unexpected characteristic for furans. The procedure for the isolation of the neutral oxalates was patterned after that of Adamson (16).

## SUMMARY

A total of 31 γ-amino tertiary alcohols which are to be screened for possible pharmacodynamic or chemotherapeutic activity is presented. It is anticipated that further modifications of these structures and detailed pharmacological reports will be presented in later publications.

## REFERENCES

(1) Blanton, C. D., and Nobles, W. L., THIS JOURNAL, **51**, 878(1962).
(2) *Ibid.*, **52**, 46(1963).
(3) Denton, J. J., Turner, R. J., Neier, W. B., Lawson, V. A., and Schedi, H. P., *J. Am. Chem. Soc.*, **71**, 2048(1949).
(4) Denton, J. J., Schedi, H. P., Neier, W. B., and Lawson, V. A., *ibid.*, **71**, 2054(1949).
(5) Denton, J. J., and Lawson, V. A., *ibid.*, **72**, 3279(1950); Denton, J. J., Schedi, H. P., Lawson, V. A., and Neier, W. B., *ibid.*, **72**, 3795(1950).
(6) Denton, J. J., U. S. pat. 2,716,121(August 23, 1955); through *Chem. Abstr.*, **50**, 5770(1956).
(7) Denton, J. J., U. S. pat. 2,723,269(November 8, 1955); through *Chem. Abstr.*, **50**, 13099(1956).
(8) Denton, J. J., U. S. pat. 2,725,399(November 29, 1955); through *Chem. Abstr.*, **50**, 9446(1956).
(9) Denton, J. J., Lawson, V. A., Neier, W. B., and Turner, R. J., *J. Am. Chem. Soc.*, **71**, 2050(1949).
(10) Denton, J. J., Neier, W. B., and Lawson, V. A., *ibid.*, **71**, 2053(1949).
(11) Cunningham, R. W., Harned, B. K., Clark, M. C., Cosgrove, R. R., Daugherty, N. S., Hine, C. H., Vessey, R. E., and Yuda, N. N., *J. Pharmacol. Exptl. Therap.*, **96**, 151(1949); through *Chem. Abstr.*, **43**, 7581(1949).
(12) Harder, A. and Prelicz, T., *Schweiz. Med. Wochschr.*, **86**, 335(1956), through Burger, A., "Medicinal Chemistry," Interscience Publishers, Inc., New York, N. Y., 1960, p. 504.
(13) Adamson, D. W., Barrett, P. A., and Wilkinson, S., *J. Chem. Soc.*, **1951**, 52.
(14) Adamson, D. W., *ibid.*, **1949**, S144.
(15) *Ibid.*, **1950**, 885.
(16) Adamson, D. W., and Billinghurst, J., *ibid.*, **1950**, 1039.
(17) Adamson, D. W., Brit. pat. 689,234(March 25, 1953); through *Chem. Abstr.*, **48**, 4008(1954).
(18) Burckhalter, J. H., and Johnson, S. H., *J. Am. Chem. Soc.*, **73**, 4827(1951).
(19) Pohland, A., and Sullivan, H. R., *ibid.*, **75**, 4458(1953).
(20) Morrison, A. L., and Rinderkneckt, H., *J. Chem. Soc.*, **1950**, 1510.
(21) Gilman, H., and Catlin, W. E., "Organic Syntheses," Coll. Vol. I, John Wiley & Sons, Inc., New York, N. Y., 1951, p. 471.
(22) Pohland, A., and Sullivan, H. R., *J. Am. Chem. Soc.*, **77**, 3400(1955).
(23) Roger, F. C., and Nobles, W. L., THIS JOURNAL, **51**, 272(1962).
(24) Allen, C. F. H., and Gates, J. W., "Organic Syntheses," Coll. Vol. III, John Wiley & Sons, Inc., New York, N. Y., 1955, p. 140.
(25) Profft, E., *Chem. Tech. (Berlin)*, **3**, 210(1951); *ibid.*, **4**, 241(1952); *ibid.*, **5**, 13(1953); through *Chem. Abstr.*, **46**, 688(1952); *ibid.*, **47**, 10532(1953); *ibid.*, **48**, 7608(1954).
(26) Bockstahler, E. R., and Wright, D. L., THIS JOURNAL, **46**, 542(1957).
(27) Adams, R., and Noller, C. R., "Organic Syntheses," Coll. Vol. I, John Wiley & Sons, Inc., New York, N. Y., 1951, p. 109.

