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.

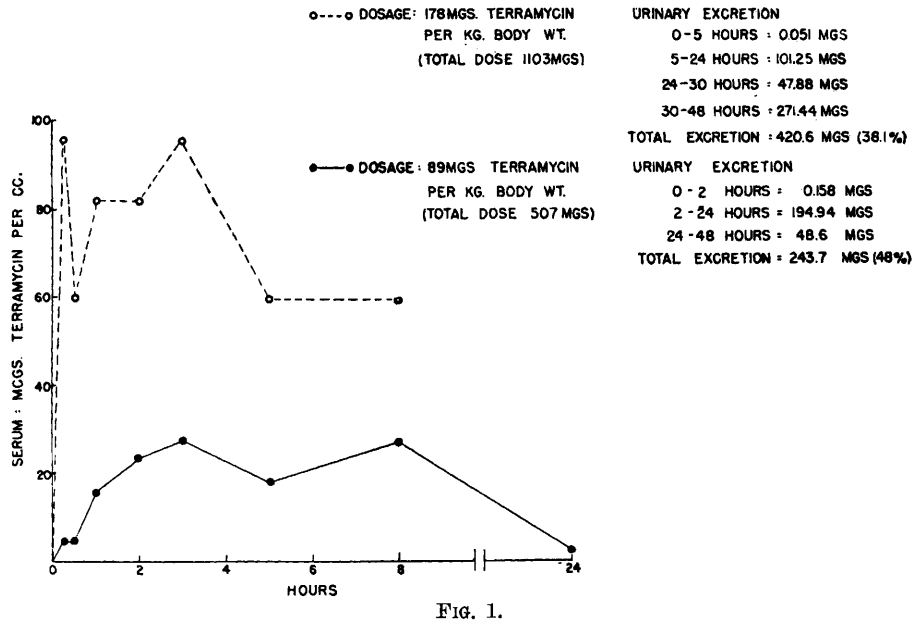

FIG. 1.

The absorption and excretion of terramycin following single intramuscular injections of terramycin sodium salt in dogs.

tubes were used, containing 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml, 0.6 ml, and 0.8 ml of the diluted serum. The amount of fluid per tube was adjusted to a volume of 0.8 ml by the addition of 25% serum broth. Two-tenths ml of diluted culture was added to each tube and mixed well. *Streptococcus hemolyticus,* strain C203Mv, was used throughout; a 10^{-5} dilution of a 15-hour blood broth culture gave uniform and satisfactory results. All tests were incubated at 37°C for 18 to 24 hours. The end point was accepted as the least amount of serum capable of inhibiting the growth of the organism as evidenced by absence of gross turbidity at the end of the incubation period. The concentration of terramycin in urine was determined by a modification of this procedure. Plain broth was used in place of serum broth for all dilutions throughout the test. Higher dilutions were frequently necessary (1:1800, 1:5400, etc.). Incubation was carried out at 37°C for a period of 24 to 30 hours or longer, depending upon the rate at which the culture grows in the urine. As above, the end point was accepted as the least amont of urine capable of inhibiting growth of the test organism as evidenced by absence of gross turbidity.

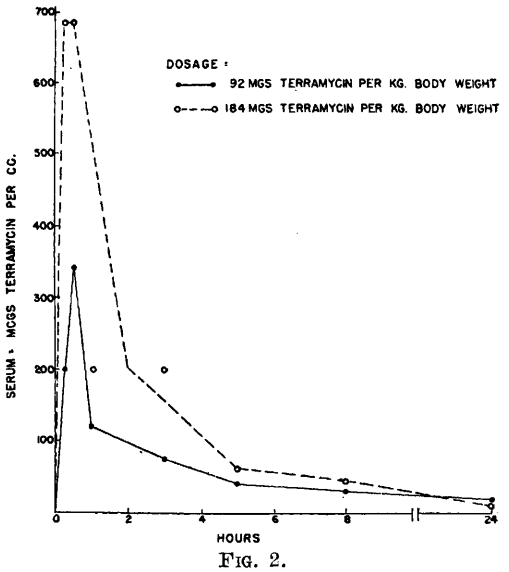

FIG. 2.

