Our observations, that the exhaustion number of dystrophic chicks can be raised to normal values by oxygen therapy, are consistent with the view that dystrophic tissues are deficient in oxygen and are, therefore, most likely in a more anaerobic state than normal tissue.

Summary. Spectrophotometric analyses of myoglobin from crude muscle extracts of chicken breast muscle show an increase (35%) of reduced myoglobin, and a decrease (17%) of metmyoglobin in dystrophic muscle from hereditary dystrophic chickens when compared with normal controls. This may reflect a disruption of intracellular oxygen supply and demand.

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α-6-Deoxyoxytetracycline I. Some Biological Properties. (31338)

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Objectives in the search for new tetracycline antibiotics continue to stress greater *in vivo* effectiveness and other novel properties. Preliminary evaluation of one of the more recent, *i.e.*, α-6-deoxyoxytetracycline *(1), indicated marked chemotherapeutic advantage, after oral administration, against experimental infections in mice, even though *in vitro* studies had demonstrated equivalence with several other known tetracyclines. Supplementary studies in the dog demonstrated high and prolonged anti-streptococcal activity in their sera after oral administration. Schach Von Wittenau and Yeary(2) emphasized oral absorption, as well as tissue distribution, as a function of the lipoid-solubility of this new antibiotic in their studies in the dog.

A more extensive laboratory evaluation was prompted by these preliminary studies. Therefore, the present communication will document some general biological properties of this antibiotic as demonstrated by *in vitro* studies. Data from animal protection tests also will be presented and these serve to suggest potential clinical advantage for this new tetracycline.

Materials and methods. The antibiotics, *i.e.*, α-6-deoxyoxytetracycline (DOOTC), 6-methylene oxytetracycline (MOTC)†, 6-demethylchlortetracycline (DMCT)‡, tetracycline (TC),§ and oxytetracycline (OTC)‖, were used in these studies as the hydrochlorides and were of research quality.

All *in vitro* susceptibility tests were done

* Generic name, doxycycline.

† Registered trade mark of Chas. Pfizer & Co., Inc., is Rondomycin.

‡ Registered trade mark of Lederle Laboratories, American Cyanamid Co., Inc., is Declomycin.

§ Registered trade mark of Chas. Pfizer & Co., Inc., is Tetracyn.

‖ Registered trade mark of Chas. Pfizer & Co., Inc., is Terramycin.

by the same basic method unless specifically stated otherwise. The test was a 2-fold, serial dilution of the antibiotic in brain heart infusion broth (Difco). The inoculum consisted of 0.5 ml of a 1×10^{-3} dilution of an overnight culture incubated at 37°C. As is now routine in this laboratory, the test was carried out in plastic DisPoso trays (Linbro Chemical Co., New Haven, Conn.) instead of glass tubes. Total final volume in each cup was 1 ml. The test is read visually, *i.e.*, the MIC is the lowest concentration of antibiotic preventing visual turbidity after overnight incubation at 37°C. Data presented are the average and the standard deviation calculated from 5 separate trials.

Experimental systemic infections in the mouse were produced by the intraperitoneal inoculation of standardized cultures of *Streptococcus pyogenes* C 203, *Pasteurella multocida*, and *Staphylococcus aureus* 5 mp. The severity of infection was consistently at 1 to 10 LD_{100}, *i.e.*, Lethal $Dose_{100\ per\ cent}$: 1 to 10 times the number of organisms needed to kill 100% of the mice under the conditions of the experiment. The *S. pyogenes* C 203 and *P. multocida* inocula were as broth cultures; *S. aureus* 5 mp inoculum was suspended in 5% hog gastric mucin. DOOTC and the other tetracyclines were administered in a diluent consisting of water and 1% carboxymethylcellusol. Initially, dose-response curves were obtained for a multiple dosing regimen in which each antibiotic was administered either orally or subcutaneously at 0.5 hour post infection and repeated 4, 24, and 48 hours later. A single dose regimen, *i.e.*, oral administration, 0.5 hour post infection, was studied also to relate how much activity after multiple dosing could be attributed to the initial dose, and whether or not the order of antibiotic activity would be altered. At the end of 4 days, living mice were counted and per cent alive calculated. These values were converted to probits and a PD_{50} (mg/kg) value with the 95% confidence limits calculated(3).