# Antibiotic Therapy of Experimental Leptospiral Infection in Chick Embryos II

## Comparison of the Action of Demethylchlortetracycline and Three Other Tetracyclines With and Without Ascorbic Acid on *Leptospira icterohaemorrhagiae*

By S. F. QUAN*, M. I. GOLDENBERG, and C. W. ABBOTT

**Saline and antibiotic solutions, with and without ascorbic acid, were injected into 7-day-old chick embryos inoculated with *Leptospira icterohaemorrhagiae* 1 day after infection. All inoculations were made into the yolk sac. The treated embryos were observed for prolongation of life and for survival rates over a period of 12 to 14 days. Demethylchlortetracycline was about twice as active as tetracycline and oxytetracycline and about 10 times as active as chlortetracycline. Ascorbic acid alone did not influence the course of the infection and did not affect their therapeutic activity when given with antibiotics.**

DEMETHYLCHLORTETRACYCLINE (DMCT), a recently introduced commercial antibiotic, is reported to have advantages over the older tetracycline antibiotics with respect to chemical stability, antibacterial activity, and efficiency of maintaining an effective serum level (1–4).

The chemical structure of DMCT is identical to that of chlortetracycline with the absence of a methyl group in the 6 position of the tetracycline molecule. While DMCT has not been reported to have been tested in the chemotherapy of leptospirosis, chlortetracycline (CT), oxytetracycline (OT), and tetracycline (TC) have been effective against experimental leptospiral infections in chick embryos, hamsters, guinea pigs, dogs, and cattle (5–10). Furthermore, Howarth (11) was successful in clearing swine carriers of *Leptospira pomona* with CT and OT, while Stoenner and his associates (12) eliminated *L. ballum* from a naturally infected colony of mice using CT.

Received February 12, 1963, from the Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Technology Branch, San Francisco Field Station, San Francisco, Calif.
Accepted for publication September 16, 1963.
*Deceased February 29, 1964.

Also, CT and OT have been used with success against leptospiroses in man (10, 13).

In our previous study (14) we found the activities of OT and TC about the same, but many times more active than CT. This finding was similar to that of Kiser, *et al.* (15), who reported TC more active than CT against *L. icterohaemorrhagiae* and *L. pomona* in chick embryos. However, in both of these studies the more active tetracycline antibiotics were administered in a solution containing ascorbic acid as a buffer, while the solutions of CT were given without buffer. It was not known whether ascorbic acid has an effect on the infection or the therapeutic quality of the drugs.

This report is concerned with (*a*) the effect of ascorbic acid on chick embryos inoculated with a suspension of *L. icterohaemorrhagiae*, (*b*) the efficacy of DMCT in solutions with and without ascorbic acid, and (*c*) a comparison of DMCT with CT, OT, and TC in dosage ranges capable of producing a significant prolongation of mean life and protecting 50% of the infected chick embryos.

## MATERIALS AND METHODS

**Ascorbic Acid.**—Commercial packages of TC are available in vials containing 100 mg. of tetracycline hydrochloride with 250 mg. of ascorbic acid, 250 mg. TC HCl with 750 mg. ascorbic acid, and 500 mg. TC HCl with 1.5 Gm. of ascorbic acid. In this study ascorbic acid was given in the amount of 3 mg. for each milligram of tetracycline antibiotic per egg. When ascorbic acid was given alone, 4 mg. per egg was used. The latter solution was prepared from a 200 μm. stock solution which contained 1.76 Gm. of ascorbic acid, ACS grade, in 50 ml. of distilled water. The pH of this solution was 2.5. It was sterilized by filtration, stored in air-tight vials in the refrigerator, and used during a period of 2 months with good results. For use, 4.0 ml. of the stock solution was partly neutralized with 0.35 ml. of 2 *N* NaOH and diluted with 2.7 ml. of sterile physiological saline. Each embryo was given 0.2 ml. of this solution as the control treatment with ascorbic acid.

