gests that the role and significance of courtship disruption varies among lek-breeding species. For contrasting levels of disruption in otherwise similar species, see A. Lill, *Z. Tierpsychol.* **36**, 1 (1974); *Adv. Ethol.* **18** (1976). For data on disruption in lek birds, see J. P. Kruijt and J. A. Hogan, *Ardea* **55**, 203 (1967); R. H. Wiley, *Anim. Behav. Monogr.* **6**, 87 (1973); R. J. Robel and W. B. Ballard, *Am. Zool.* **14**, 121 (1974); P. E. Lemnell, *Ornis Scand.* **9**, 146 (1978); J. P. Myers, *Am. Birds* **33**, 823 (1979).

5. P. W. Trail, thesis, Cornell University, Ithaca, N.Y. (1984); *Am. Birds*, in press.
6. Mating males were divided into three classes: high success (performed >10 percent of all matings in a given year; $n = 6$), moderate (>5 and ≤10 percent of the matings; $n = 13$), and low (≤5 percent of the matings; $n = 15$). There were no statistically significant differences between the mean amount of disruption performed by males in these three classes. Neither disruption nor mate choice is directly affected by dominance in *Rupicola*. See P. W. Trail (*Natl. Geogr. Res.*, in press) on territoriality and dominance in this species.
7. Male *Rupicola* gain adult plumage as 3-year olds. Although younger males and females sometimes disrupt courtship, they never interrupt matings and are not included in this discussion.
8. Copulation in *Rupicola* typically lasts 10 to 15 seconds and is terminated by the female. I classified any copulation terminated by an intruding male as interrupted, regardless of its duration.
9. The mean number of courtship visits per female during this study was 8.7 ± 8.1 ($n = 72$ mating bouts by banded females). A persistent disrupter thus interrupted approximately half of the female's visits.
10. This research was made possible through the cooperation of STINASU, the Foundation for Nature Preservation in Suriname. I thank D. Clark, G. Farley, K. Fristrup, L. Kellogg, B. McCaffery, D. Smith, and G. Tabor for help in the field. This report was greatly improved by comments from J. Bradbury, S. T. Emlen, D. Koutnik, A. S. Rand, and N. G. Smith. Financial support was provided by the National Geographic Society, NSF grant BNS 79-11231, the National Academy of Sciences Henry Fund, the Harris Foundation, the Chapman Fund, and Sigma Xi. This report was prepared while P.W.T. was a postdoctoral fellow at the Smithsonian Tropical Research Institute.

* Present address: Department of Ornithology and Mammalogy, California Academy of Sciences, Golden Gate Park, San Francisco 94118.

23 August 1984; accepted 30 November 1984

## Continued Expression of Neonatal Myosin Heavy Chain in Adult Dystrophic Skeletal Muscle

Abstract. *The expression of myosin heavy chain isoforms was examined in normal and dystrophic chicken muscle with a monoclonal antibody specific for neonatal myosin. Adult dystrophic muscle continued to contain neonatal myosin long after it disappeared from adult normal muscle. A new technique involving western blotting and peptide mapping demonstrated that the immunoreactive myosin in adult dystrophic muscle was identical to that found in neonatal normal muscle. Immunocytochemistry revealed that all fibers in the dystrophic muscle failed to repress neonatal myosin heavy chain. These studies suggest that muscular dystrophy inhibits the myosin gene switching that normally occurs during muscle maturation.*

Myosin heavy chain (MHC) has been shown to undergo an isoform transition from embryo to neonate to adult in a variety of muscle systems (*1*). Since these MHC isoforms appear to be products of separate genes, a precise program of gene switching must be maintained during normal muscle development. Avian muscular dystrophy, like Duchenne's muscular dystrophy, is a single-gene disorder of myogenic origin (*2*). Muscles in dystrophic individuals often have many characteristics of immature muscle (*3*), suggesting that the disease is a failure of normal developmental regulation. To explore this possibility, I prepared a monoclonal antibody that reacts with the neonatal MHC but not the embryonic or adult MHC of the chicken pectoralis major (PM). I found that virtually all fibers in the PM of 6-month and 1-year-old dystrophic chickens reacted with this antibody, while no fibers in the PM of normal birds of these ages reacted. Furthermore, by using an "immunofingerprinting" technique, I was able to demonstrate that the immunoreactive myosin in the adult dystrophic PM was identical to that in the neonatal normal PM.

