Relative Distributions of Myoglobin Derivatives in Breast Muscle of Chickens with Hereditary Muscular Dystrophy.* (31337)

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(Introduced by H. Herrmann)
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Considerable biochemical research has been reported concerning hereditary muscular dystrophy(1,2,3). However, comparatively little information is available concerning myoglobin in this myopathy. Perkoff and Tyler(4) investigated myoglobin from normal and dystrophic human patients and found no differences in their absorption spectra or in their electrophoretic patterns, although they did find a quantitative reduction of myoglobin in dystrophic samples as compared to normal ones. In light of reports indicating a substantial reduction in non-collagen protein from dystrophic tissue of other animals(1,5,6,), it is likely that the reported reduction in myoglobin is a reflection of protein catabolism and/or leakage from dying muscle cells. Whorton *et al*(7) reported spectrophotometric differences in metmyoglobin of patients with Duchenne-type muscular dystrophy. We have not detected abnormal myoglobin absorption spectra in crude extracts of dystrophic chicken muscle. We have, however, detected significant differences in the relative percentages of reduced myoglobin and metmyoglobin in dystrophic chicken muscle as compared to the same fractions in normal chicken muscle.

Materials and methods. The source of muscle in our experiments was the superficial pectoralis of 8-week-old chickens. The dystrophic birds used were from our own stock which was started from chicks supplied by Dr. Louis Pierro of the Department of Animal Genetics, University of Connecticut. Samples from 30 chickens, 15 males and 15 females, were analyzed in groups of 3; each group consisting of one normal chicken as well as one heterozygous and one homozygous for the dystrophic gene. The 3 birds used in each group were killed at random, and were of the same sex and of identical age.

The birds were exsanguinated by severing the neck arteries. After 10 minutes, the superficial pectorals were excised, minced with scissors, and any remaining blood trapped in vessels was removed. This procedure ensures the removal of hemoglobin. The tissue was then homogenized with approximately 10 ml of ice cold distilled water in a Waring blendor and centrifuged at 15,000 $\times$ *g* for 25 minutes. The pH of the supernatant was invariably in the range of 5.9-6.3. Three ml samples were then read on a Bausch and Lomb 505 recording spectrophotometer through the range of 450-620 mμ.

Some hemoglobin and cytochrome *c* are undoubtedly present in the extract, but, quantitatively, they should be insignificant in relation to myoglobin since hemoglobin is removed as noted above, and most of the cytochrome *c* is removed with the mitochondria. Because this technique is designed only to give relative percentages and not quantitative values, and since all samples were treated identically, any introduction of error by contamination would be uniform and would not affect relative values.

Calculations of relative percentages of the 3 myoglobin derivatives, reduced myoglobin (Mb), metmyoglobin (MMb), and oxymyoglobin (O_2Mb), were made according to the absorbency ratio method of Broumand *et al* (8). This method employs the wavelengths of isobestic points of the molar extinction coefficient curves of the 3 myoglobin derivatives. The extinction coefficients used are those of Bowen(9) for horse heart myoglobin. Broumand *et al*(8) have shown that the isobestic points of the absorption curves for beef myoglobin derivatives are the same as those

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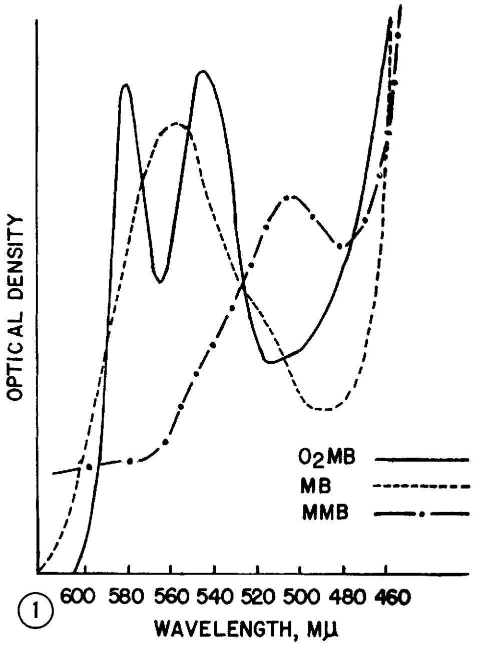