**Infection.**—The infectious agent, the test animals, and the methods of infecting and treating the embryos have been described in detail in a previous paper (14). Briefly, the method used to infect the eggs was as follows. A fresh culture of *L. icterohaemorrhagiae*, strain LT-331, was transplanted from Fletcher's to Stuart's medium and grown for 7 days at 28°, then diluted 1–10 with 1% peptone water for infecting eggs. Fertile White Leghorn eggs were purchased unincubated from a commercial hatchery. On arrival they were incubated for 7 days, then candled before they were inoculated in the yolk sac with 0.2 ml. of spirochetal suspension. On the following day the infected embryos were candled, randomized, and then treated. The treated eggs were incubated upright, and candled daily for the next 12–14 days to check their viability.

**Antibiotics.**—Sterile powders of the hydrochloride salts of the antibiotics were used in this study. The powders were kept free of moisture in the refrigerator. For each test, fresh solutions were prepared using sterile physiological saline.

Antibiotic solutions containing ascorbic acid were prepared by adding appropriate volumes of the ascorbic acid stock solution to weighed samples of the antibiotic, so that for each milligram of antibiotic hydrochloride there was 3 mg. of ascorbic acid.

Treatment of infected embryos was effected by inoculating 0.2 ml. of the appropriate dilutions of antibiotics, with or without ascorbic acid, into the yolk sac on the day following infection. Each embryo was treated only once.

**Analysis of Results.**—All of the results were analyzed on the basis of the mean life of groups of animals. For purposes of calculating mean life in groups having survivors at the end of the observation period (14 days), surviving animals were considered dead on the 18th day. Also, because deaths occurring before the 5th day have been shown to be due to trauma rather than leptospiral infection, survival measurements were based on the number of viable infected embryos observed on the 4th day. Additionally, the survival times of various treatment groups were analyzed for time-per cent effect by the method of Litchfield (16), and for dose-survival effects by the method of Litchfield and Wilcoxon (17). By the latter method, the survival percentages were based on numbers surviving on the 12th day, as we have found previously that deaths became irregular again beginning on the 12th day. These methods of analysis are identical to those used in our previous paper (14).

The statistical analysis of the effect of ascorbic acid was made on pairs of parameters in which one member of the pair was given ascorbic acid and the other was not. Differences in mean life were considered significant at the 5% level using the Student *t* test. Differences of the $ET_{50}$'s and of the slope functions were compared, again by the methods of Litchfield, and Litchfield and Wilcoxon (16, 17).

## RESULTS

The results of treatments, with and without ascorbic acid, in 12 pairs of infected groups of embryos are shown in Table I. Four of the pairs were treatment controls in which the infected animals were treated with saline or a saline solution of ascorbic acid; the other eight pairs were treated with solutions of antibiotics with or without ascorbic acid.

Analysis of the average life of the controls, as separate pairs or as a total without regard to ascorbic acid, showed that ascorbic acid had no influence on the average life of the embryos inoculated with leptospires. The average life of the saline-treated controls was 6.55 days; of ascorbic acid-treated, 6.38 days; of all controls taken together, 6.46 days. There is no significant difference among these averages even at the 1% level. Furthermore, they do not differ significantly from a mean life of 6.44 days, the average of 25 similar tests conducted over a 3-year period reported previously (14).

