it would not be surprising if these doses produced intracellular concentrations of DMSO sufficient to inhibit ADH. Dimethyl sulfoxide is hepatotoxic in experimental animals (*8*) and in humans (*9*). Its use has been associated with hyperbilirubinemia and elevated serum transaminase levels. Under certain circumstances, DMSO can potentiate the toxic effects of ethanol (*10*). It is not known whether inhibition of liver ADH underlies these effects or any of the other biological activities of DMSO.

ROBERT L. PERLMAN
J. WOLFF
National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014

References and Notes

1. C. D. Leake, Ed., "Biological actions of dimethyl sulfoxide," *Ann. N.Y. Acad. Sci.* **141**, Art. 1, p. 1 (1967).
2. Horse liver ADH was purchased from Worthington Biochemical Corp., and NAD and NADH from Sigma Chemical Co.; DMSO and butyraldehyde were obtained from Eastman Kodak Co. The butyraldehyde was freshly distilled with one drop of concentrated H_2SO_4 before use.
3. P. D. Boyer and H. Theorell, *Acta Chem. Scand.* **10**, 447 (1956).
4. D. H. Rammler, *Ann. N.Y. Acad. Sci.* **141**, 291 (1967).
5. F. A. Cotton and R. Francis, *J. Amer. Chem. Soc.* **82**, 2986 (1960).
6. A. Witter, *Acta Chem. Scand.* **14**, 1717 (1960).
7. C. W. Denko, R. M. Goodman, R. Miller, T. Donovan, *Ann. N.Y. Acad. Sci.* **141**, 77 (1967).
8. E. R. Smith, Z. Hadidian, M. M. Mason, *ibid.*, p. 96.
9. D. M. Bachman, D. R. Bottomly, Y. Shimazaki, *Arthritis Rheum.* **7**, 725 (1964).
10. H. J. Mallach, *Ann. N.Y. Acad. Sci.* **141**, 457 (1967).

23 February 1968

Microcirculation: Loss of an Enzyme Activity in Chickens with Hereditary Muscular Dystrophy

Abstract. *Histochemical localization of an alkaline phosphatase, with α-naphthyl phosphate used as substrate, shows that activity in breast muscle from normal chickens is restricted to the microvasculature. In chickens with hereditary muscular dystrophy, this enzyme activity disappears from capillaries and small arterioles before degeneration of muscle fibers is detectable. This loss is retarded in myopathic chickens that have received oxygen therapy.*

Continuous oxygen therapy results in a functional improvement during early stages of myopathy (*1*) in chickens with hereditary muscular dystrophy. Birds which show a functional improvement have muscle which appears more normal than dystrophic, as viewed histologically (*2*). Patterns of soluble protein and lactic dehydrogenase isoenzymes are also shifted toward normal (*3*). Because of apparent vascular abnormalities in dystrophic muscle, as seen both grossly and histologically (*2*), we further investigated the microcirculation in this myopathy. Normally, the control of blood flow to skeletal muscle is under greater control and is more closely associated with local metabolic activity than it is in any other organ except the heart (*4*). It has been suggested that an alkaline phosphatase located in the microvasculature of skeletal muscle may function in the autoregulation of blood supply (*5*). Our results show that there is an early loss of this enzyme activity in the microvasculature of dystrophic muscle, and they support the concept that an early vascular lesion contributes significantly to the progress of the myopathy.

We used the modified azo dye technique of Gomori (*6*), since its use in the study of this enzyme activity in muscle arterioles and capillaries has been thoroughly described (*5*, *7*). The enzyme (or enzymes) that hydrolyzes α-naphthyl phosphate in skeletal muscle is localized almost entirely in the endothelial cells of small arterioles and capillaries (*5*, *7*).

Normal chicks and those with hereditary dystrophy (*8*) were raised in a 70 percent oxygen environment from 1 day of age (*1*, *2*). At least three chicks of each genotype raised in such an environment, and an equal number raised in the normal atmosphere, were examined at 1, 2, 3, 4, 5, 7, and 11 weeks of age. Pieces of breast muscle (1 cm^2) were dissected from the midsternal region and rapidly frozen in a mixture of dry ice and acetone. In some cases, sections were also taken through the distal third of the gastrocnemius. The samples were then cross-sectioned at 16 μ in a cryostat at −20°C and incubated for 30 minutes at 20°C (*9*).