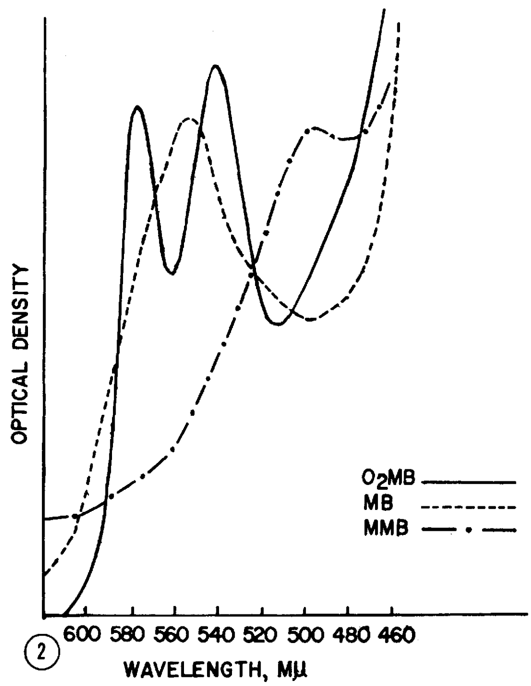

FIG. 1. Absorption curves for O_2Mb, Mb, and MMb in crude muscle extract from beef.
FIG. 2. Absorption curves for O_2Mb, Mb, and MMb in crude muscle extract from chicken.

for horse heart myoglobin. The absorption curves for chicken myoglobin derivatives likewise show the same isobestic points (Fig. 1 and 2). Although it is possible that the specific molar extinction coefficients for chicken myoglobin are not the same as those of beef heart myoglobin, we are making only a relative comparison of Mb, MMb, and O_2Mb from 3 genotypes of chickens. Any quantitative errors introduced would not affect these relative values. The O_2Mb values were obtained by subtraction of the sum of the experimentally determined Mb and MMb values from 100.

Results. The data in Table I show that in comparison to normal muscle, dystrophic muscle has a significantly higher percentage of Mb and a lower percentage of MMb. The change in the relative percentages of Mb and MMb in dystrophic breast muscle appears to be due to an increase in the amount of Mb and a corresponding decrease in MMb. No significant differences are apparent in O_2Mb. The Mb and MMb values for the carrier (heterozygous) chickens are either intermediate or more like the normal values. The O_2Mb values for all 3 genotypes are considerably lower in magnitude than are corresponding values for the Mb and MMb.

Variability among the values of O_2Mb is very large compared to the variations exhibited in the Mb or MMb. It should be emphasized here that O_2Mb values are not determined directly from the absorption spectrum as are the Mb and MMb values, but are obtained by subtraction of the sum of the latter two values from 100. Hence, any experimental error in the determination of Mb and MMb may be compounded in the O_2Mb data due to the method of O_2Mb determination. Since the values for the O_2Mb derivative are low, experimental error of only a few percent could lead to a large deviation from the actual concentration. It appears that most of the O_2Mb variability is due to a corresponding and opposite variability in Mb. This has been substantiated by correlation tests within genotypes between the various derivatives which show significantly high negative values between Mb and O_2Mb in all 3 genotypes, whereas the correlation values between MMb and O_2Mb, and between Mb

TABLE I. Relative Percentages of Myoglobin Derivatives in Chicken Breast Muscle.