The slope functions of the time-per cent effect curves of the control pairs were tested for parallelism by calculating their ratios (16). The slope function

TABLE I.—EFFECT OF ASCORBIC ACID ON ANTIBIOTIC THERAPY OF CHICK EMBRYOS EXPERIMENTALLY INFECTED WITH *L. icterohaemorrhagiae*

| Test Date | Antibiotic | Dose/Egg μm. | Dose/Egg mg. | No. Eggs Each | Survival — Without Ascorbic Acid — Mean Life | ET$_s$ | S[a] | Survival — With Ascorbic Acid — Mean Life | ET$_{50}$ | S[a] | Sig. Dif.[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2-23 | Control | ... | ... | 20 | 6.6 | 6.1 | 1.11 | 6.1 | 5.5 | 1.16 | + |
| 3-16 | (saline) | ... | ... | 20 | 6.2 | 5.8 | 1.21 | 6.4 | 6.2 | 1.17 | 0 |
| 4-6 | | ... | ... | 18 | 6.5 | 6.0 | 1.08 | 6.4 | 6.1 | 1.15 | 0 |
| 4-27 | | ... | ... | 18 | 6.9 | 6.1 | 1.15 | 6.6 | 6.2 | 1 16 | 0 |
| 4-27 | Demethylchlor- | 0.05 | 0.03 | 14 | 8.2 | 7.7 | 1.15 | 7.3 | 7.0 | 1.18 | 0 |
| 4-27 | tetracycline | 0.5 | 0.3 | 14 | 14.4 | 15.6 | 1.64 | 14.8 | 14.4 | 1.35 | 0 |
| 4-6 | Oxytetracycline | 0.2 | 0.09 | 18 | 11.6 | 10.5 | 1.43 | 11.4 | 11.8 | 1.36 | 0 |
| 4-6 | | 1.0 | 0.5 | 18 | 14.8 | 15.0 | 1.45 | 14.2 | 15.0 | 1.60 | 0 |
| 3-16 | Tetracycline | 0.2 | 0.08 | 14 | 10.8 | 10.5 | 1.48 | 12.0 | 11.6 | 1.27 | 0 |
| 3-16 | | 1.0 | 0.5 | 14 | 13.5 | 13.5 | 1.60 | 14.2 | 14.9 | 1.27 | 0 |
| 2-23 | Chlortetra- | 1.0 | 0.5 | 19 | 10.0 | 8.7 | 1.22 | 10.2 | 9.8 | 1.16 | 0 |
| 2-23 | cycline | 2.5 | 1.3 | 19 | 13.6 | 14.6 | 1.47 | 14.2 | 14.6 | 1.30 | 0 |

[a] S = Slope function (an equivalent of the standard deviation). [b] Significant difference at 5% level; 0 = none of three parameters, + = of ET$_{50}$.

ratios showed that these curves were all parallel within experimental error, and therefore not different from one another. The ET$_{50}$'s were compared by computing the reaction time ratio, *i.e.*, the ratio of the ET$_{50}$'s. In these comparisons of the four control pairs, only one pair showed a significant difference. This difference was found only in the test of 2-23, the first test of this series. The comparative test used is especially sensitive to the accumulation of daily effects. When the 2-23 control pair was analyzed by a *t* test comparing the daily differences, these paired results were also significantly different. As these four pairs of controls were analyzed for differences by three parameters, 12 comparisons were made. Since the addition of ascorbic acid gave no other significant difference, the single exception in 12 observations was given little weight in the over-all evaluation of the action of ascorbic acid on the infection. The average life of none of these control pairs, as already indicated above, was significantly different.

Among the antibiotic-treated embryos, the differences in mean life of embryos with and without ascorbic acid varied from 0.2 days for the pair treated with 1.0 μm. CT to 1.2 days for the pair treated with 0.2 μm. TC. The difference between the ET$_{50}$'s of these pairs varied from zero days for 2.5 μm. CT and 1.0 μm. OT to 1.4 days for 1.0 μm. TC. The difference between the slope functions varied from 0.03 for the 0.05 μm. DMCT pair to 0.33 for the 1.0 μm. TC pair. The differences in this function were less among groups of animals treated with smaller dosages of each of the antibiotics (where less than 15% of the embryos survived the observation period of 14 days) than among those groups treated with larger doses (where about 50% of the embryos survived for 14 days). The range of the differences for the former groups varied from 0.03 for 0.5 μm. DMCT to 0.21 for 0.2 μm. TC, and for the latter groups, from 0.15 for 1.0 μm. OT to 0.33 for 1.0 μm. TC. The wide range of these parameters showed that the experimental error in these drug treatments was large. Nevertheless, within experimental error, statistical analyses showed that ascorbic acid did not have an effect on the therapeutic action of the drugs.