Concentrations of terramycin in serum following single intravenous injections of terramycin sodium salt in dogs.

Crystalline terramycin was used as standard‡ for both serum and urine assays. Concentrations of 2.0, 1.0, 0.5, and 0.1 μg of terramycin per ml of broth§ gave satisfactory results. The sensitivity of the test organism

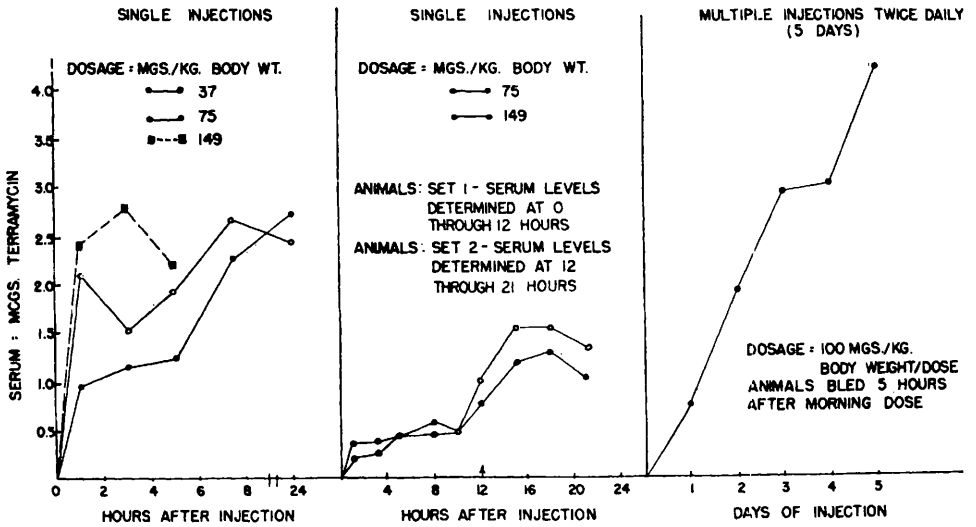

FIG. 3.

Concentrations of terramycin in serum following intramuscular administration in rabbits. (Average of 3 to 8 animals per dosage level.)

(C203Mv) to crystalline terramycin under the conditions of this test was generally 0.1 to 0.15 μg per ml.

Results. The results of a limited series of observations on the absorption and excretion of terramycin in rabbits and dogs are illustrated in Fig. 1 to 5.

Dogs. Following the intramuscular injection of the sodium salt of terramycin to dogs, in dosages of 89 to 178 mg per kg, terramycin was absorbed rapidly into the circulatory system. Significant concentrations were detected in the serum of injected animals within 15 to 30 minutes and peak concentrations were reached one quarter to three hours after injection. The rate at which the concentration in the blood stream reached its maximum varied with the dosage administered. Following intramuscular injection of a dosage equivalent to 89 mg per kg body weight, terramycin was demonstrated in the blood stream for a period of at least 24 hours. After intramuscular injection of 89 and 178 mg of pure terramycin per kg body weight of dogs terramycin appeared in the urine in biologically active form within 2 hours. Large quantities were recovered as late as 30 to 48 hours after administration. In the small series of dogs tested, the total excretion ranged from 38 to 48% of the administered dose. After intravenous injection in dogs, maximum concentrations of terramycin were detected in serum within 15 to 30 minutes. This was followed by a rapid decrease in the concentration present in the serum, and approximately 70% of the total dose administered was removed from the blood stream within 1 to 2 hours after injection. Low concentrations were detected, however, in the serum for as long as 24 hours after intravenous administration.

Rabbits. Extensive studies were carried out in rabbits to compare the rate of absorption of orally and parenterally administered terramycin and to determine differences, if

‡ All studies reported herein were carried out using, as standard, pure terramycin (amphoteric) to which a potency of 1000 μg per mg had been assigned. This was a dihydrate compound. The current master standard is an anhydrous preparation of terramycin having a potency established at 1000 μg per mg; the working standard now in use is terramycin dihydrate having a designated potency of 925 μg per mg.

