from those of Rammelkamp, Kirby, and other investigators in that the increments are small, the amount of serum in each tube is constant and the concentration of organisms is small. Three dilutions of each serum sample were made as follows: (1) a 1:4 dilution in broth; (2) a 1:20 dilution in 25% normal rabbit (or pooled human) serum broth; (3) a 1:120 dilution in 25% normal serum broth; and (4) a 1:600 dilution in 25% normal serum broth. If it was expected that the concentration of terramycin per ml of serum may be low, undiluted serum likewise was tested. When dilutions were prepared in this manner a concentration of 25% serum existed in all except a portion of the undiluted serum series.†

For each dilution of serum, a series of 6

1. Hobby, G. L., *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 503.

* The nature of the 2 forms of terramycin sodium salt has been discussed previously.(1)

2. Tompsett, R., *et al.*, *J. Bact.*, 1947, v53, 581.

† No correction has been made for the high concentration of serum in these tubes. It is assumed that the inhibition of the growth of *Streptococcus hemolyticus* in these tubes is indicative of the presence of terramycin; it is recognized, however, that the concentration in the serum cannot be measured accurately by the method used herein.