Results and discussion. Minimum inhibitory concentrations (MIC) of DOOTC and 4 other tetracyclines against laboratory strains of Gram-positive and Gram-negative bacteria are presented in Table I and IA. Six Gram-positive bacteria, representing 4 different genera, were susceptible to DOOTC in a concentration range of 0.04 to 0.27 μg/ml. The average values obtained for the Gram-positives generally show DOOTC, MOTC, and DMCT to have greater activity than either TC or OTC. DOOTC, MOTC, and DMCT appear equivalent, *e.g.*, DOOTC has the lowest average MIC against 2 of the 7 Gram-positive bacteria; MOTC, against 3; and DMCT, against 2. Actual differences in the average MIC values represent even less than a single dilution in the 2-fold technic.

As indicated by data for the last two organisms in Table I, the presence of 20% human serum in the medium lowers the activity of DOOTC as well as that of the other tetracyclines. Using values of *S. aureus* as a rough approximation of serum inactivation, *e.g.*,

$$\frac{\text{MIC in serum} - \text{MIC in broth}}{\text{MIC in serum}} \times 100 =$$

Per Cent Inactivation, the following are obtained: DOOTC 78%, MOTC 81%, DMCT 87%, TC 55% and OTC 40%. These are in fairly good agreement with binding values determined in a variety of methods by other workers(2,4).

DOOTC was active in a concentration range of 0.31 to 4.37 μg/ml against 8 of the 9 representative Gram-negative genera shown in Table IA. DOOTC has activity against these bacteria comparable to TC and is generally more active than is OTC. DOOTC generally appeared to be somewhat less active against these laboratory strains than was either MOTC or DMCT. Such differences usually represented 1 or 2 tubes in the 2-fold technic. DOOTC was inactive, as were the other tetracyclines, against *Proteus vulgaris* 59. However, a number of strains of *Proteus morgani* were susceptible to these tetracyclines. Data for Strain 73, as being representative, are given in Table IA. All of the tetracyclines demonstrated a degree of *in vitro* activity against *Pseudomonas aeruginosa* L 173; however, this strain is now considered atypical of the genus.

In addition to the susceptible laboratory strains listed in Tables I and IA, compara-

tive MIC values for DOOTC and the other tetracyclines for 20 recently, clinically isolated *S. aureus* and 12 *E. coli* strains were determined. DOOTC demonstrated activity against all 20 strains of *S. aureus* in a concentration range of 0.06 to 0.11 μg/ml. Comparable ranges were 0.04 to 0.09 for MOTC; 0.04 to 0.10 for DMCT; 0.09 to 0.21 for TC; and 0.15 to 0.43 for OTC. There appeared to be no significant difference between DOOTC, MOTC, and DMCT, *e.g.*, the average MIC value was 0.08 μg/ml for DOOTC; 0.06, for MOTC; and 0.07, for DMCT. Differences between these average values are represented by less than a single dilution in the 2-fold technic. Comparative average values for *S. aureus* for TC were 0.14 and 0.26 for OTC. These represent, respectively, approximately a single to 2 dilutions in the technic from the DOOTC values.

TABLE I. *In vitro* Activity of Doxycycline and Other Tetracyclines Against Gram-Positive Bacteria.

Organism	Avg MIC (μg/ml) and standard deviation				
	DOOTC	MOTC	DMCT	TC	OTC
Staphylococcus aureus No. 5	.19 ± .01	.13 ± .05	.11 ± .09	.21 ± .10	.55 ± .19
" " A/R 400*	8.75 ± 3.06	50 ± 0	35 ± 13	60 ± 20	>100 ± 0
Streptococcus pyogenes 8668	.04 ± .03	.03 ± .01	.04 ± .03	.06 ± .04	.07 ± .06
" " C203	.04 ± .03	.04 ± 0	.03 ± .01	.04 ± .01	.11 ± .09
" *faecalis* A121	.17 ± .04	.15 ± .04	.19 ± 0	.39 ± 0	.47 ± .17
Diplococcus pneumoniae I	.02 ± .01	.04 ± .03	.03 ± .01	.09 ± .08	.07 ± .06
Erysipelothrix insidiosa	.27 ± .09	.23 ± .09	.27 ± .09	.35 ± .09	.78 ± 0
Medium + 20% human serum					
Staphylococcus aureus No. 5	.86 ± .52	.70 ± .17	.86 ± .35	.47 ± .17	.93 ± .35
Streptococcus pyogenes 8668	.78 ± 0	.62 ± .17	.62 ± .17	.93 ± .35	.70 ± .17

* Resistant to tetracyclines, streptomycin, erythromycin, and penicillin.