§ In all instances, standard was dissolved in serum broth and subsequently Seitz filtered to ensure sterility. As discussed in a previous communication(1), filtration of small volumes of dilute aqueous solutions is not recommended.

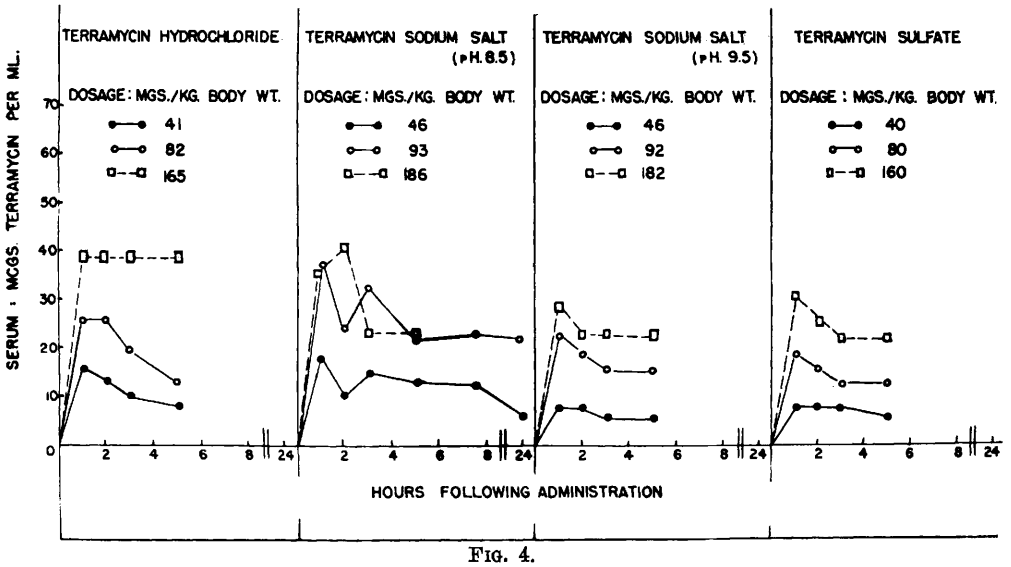

FIG. 4.

Concentrations of terramycin in serum following single intramuscular injection in rabbits. (Average of 3 to 8 animals per dosage level.)

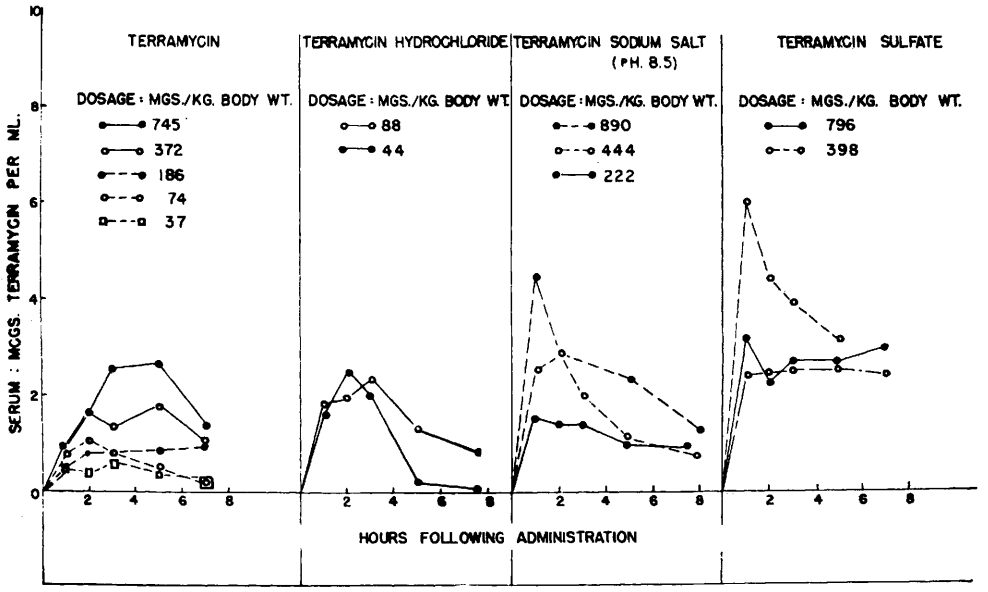

FIG. 5.