Table II shows the chemotherapeutic effect of various dosages of DMCT in chick embryos inoculated with leptospires, without regard to the effect of ascorbic acid. DMCT given in a dose of 0.05 μm. prolonged the average life of the embryos by 1.5 days and extended the ET$_{50}$ by 1.6 days. The baseline for comparing prolongation of life was established in our previous studies at from 3 to 5 days. In the present study, this level of prolongation of life was found at a dosage of 0.1 μm. of DMCT. With increasing dosages of this drug, not only was there an increase in life, but also the number and

TABLE II.—EFFECT OF TETRACYCLINE ANTIBIOTICS ON THE SURVIVAL OF CHICK EMBRYOS INOCULATED WITH *L. icterohaemorrhagiae*

| Compd. | Dose/Egg μm. | Dose/Egg mg. | No. Tests | Survivors on Day 1 | 4 | 8 | 9 | 12 | 13 | 14 | Survival — Mean Days | ET$_{50}$ | S | Prolonged Life Mean Days | ET$_{50}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Demethyl- | 1.0 | 0.6 | 1 | 13 | 10 | 10 | 10 | 9 | 9 | 5 | 15.7 | ... | ... | 9.3 | ... |
| chlortetra- | 0.5 | 0.3 | 3 | 37 | 29 | 25 | 24 | 20 | 16 | 14 | 14.0 | ... | ... | 7 6 | ... |
| cycline | 0.25 | 0.15 | 3 | 41 | 36 | 32 | 31 | 27 | 19 | 13 | 13.9 | 16 0 | 1.68 | 7.5 | 10.2 |
| | 0.2 | 0.12 | 1 | 14 | 13 | 11 | 11 | 9 | 6 | 4 | 13.6 | 13.2 | 1.41 | 7.2 | 7.4 |
| | 0.1 | 0.06 | 5 | 63 | 44 | 35 | 28 | 9 | 5 | 0 | 10.3 | 10.1 | 1.34 | 3.9 | 5.3 |
| | 0.05 | 0.03 | 3 | 39 | 33 | 9 | 1 | 0 | 0 | 0 | 7.9 | 7.4 | 1.13 | 1.5 | 1.6 |
| Controls | ... | ... | 8 | 153 | 124 | 1 | 0 | 0 | 0 | 0 | 6.4 | 5.8 | 1.11 | 0 | 0 |
| Tetracycline | 1.0 | 0.5 | 2 | 28 | 20 | 19 | 17 | 15 | 11 | 6 | 13.9 | 14.1 | 1.48 | 7.6 | 8.1 |
| | 0.2 | 0.09 | 2 | 28 | 25 | 20 | 20 | 10 | 6 | 1 | 11.4 | 10.9 | 1.39 | 5.1 | 4.9 |
| Controls | ... | ... | 2 | 40 | 30 | 0 | 0 | 0 | 0 | 0 | 6.3 | 6.0 | 1.22 | 0 | 0 |
| Oxytetra- | 1.0 | 0.5 | 2 | 36 | 30 | 28 | 26 | 21 | 15 | 15 | 14.5 | 15.0 | 1.51 | 8.1 | 9.0 |
| cycline | 0.2 | 0.09 | 2 | 36 | 31 | 28 | 26 | 10 | 3 | 2 | 11.2 | 11.8 | 1.39 | 4.8 | 5.8 |
| Controls | ... | ... | 2 | 36 | 27 | 0 | 0 | 0 | 0 | 0 | 6.4 | 6.0 | 1.14 | 0 | 0 |
| Chlortetra- | 2 5 | 1.3 | 2 | 38 | 28 | 27 | 27 | 21 | 16 | 7 | 13.9 | 14.6 | 1.40 | 8.1 | 8.9 |
| cycline | 1.0 | 0.5 | 2 | 38 | 33 | 28 | 18 | 3 | 2 | 2 | 10.2 | 9.4 | 1.26 | 4.4 | 3.5 |
| Controls | ... | ... | 2 | 41 | 34 | 0 | 0 | 0 | 0 | 0 | 5 8 | 5.7 | 1 16 | 0 | 0 |