DOOTC = α-6-deoxyoxytetracycline; MOTC = 6-methylene oxytetracycline; DMCT = 6-demethylchlortetracycline; TC = tetracycline; OTC = oxytetracycline.

TABLE IA. *In vitro* Activity of Doxycycline and Other Tetracyclines Against Gram-Negative Bacteria.

Organism	Avg MIC (μg/ml) and standard deviation				
	DOOTC	MOTC	DMCT	TC	OTC
Aerobacter aerogenes 2	2.54 ± 1.3	.94 ± .23	.35 ± .11	1.66 ± .67	2.91 ± 1.3
Escherichia coli 266	1.74 ± .66	.43 ± .19	.33 ± .17	.73 ± .3	1.09 ± .20
Proteus vulgaris 59	>100 ± 0	>100 ± 0	90 ± 22.2	>100 ± 0	>100 ± 0
Proteus morgani 73	4.37 ± 1.4	2.18 ± .69	.78 ± 0	2.19 ± .69	2.18 ± .69
Pseudomonas aeruginosa L173	22.5 ± 5.6	6.25 ± 0	3.12 ± 0	10 ± 2.8	6.25 ± 0
Salmonella typhosa 344	1.56 ± 0	1.4 ± .35	.55 ± .17	1.56 ± 0	3.12 ± 0
Klebsiella pneumoniae 132	1.87 ± .09	1.56 ± 0	.55 ± .17	1.56 ± 0	2.5 ± .69
Shigella sonnei	2.18 ± .69	1.56 ± 0	1.40 ± .35	2.49 ± .69	4.98 ± 1.39
Vibrio comma	.31 ± .08	.29 ± .09	.17 ± 0	.58 ± .61	.55 ± .17
Pasteurella multocida	.20 ± .04	.16 ± .04	.16 ± .02	.31 ± .04	.47 ± .26

DOOTC = α-6-deoxyoxytetracycline; MOTC = 6-methylene oxytetracycline; DMCT = 6-demethylchlortetracycline; TC = tetracycline; OTC = oxytetracycline.

DOOTC demonstrated activity against the group of 12 *E. coli* isolates in a concentration range of 0.49 to 2.81 μg/ml. Comparable ranges were 0.70 to 1.87 for MOTC; 0.62 to 0.94 for DMCT; 0.94 to 3.12 for TC; and 1.56 to 3.12 for OTC. Overall average MIC values were 1.34 μg/ml for DOOTC; 1.33 for MOTC; 0.76 for DMCT; 1.58 for TC; and 1.97 for OTC. Differences between these average values of DOOTC and the other antibiotics are represented by less than 1 to about 2 dilutions in the technic.

The relationship between bacteriostatic and bactericidal concentrations of DOOTC and the other tetracyclines for 12 of the 20 clinical isolates of *S. aureus* was studied in the following procedure. After overnight incubation at 37°C, MIC's (bacteriostatic concentration) in the usual 10 cup, 2-fold dilution series were determined. The MIC concentration as well as all other negative cups (no visible growth) were then streaked out with a standard loop onto the surface of antibiotic-free brain heart agar. After an additional overnight incubation period, the plates were read for the presence or absence of growth. Any cup which, after streaking, showed 10 or fewer colonies was considered to contain a bactericidal concentration of antibiotic. Such a low count represented greater than a 99.9% kill(5). The average number of dilution cups representing the difference between bacteriostatic and bactericidal concentrations was almost consistently 3.4 two-fold dilutions for all tetracyclines. Using the average MIC value of DOOTC (.083 μg/ml) for the 20 *S. aureus* strains mentioned above as an example, this 3.5 two-fold dilution differential would place the bactericidal concentration at about 0.9 μg/ml. This concentration of DOOTC, as free-drug in the serum, is readily attainable in the dog with relatively low oral doses(6).