Concentrations of terramycin in serum following single doses administered orally in rabbits. (Average of 3 to 8 animals per dosage level.)

any, in the rates of absorption of terramycin and its salts. It is recognized that the rabbit is not the optimum animal for absorption studies. Large scale experiments carried out on the absorption of orally and parenterally administered penicillin indicated, however, that significant results may be obtained in this species of animal provided the tests are carried out under standardized conditions. To obtain comparable blood levels approximately 6 times as much penicillin is required in rabbits as in man; consequently, it is believed that the ratio of terramycin dosages may be comparably different. Following the

intravenous administration of 46 mg of pure terramycin per kg body weight, the average concentration of terramycin detectable in the serum at one hour after administration was 24 μg per ml; an average of 5 μg per ml of serum was detectable at 5 hours after injection.

High concentrations of terramycin were observed in the serum of rabbits following the intramuscular administration of terramycin hydrochloride or terramycin sodium salt. The concentrations obtained following intramuscular administration of pure terramycin were low, indicating slow absorption which may have been due, at least in part, to its extremely low solubility in aqueous solutions. On repeated intramuscular injection of terramycin, a slight increase in the concentration of antibiotic in the serum was noted; the increase was not sufficient, however, to account for the dosage used. In all instances, terramycin sulfate gave irregular results. This may have been due to the rapid rate at which pure terramycin precipitates from solutions of terramycin sulfate. Absorption studies in a large series of rabbits have indicated that terramycin is absorbed readily from the gastro-intestinal tract. Following oral administration, high concentrations were detected in the blood within one hour. Although higher concentrations were attained in the serum of rabbits following administration of terramycin by the oral than by the intramuscular route, most satisfactory results were attained on oral administration by use of terramycin hydrochloride or sodium terramycin.

Discussion and summary. Studies on the absorption and excretion of terramycin in man will be reported by Werner and his associates(3) at an early date. The results obtained by these investigators agree, in general, with those reported herein. Terramycin and its salts are absorbed readily following either oral or parenteral administration. Terramycin in the amphoteric form is a highly insoluble compound. It is absorbed following oral or parenteral administration at an exceedingly slow rate. A comparative study of the chemotherapeutic action of terramycin and its salts in experimental infections in mice(1) have suggested that the slow absorption of this form of terramycin may be a valuable asset in the treatment of certain infections, provided a dosage form can be prepared which will permit its parenteral administration to humans. Studies are in progress, furthermore, to determine the extent to which this highly insoluble form of terramycin is excreted in the feces.

Most satisfactory results have been obtained to date on oral administration of terramycin hydrochloride, a highly soluble salt. The high concentrations of terramycin which may be detected in the serum of animals following oral administration suggests a parallelism between terramycin and aureomycin and chloramphenicol. In contrast to aureomycin and chloramphenicol, however, a high percentage of the terramycin administered is excreted in a biologically active form. Terramycin hydrochloride in aqueous solution has a pH of 1.5; its administration, parenterally in aqueous solution, is not feasible. Preliminary observations suggest, however, that the intravenous administration of this form of terramycin in an appropriate buffer should be possible.

The rate and degree of absorption of terramycin and its salts suggest that terramycin, a highly active antimicrobial agent, should be readily effective in the treatment of infections due to terramycin-sensitive microorganisms. Based on the experimental data available to date, terramycin offers promise as a chemotherapeutic agent for infections in man.

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3. Werner, C., *et al.*, 1950, in press.