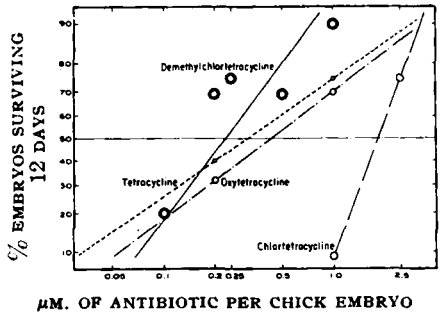


Fig. 1.—Dose-survival rates of chick embryos experimentally infected with *L. icterohaemorrhagiae* and treated with tetracyclines.

percentage of survivors were increased. At the 1.0-μm. per egg dose, 10 of the embryos survived 4 days; of these nine survived 12 days.

Also given in Table II are survival data for embryos treated with three other tetracyclines in doses which can be used to compare them with DMCT. At a dose of 0.2 μm. per egg, the prolongation of mean life and extension of the $ET_{50}$ for DMCT were 7.2 and 7.4 days, respectively; for TC, 5.1 and 4.9 days, and for OT, 4.8 and 5.8 days. At 1.0 μm. the prolongation of mean life for DMCT, OT, and TC was 9.3, 8.1, and 7.6 days, respectively. The weakest activity was shown by CT. Chemotherapeutic activity by the other three antibiotics at levels of 0.2 μm. and 1.0 μm. was approximated by CT at dosages of 1.0 μm. and 2.5 μm., respectively. These data indicate that DMCT was slightly more active than OT and TC and several times more active than CT against *L. icterohaemorrhagiae* in chick embryos.

Figure 1 shows the percentages of embryos surviving on the 12th day after infection in relation to the dose of DMCT. For various reasons already discussed in the first paper of this series, the dose-survival effect data in these tests were heterogeneous. The dose-effect line is drawn by inspection in terms of best fit. Accordingly, the $ED_{50}$ for DMCT is about 0.25 μm. On the other hand, only two doses of the other tetracyclines were studied. The dose-effect lines for these antibiotics were drawn through the two points marking the survival rates of the doses on the 12th day. These lines indicate $ED_{50}$'s of 0.30, 0.40, and 1.9 μm. for TC, OT, and CT, respectively. These lines and the $ED_{50}$'s show that DMCT was slightly more active than TC and OT, and all three were more active than CT by five to eightfold.

## DISCUSSION AND CONCLUSIONS

Ascorbic acid given in 4-mg. quantities, or about 23 μm. per egg, had no apparent influence on the survival of chick embryos inoculated with *L. icterohaemorrhagiae*. In addition, the therapeutic action of the antibiotics DMCT, CT, OT, or TC was not affected by the incorporation of 3 mg. of ascorbic acid for each milligram of antibiotic used. The preparation of solutions of tetracyclines was facilitated by the addition of ascorbic acid. These observations conform to the known properties of ascorbic acid, which is primarily a vitamin and is often used as a buffer and an antioxidant.

Although the dose-effect curves of TC, CT, and OT were drawn from just two points for each drug in this study, the activities found are in good agreement with those reported previously for these drugs when they were studied in many dosages over a wide range of levels (14). In that study none of the embryos survived after treatment with 0.1 μm. of OT or TC; the $ED_{50}$ of these two compounds was 0.35 μm. In the present study (Fig. 1) the curves for OT and TC predicted the survival of 20 and 25% of the embryos at a dose of 0.1 μm. and $ED_{50}$'s of 0.3 and 0.4 μm., respectively. When the results of past and present studies are considered together, the activities of OT and TC may be interpreted as the same, and one line can be drawn to represent their dose-effect. This line should be drawn through an $ED_{50}$ of 0.35 μm. and parallel to the line of DMCT to reflect little or no survival at the 0.1-μm. dose. Similarly, the line for CT should be redrawn to encompass the previous findings. For the new CT line, the slope would again be that of the DMCT line, with the $ED_{50}$ at about 3.0 μm. Drawing all of these dose-effect curves with the same slope as that of DMCT infers that all of these compounds act by the same mechanism.