S. aureus A/R 400 listed in Table I is a multi-resistant strain, resistant to some tetracyclines, penicillin, streptomycin, and erythromycin. As indicated in Table I, DOOTC has greater *in vitro* activity against this strain than do the other tetracyclines, *e.g.*, DOOTC was approximately 7 times more active than was TC. To determine the frequency and extent of the above finding, additional tetracycline-resistant isolates of *S. aureus* and *E. coli* were studied. The average MIC of DOOTC for 10 such staphylococci was 22.5 μg/ml. The MIC of each against TC was $>$ 100 μg/ml. *E. coli* cultures varied considerably in their response to DOOTC. Of 11, 2 were susceptible to 3.12 μg/ml; 7 were susceptible to 31.2 μg/ml, and 2 were resistant to $>$ 100 μg/ml. In contrast, all were resistant to $>$ 100 μg/ml of TC. Although not tested against all cultures, 2 *E. coli* strains were resistant to $>$ 100 μg/ml of MOTC and DMCT; 2 were susceptible to 6.25 μg/ml of MOTC and 3.12 μg/ml of DMCT. These data indicate wide variations in differences in degree and susceptibility as related to cross-resistance among various tetracyclines. Thus, although DOOTC can demonstrate *in vitro* activity against a variety of tetracycline resistant bacteria, such activity can be considered as only partial and does not approach that shown against 'normal' (Table I and IA) strains.

The *in vitro* activity of DOOTC and the other tetracyclines against experimental infections in the mouse is summarized in Table II. As indicated by the data in I of Table II, after multiple subcutaneous therapy the overall impression is one of equivalence of the 4 tetracyclines in the experimental protocol utilized. An exception is that MOTC was somewhat more active than the other tetracyclines against the experimental *S. pyogenes* C203 infection. In every other instance, PD_{50} values and their confidence limits overlapped for all tetracyclines.

DOOTC, after either single or multiple oral dosage (II and III, Table II) is significantly more active against these infections than are MOTC, DMCT, and TC. Data presented in II, Table II for multiple oral therapy show DOOTC to have significant superiority over MOTC, DMCT, and TC with a single exception. Although the PD_{50} values of DOOTC for the experimental *P. multocida* infection is 6.6 mg/kg and 9.8 mg/kg for DMCT, there is an overlapping of confidence limits. Similarly, data obtained after single oral dosage again show the significantly greater activity of DOOTC. A single exception was noted,

TABLE II. Chemotherapeutic Activity of Doxycycline and Other Tetracyclines After Various Therapeutic Regimens.

	Antibiotic	PD_{50} (mg/kg) and 95% confidence limits		
		S. pyogenes C203	*P. multocida*	*S. aureus* 5 mp
I. Multiple subcutaneous therapy*	DOOTC	.98 (.83–1.16)	3.9 (3.33– 4.56)	1.0 (.78– 1.28)
	MOTC	.66 (.56– .77)	2.6 (2.34– 2.88)	1.19 (.93– 1.52)
	DMCT	1.33 (1.13–1.57)	3.0 (2.34– 3.84)	1.63 (1.09– 2.44)
	TC	1.85 (1.42–2.4)	3.6 (2.88– 4.5)	1.23 (1.03– 2.04)
II. Multiple oral therapy*	DOOTC	1.6 (.84–2.86)	6.6 (5 – 8.7)	2.55 (1.96– 3.3)
	MOTC	3.9 (3.14–4.84)	13 (10.8 –15.6)	4.5 (3.73– 6.07)
	DMCT	6.7 (5.63–7.97)	9.8 (7.84–12.25)	6 (4.6 – 7.8)
	TC	6.25 (5.25–7.44)	22 (18.3 –26.4)	5.81 (4.2 – 8.13)
III. Single oral therapy†	DOOTC	1.38 (.95–1.81)	6.5 (3.62– 9.38)	4.8 (2.9 – 6.7)
	MOTC	3.1 (2.09–4.11)	23 (15.9 –30.01)	9.5 (5.98–13.02)
	DMCT	4.6 (2.95–6.25)	26 (13 –39.0)	11.0 (7.0 –15)
	TC	5.4 (3.52–7.28)	32 (20.03–43.97)	14 (9.0 –19.0)

* Dosage at 0.5, 4, 24, and 48 hr post infection.
† Dosage at 0.5 hr post infection.

DOOTC = α-6-deoxyoxytetracycline; MOTC = 6-methylene oxytetracycline; DMCT = 6-demethylchlortetracycline; TC = tetracycline.

i.e., although the PD_{50} value for DOOTC was approximately half that of MOTC against the experimental *S. aureus* 5 mp infection, their confidence limits overlap (III, Table II).