		Reduced myoglobin (Mb)			Metmyoglobin (MMb)			Oxymyoglobin (O_2Mb)		
Group No.	Sex	Normal	Carrier	Dys-trophic	Normal	Carrier	Dys-trophic	Normal	Carrier	Dys-trophic
1	♀	24.1	37.0	47.6	57.0	51.0	45.4	18.9	12.0	7.0
2	♀	33.8	40.3	40.3	57.7	53.9	45.4	8.5	5.8	14.3
3	♀	28.0	42.5	40.9	57.2	47.5	46.8	14.8	10.0	12.3
4	♂	33.8	38.7	40.3	53.9	51.0	41.5	12.3	10.3	18.2
5	♀	31.9	35.9	39.8	53.9	52.5	48.0	14.2	11.6	12.2
6	♂	39.2	37.0	54.0	54.5	53.1	44.8	6.3	9.9	1.2
7	♂	37.6	30.2	45.3	48.7	46.9	44.0	13.7	22.9	10.7
8	♂	38.8	35.9	43.7	55.9	51.7	47.4	5.3	12.4	8.9
9	♂	34.9	37.6	55.0	53.0	51.8	41.5	12.1	10.6	3.5
10	♀	31.3	35.3	43.7	47.5	51.5	43.5	21.2	13.2	12.8
Avg	♂	36.9 ±2.4*	35.9 ±3.3	47.7 ±6.5	53.2 ±2.7	50.9 ±2.4	43.8 ±2.5	9.9 ±3.8	13.2 ±5.5	8.5 ±6.7
Avg	♀	29.8 ±3.8	38.2 ±3.1	42.5 ±3.2	54.7 ±4.3	51.3 ±2.4	45.8 ±1.7	15.5 ±4.9	10.5 ±2.9	11.7 ±2.8

* Mean ± standard deviation.

and MMb, are small and significant only in the carrier chickens. Since MMb is nonfunctional in the transport of oxygen due to its oxidation state, and since Mb and O_2Mb are readily interconvertible, such correlation values are not unexpected.

Statistical analysis of the data by a set of orthogonal comparisons (Table II) shows that the differences between all 3 genotypes in the Mb and MMb fractions are significant with the exception of the normal *vs* carrier comparison of the Mb fraction which, however, does approach significance at the 5% level.

Discussion. The data presented here show that dystrophic chicken breast muscle contains a significantly higher relative level of Mb and a lower level of MMb than does normal tissue. The reason for these value changes is not known. However, one possibility is that in degenerating tissue, conditions are more anaerobic than normal, resulting in less oxidation of Mb to MMb. That dystrophic cells are in a more anaerobic or reducing state than are the cells of normal tissue is suggested by McCaman's report(2) that in dystrophic muscle there is an apparent shift of glucose metabolism from the glycolytic pathway to the hexose monophosphate shunt pathway and also by the observations (2,10,11) of increased fat synthesis in dystrophic tissue.

TABLE II. Sets of Orthogonal Comparisons Between Genotypes.

Genotype	Mb	MMb	O_2Mb
Normal *vs* dystrophic	43.91†	54.05†	2.30
Carrier *vs* dystrophic	20.56†	25.58†	1.04
Normal *vs* carrier	3.38	5.26*	.25

* Significant at 5% level.
† Significant at 1% level.

Data from one of our laboratories (C.R.A. and R.G.S.) also support the view expressed above. We have found that dystrophic chicks which have received O_2 therapy for 4 weeks have exhaustion numbers equal to normal controls. These observations suggest that intracellular O_2 supply and demand may be an important aspect of this myopathy.

It is of interest that in hereditary muscular dystrophy of the chicken, breast muscle is affected before red muscles and more severely (10). Chandra-Bose *et al*(12) have shown that the white fibers of chicken breast muscle contain little, if any, myoglobin. Since myoglobin facilitates oxygen transport(13), and since chicken breast muscle is composed mainly of white fibers, oxygen transport in this fiber type must depend primarily upon a simple diffusion of unbound intracellular oxygen. Further, because of the lack of myoglobin, white breast muscle has no means of oxygen storage. Hence this tissue would be at a metabolic disadvantage compared to the red muscle fibers in the event of a decrease in the concentration of unbound, intracellular oxygen.

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.