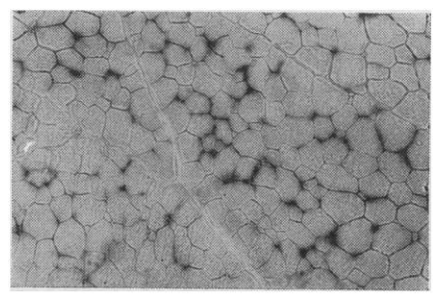
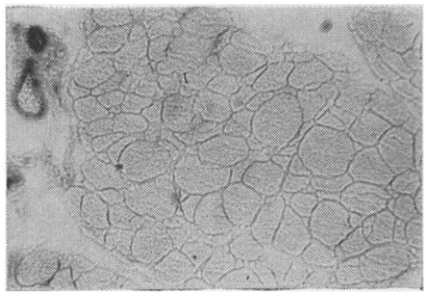


Fig. 1. Alkaline phosphatase activity in the microvasculature of breast muscle from a 4-week-old normal chicken (× 390).

Throughout the age range studied, there appeared to be no quantitative or qualitative differences in enzymatic activity between normal chickens raised in 21 percent oxygen and normal chickens raised in 70 percent oxygen. At 1 and 2 weeks of age, two types of staining were visible. A very dark, but sharply defined, stain was present at the location of small arterioles and capillaries between muscle fibers. Sections containing larger vessels showed that the stain was confined to the endothelial cells. Some vessels were not stained, but these were venules or the distal ends of capillaries (*5*, *7*). Sections from 1-week-old and some 2-week-old chicks had, in addition to the vascular reaction, a somewhat lighter, but very diffuse, stain between and around the muscle fibers. This stain was usually heaviest around areas of connective tissue. Newly formed collagen fibers have a high alkaline phosphatase activity (*10*), as do proliferating cells and those with high metabolic activity (*11*). This diffuse stain, which was seen for a short period of time right after hatching, likely resulted from either or both of these sources. After 2 weeks of age, the reaction consisted only of the type which was confined to the blood vessels (Fig. 1).

The staining pattern was strikingly different in the dystrophic muscle. The diffuse stain seen in muscle from chicks 1 and 2 weeks old was much more intense than normal and persisted in most sections from dystrophic chicks up to 4 weeks old. It was essentially absent from muscle of chicks more than 4 weeks old, although some faint staining was occasionally seen in some localized areas at later ages. The number of blood vessels that stain in breast muscle from 1-week-old dystrophic chicks appeared to be less than that in normal muscle, but this cannot be said with certainty because the heavy diffuse stain in the dystrophic muscle may have masked some staining of vessels. Staining of small vessels was virtually absent from muscle of chicks more than 2 weeks old (Fig. 2). Larger arterioles did retain the



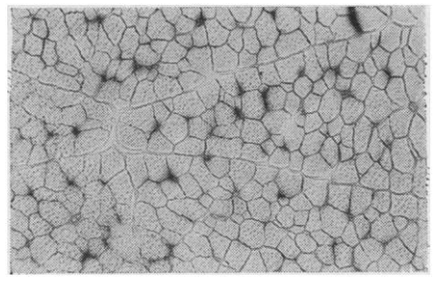


Fig. 2 (left). Absence of alkaline phosphatase activity in the microvasculature of breast muscle from a 4-week-old chicken with hereditary muscular dystrophy (× 390). Fig. 3 (right). Alkaline phosphatase activity in the microvasculature of breast muscle from a 4-week-old chicken, with hereditary muscular dystrophy, raised in 70 ± 5 percent oxygen (× 390).

enzyme activity in their endothelial linings. This was true at all ages and was comparable to the activity of vessels of the same size in normal tissue.

Cross sections taken through the distal third of the normal gastrocnemius at 5 and 6 weeks of age indicated that the distribution of blood vessels was directly correlated with the type of muscle fiber. Red fibers were predominant on one side of this muscle, whereas white fibers were in the majority on the other. Whereas there was a high concentration of vessels in the side with red fibers, considerably fewer were present in the other side.

Corresponding sections taken from dystrophic gastrocnemii showed that alkaline phosphatase activity was absent on the side of the muscle where the white fibers are located, but was present in areas where red fibers are predominant. The white fibers in the gastrocnemius show the same degenerative changes as breast muscle fibers, whereas the red fibers are apparently not affected (*12*).

Muscle from all dystrophic chicks treated with oxygen reacted more than untreated dystrophic controls, but in some cases this could be attributed to more of the diffuse reaction rather than to increased staining of the vessels. There was always a direct correlation between vessel activity and fiber morphology. In treated dystrophics, which maintained good cellular structure, there was a good phosphatase reaction in the vessels (Fig. 3). By 5 weeks of age, staining of vessels was diminished in some treated dystrophic chicks, and there were some degenerative changes. After 8 weeks of age, the vessel reaction was essentially absent in treated dystrophics. This finding agrees with the report that the favorable response to oxygen therapy is temporary (*3*).