It should be pointed out that, as shown by MIC values (Table I, and IA), the activity of DOOTC and the other tetracyclines against the organisms used to establish the experimental infections was quite comparable. Thus, the *in vivo* data, emphasizing the greater chemotherapeutic efficacy of DOOTC, is not merely one reflecting greater activity *per se* but one likely to be intimately associated with greater absorption after oral administration and other pharmacodynamic properties, *i.e.*, longer half-life, etc.(2,6). Indeed, where absorption after oral administration is not a factor, *e.g.*, when administered subcutaneously, chemotherapeutic activity of these tetracyclines was equivalent. It is only after oral administration that DOOTC shows superiority. Using the following ratio, therefore, as a tacit indication of absorption *via* the oral route, $\frac{PD_{50}, \text{ multiple oral dosage}}{PD_{50}, \text{ multiple subcutaneous dosage}}$, DOOTC shows values of 1.6, 1.6, and 2.5, respectively, in the experimental *S. pyogenes* C 203, *P. multocida*, and *S. aureus* 5 mp infections. A ratio of 1, of course, would indicate as much efficiency after oral as after subcutaneous administration. Similar values for MOTC were 5.9, 5.0, and 3.8; for DMCT 5.0, 3.3, and 3.7; and 3.4, 6.1, and 4.7 for TC. Such values indicate approximately a 2 to 4 times greater oral absorption for DOOTC than for the other tetracyclines. An obvious extrapolation of these data is that considerably lower dosage of DOOTC would be required orally to treat infections in man.

Summary. 1. DOOTC demonstrated greater activity after oral administration than did MOTC, DMCT, and TC against 3 types of experimental infections in mice. DOOTC superiority after oral administration was demonstrated by a comparison of chemotherapeutic efficacy produced in 3 different dosage regimens. 2. A variety of *in vitro* studies, in contrast, indicated general equivalence with MOTC and DMCT but greater activity than TC and OTC.

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Influence of Adrenal Cortex on Synthesis of a_2-AP Globulin of Rat Serum.* (31339)

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The appearance of a new protein in the serum of the albino rat during the acute phase of inflammatory states has been reported by several investigators(1). The protein which we have designated a_2-AP (acute phase) globulin(1) is a carbohydrate-containing euglobulin of high molecular weight(1,2) and is synthesized by the liver(3). Since noxious stimuli which evoke the appearance of the protein also cause enhanced levels of corticosteroids (4,5), it was of interest to determine the influence of the adrenal glands on the formation of the protein. In the present study, the effects of adrenalectomy and of cortisol administration on the synthesis of a_2-AP globulin were investigated.

Materials and methods. Young, adult, male rats of Sprague-Dawley origin, weighing from 360 to 400 g, were housed singly in suspended-type wire cages. They were maintained on Purina laboratory chow and tap water. Adrenalectomized rats were supplied with isotonic saline. Paired-feeding was not deemed necessary, since it has been demonstrated that the response of the acute phase reactants of rat serum to the phlogogenic agent used in the present study is not affected by nutritional status(6).

Bilateral adrenalectomy was performed under ether anesthesia employing a dorsal approach. Two incisions were made and the wounds were closed with 2 stitches and sutures each. Sham-operated rats were subjected to the same surgical procedures with the exception that the adrenals were left intact. All rats were allowed to rest 10 to 14 days following surgery.

Thirteen of the groups listed in Table I were injected by the intramuscular route with cortisol† or with pyrogen-free saline. Daily injections were made for 4 days and the rats were bled on Day 5. An inflammatory response was induced in 11 groups by subcutaneous injection of 0.5 ml of a sterile solution of spirits of turpentine, N.F., in corn oil (v/v) at the midline of the back. The rats were anesthetized with ether prior to challenge. Animals were bled 48 hours later by cardiac puncture under ether anesthesia. For the groups which received hydrocortisone or saline, the turpentine challenge was made on Day 3 of the treatment schedule.

The relative concentration of a_2-AP globulin in each of the samples with respect to a standard reference serum was determined by the single radial immunodiffusion procedure described by Fahey and McKelvey(7). The standard was a pooled serum obtained from 25 male rats which had been injected 48 hours previously with 1.0 ml of the turpentine in corn oil solution described above. The concentration of a_2-AP globulin in this serum was arbitrarily established at 100 units per 100 ml. Preparation of antiserum against the protein for use in the immunodiffusion analysis has been previously reported(1). Hematocrit values were determined in Wintrobe tubes. Analysis of the data was made by standard statistical methods employing the t test(8).

A 3-letter code was used to designate the

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† Cortef® (hydrocortisone), Upjohn Co., Kalamazoo, Mich.