Although there is yet no proof that the chemotherapeutic activity of these drugs is based on a common mechanism, the following factors make this thesis reasonable. (*a*) The same chemical structure, that of tetracycline, is basic for each of these drugs. (*b*) Sweeney, *et al.* (2), found DMCT and CT to have the same antibacterial activity, while other investigators found only minor differences between these tetracyclines (4, 18). (*c*) Eidus, *et al.* (19), found the distribution of all of these compounds to be similar in mouse organs. (*d*) The *in vivo* action of OT and CT on experimental leptospirosis in hamsters was similar, with but minor differences (7, 20). In chick embryos, we have observed in this study the action of DMCT, OT, and TC to be similar and, in the previous study, the similarity of the actions of OT and TC (14).

On the other hand, differences in the activity of the various tetracycline analogs have been reported, although in some cases the differences were minor. These differences in activity are expected, and may be due to the various chemical constituents present or absent on the basic tetracycline structure. In DMCT the presence of the chloro substituent may give the compound greater activity, and the absence of the methyl group may stabilize the molecule. Thus, DMCT has been reported as the most active and most stable of the tetracyclines, whereas CT has been the least stable.

The descending order of biological activity most often listed for these compounds is: DMCT, OT, TC, and CT. As stated above, some of these differences were minor both *in vitro* and *in vivo*. In studies where stability may not have been a factor, the activity of CT was equal to or better than that of DMCT. Sweeney, *et al.* (2), found this equality of action in their antibacterial studies, while Kunin, *et al.* (3), reported similarities in renal clearance and serum binding. Eidus and his associates (19) observed both orders of activity. These investigators reported that the *in vitro* antibacterial spectrum and effectiveness of DMCT and CT resembled one another. In a prophylactic protection test against intraperitoneal challenge of *Diplococcus pneumoniae*,

CT was slightly more effective than DMCT, and each was significantly more effective than OT, TC, and *N*-pyrrolidino-methyl-tetracycline. However, in their study with tubercle bacilli they rated DMCT four times as active as OT and 16 times as active as CT. These latter results approximate those of the present study; DMCT was about two times as active as OT and 10 times as active as CT.

From the above it is clear that the observed activity of DMCT does not only depend upon the chemical structure of the compound alone, but also on the pharmacodynamics of the compound as well as the experimental procedures. Again referring to the report of Eidus and his co-workers (19), it is noted that their results in *in vitro* and *in vivo* tests with *Diplococcus* were clearly supported by their finding that DMCT and CT were retained longer and in higher concentrations than OT, TC, and *N*-pyrrolidino-methyl-tetracycline. In addition, supporting evidence for the retention of DMCT and CT is given in the studies of renal clearance, urinary excretion, and protein-binding by Kunin, *et al.* (3), and Sweeney, *et al.* (2).

On the other hand, the results of Eidus' group with tubercle bacilli, and our results here with leptospires in chick embryos, are better aligned with half-life measurements of the tetracyclines in serum. Kunin and his associates (3) found DMCT to have the longest half-life in human serum, CT the shortest, with TC and OT intermediate. Serum half-life, however, has limited meaning. Many investigators have pointed to differences in tissue and serum concentrations (3, 19, 21). Both Kunin, *et al.* (3), and Eidus, *et al.* (19), reported the tendency of these drugs to sequestrate in body compartments, notably the liver; and Kuemmerle and Contzen (21) found high concentrations in tissues involved in inflammatory processes. Thus, when speaking of the action of these drugs, the activity and stability of the over-all process must be considered.