Apparently there is an enzymatic alteration in the microcirculation of dystrophic chicken muscle which precedes visible degeneration of muscle fibers. It is likely, however, that this change is secondary to the genetic defect, since activity is maintained in birds treated with oxygen and it is not lost in the unaffected red muscle of dystrophic chickens. It would seem that the enzyme in the vessels of dystrophic muscle is either inhibited as a result of some early biochemical change in the muscle fiber, or alternatively, perhaps it is not activated as some other alkaline phosphatases are (*13*).

Dystrophic muscle may be hypoxic (*1*, *14*). Such a condition could result from a circulatory insufficiency. After finding changes in the rate of peripheral circulation, Demos *et al.* (*15*) suggested that the microcirculation of human patients might be abnormal. Simpson and Sanderson (*16*) supported this hypothesis after measuring small increases in muscle strength of patients treated with Laevadosin, a mixture of nucleotides and nucleosides, which promotes vasodilation. Feeding semisynthetic rations containing high-quality protein and lipid increased the life span and alleviated some of the gross signs of dystrophy in mice (*17*). A defect in the normal circulation of muscle would be expected to impair its nutrition, and some compensation may be afforded by diet supplementation.

Romanul and Bannister (*5*) found that the alkaline phosphatase of the muscle vasculature is concentrated at the proximal ends of the arterioles. Although this is not the area of the vascular tree which has the highest permeability, it does have the highest contractile activity. On this basis, it was suggested that this phosphatase activity may be associated with regulation of blood flow. An abnormality in a control mechanism of the microcirculation could precipitate local areas of hypoxia, or malnutrition, or both, causing cell injury and death.

Gordon and Dowben (*18*) recently reported substantial increases in catecholamines in dystrophic mice; a serotonin antagonist will prolong the life span of dystrophic mice (*19*). Catecholamines and serotonin are vasoactive compounds. Data which show that oxygen therapy temporarily retards the progress of the hereditary myopathy in chickens (*1*) and these data which show the early loss of an enzyme located in the microcirculation of affected muscle support the concept that early vascular alterations contribute significantly to the progress of the degenerative condition.

C. R. ASHMORE
L. DOERR
R. G. SOMES, JR.
Department of Poultry Science, University of Connecticut, Storrs

References and Notes

1. C. R. Ashmore and R. G. Somes, Jr., *Proc. Soc. Exp. Biol. Med.* **122**, 1100 (1966).
2. ———, *ibid.*, in press.
3. ———, *Life Sci.*, in press.
4. H. D. Green and J. H. Kepchar, *Physiol. Rev.* **36**, 617 (1959).
5. F. C. A. Romanul and R. G. Bannister, *J. Cell Biol.* **15**, 73 (1962).
6. G. Gomori, *Microscopic Histochemistry, Principles and Practice* (Univ. of Chicago Press, Chicago, 1952), p. 185.
7. F. C. A. Romanul, *Arch. Neurol.* **12**, 497 (1965).
8. Dystrophic chicks were from our own stock which has been selected for early expression of the myopathy. White Leghorns served as normal controls.
9. The incubation medium consisted of 1×10^{-2} mole of sodium borate buffer, 8×10^{-4} mole of sodium α-naphthyl phosphate, 7×10^{-3} mole of magnesium sulfate, and 0.88 g of Fast Blue RR (Sigma Chemical Co.) per liter. The final *p*H was 8.8.
10. H. B. Fell and J. F. Danielli, *Brit. J. Exp. Pathol.* **24**, 196 (1943).
11. D. J. McWhinnie and J. W. Saunders, Jr., *Develop. Biol.* **14**, 169 (1966); H. Herrmann, *Ann. N.Y. Acad. Sci.* **138**, 49 (1966).
12. V. S. Asmundson, F. H. Kratzer, L. M. Julian, *Ann. N.Y. Acad. Sci.* **138**, 49 (1966).
13. F. Moog, *J. Exp. Zool.* **161**, 353 (1966).
14. C. R. Ashmore, R. G. Somes, Jr., F. D. Vasington, *Proc. Soc. Exp. Biol. Med.* **122**, 1104 (1966).
15. J. Demos, C. Bohuon, P. Maroteaeux, *Rev. Franc. Etud. Clin. Biol.* **5**, 707 (1960).
16. J. A. Simpson and I. D. Sanderson, in *Research in Muscular Dystrophy* (Lippincott, Philadelphia, 1965), p 35.
17. D. L. Coleman and W. T. West, *J. Nutr.* **73**, 273 (1961).
18. P Gordon and R. M. Dowben, *Amer. J. Physiol.* **210**, 728 (1966).
19. W. K. O'Steen, *Proc. Soc. Exp. Biol. Med.* **126**, 579 (1967).
20. Supported in part by grants from the Muscular Dystrophy Association of America Inc., and by PHS research grant NB-07051-01. C.R.A. is the recipient of a NIH predoctoral fellowship 7-F1-GM-20,128-01A1. Scientific contribution No. 311, Agrilculture Experimental Station (Storrs).

12 February 1968