Some clinical studies reported DMCT in 150-mg. doses to have advantages over TC in 250-mg. doses (2, 22). At variance with these reports, however, are the observations of Roberts and his associates (23). This disagreement challenged some of the former investigators to re-examine the relative merits of these two antibiotics. The additional studies not only confirmed some of the advantages of DMCT, but also provided a better understanding of how these drugs may best be used (24, 25).

From the clinical and experimental reports discussed here, DMCT appears to be a superior tetracycline analog. Being a new drug, however, its real value cannot be determined without the cumulative data concerning possible side effects (26).

## REFERENCES

(1) McCormick, J. R. D., Sjolander, N. O., Hirsch, U., Jensen, E. R., and Doershuk, A. P., *J. Am. Chem. Soc.*, **79**, 4561 (1957).
(2) Sweeney, W. M., Hardy, S. M., Dornbush, A. C., and Ruegsegger, J. M., *Antibiot. Chemotherapy*, **9**, 13(1959).
(3) Kunin, C. M., Dornbush, A. C., and Finland, M., *J. Clin. Invest.*, **38**, 1950(1959).
(4) Hirsch, H. A., and Finland, M., *Am. J. Med. Sci.*, **239**, 288(1960).
(5) Heilman, F. R., *Proc. Staff Meetings Mayo Clinic*, **23**, 569(1948).
(6) Brunner, K. T., and Meyer, K. F., *Am. J. Vet. Res.*, **11**, 89(1950).
(7) Uhlenhuth, P., and Schoenherr, K. E., *Z. Immunitaetsforsch.*, **108**, 289(1951).
(8) Orsmbee, R. A., *Proc. Soc. Exptl. Biol. Med.*, **83**, 815(1953).
(9) Ringen, L. M., and Bracken, F. K., *J. Am. Vet. Med. Assoc.*, **129**, 266(1956).
(10) Van Thiel, P. H., *Documenta Med. Geograph. Trop.*, **9**, 309(1957).
(11) Howarth, J. A., *J. Am. Vet. Med. Assoc.*, **129**, 268 (1956).
(12) Stoenner, H. G., Grimes, E. F., Thrailkill, F. B., and Davis, E., *Am. J. Trop. Med. Hyg.*, **7**, 423(1958).
(13) Gsell, O., *Schweiz. Med. Wochschr.*, **89**, 422(1959).
(14) Quan, S. F., *Zoonoses Res.*, **2**, 41(1963).
(15) Kiser, J. S., Clemente, J., and Popken, F., *Antibiot. Ann.*, (**1957–1958**), 259.
(16) Litchfield, J. T., Jr., *J. Pharmacol. Exptl. Therap.*, **97**, 399(1949).
(17) Litchfield, J. T., Jr., and Wilcoxon, F., *ibid.*, **96**, 99 (1949).
(18) Postic, B., and Finland, M., *Am. J. Med. Sci.*, **242**, 551(1961).
(19) Eidus, L., Maniar, A. C., and Greenberg, L., *Can. Med. Assoc. J.*, **86**, 366(1962).
(20) Schlipkoeter, H. W., and Gram, H., *Z. Immunitaetsforsch.*, **109**, 215(1952).
(21) Kuemmerle, H. P., and Contzen, H., *Antimicrobial Agents Ann.*, **1960**, 205.
(22) Kunin, C. M., and Finland, M., *New Engl. J. Med.*, **259**, 999(1958).
(23) Roberts, C. E., Jr., Perry, D. M., Kuharic, H. A., and Kirby, W. M. M., *Arch. Intern. Med.*, **107**, 204(1961).
(24) Sweeney, W. M., Dornbush, A. C., and Hardy, S. M., *Am. J. Med. Sci.*, **243**, 296 (1962).
(25) Kunin, C. M., *Arch. Intern. Med.*, **110**, 166(1962).
(26) Vosti, G. J., Willet, F. M., and Jawetz, E., *Clin. Pharmacol. Therap.*, **2**, 29(1961).