Myosin from 20-day-old normal chicks was prepared and MHC was electrophoretically eluted from a sodium dodecyl sulfate–polyacrylamide gel (*4*). The MHC was mixed with Freund's complete adjuvant and injected intraperitoneally into BALB/c mice, and hybridomas were prepared from the spleens of immunized mice (*5*). One clone that arose from this fusion (subseqently referred to as 2E9A) produced an antibody that reacted with myosin from the PM of neonatal chicks but not with myosin from the PM of embryonic or adult chickens. Solid-phase enzyme-linked immunoassay (ELISA) demonstrated that 2E9A reacted with myosin from the PM of 20-day-old chickens but not with myosin from the PM of 12- to 13-day-old embryos or with myosin from the PM of 1-year-old chickens (Fig. 1A). Immunoreactive myosin first was detectable shortly before hatching, increased to a maximum by 3 weeks, and then decreased until becoming undetectable by

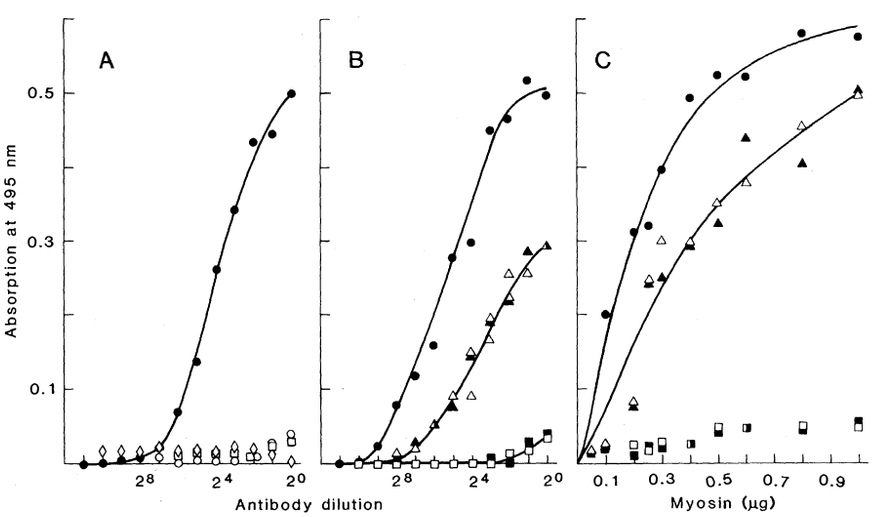


Fig. 1. Analysis by ELISA of myosins with 2E9A antibody. Myosins were plated on 96-well microtiter dishes at 0.5 μg per well (A and B) or as indicated (C). Wells were subsequently blocked with 2 percent horse serum and incubated with 50 μl of 2E9A antibody for 30 minutes at 37°C. Wells were washed three times with phosphate-buffered saline (PBS) and bound antibody was detected with the Vectastain screening kit (Vector Laboratories). Absorbance at 495 nm was measured in a microtiter plate reader. Data points are the averages of duplicates. (A) Myosin (0.5 μg) from the PM of 12-day normal embryos (○), 20-day normal chicks (●), 1-year normal adults (□), and control wells containing no myosin (◇) was reacted with 2E9A antibody diluted 1:4 in PBS. (B) Myosin (0.5 μg) from the PM of 20-day normal chicks (●), 6-month normal chickens (■), 1-year normal chickens (□), 6-month dystrophic chickens (▲), and 1-year dystrophic chickens (△) was reacted with 2E9A antibody diluted in PBS. (C) Myosin from the PM of 20-day normal chicks (●), 6-month normal chickens (■), 1-year normal chickens (□), 6-month dystrophic chickens (▲), and 1-year dystrophic chickens (△) at the indicated concentration was reacted with 2E9A antibody diluted 1:4 in PBS. The results demonstrate that 2E9A antibody reacts only with neonatal myosin during development of the PM in normal chickens but continues to react with myosin from the PM of adult dystrophic chickens.

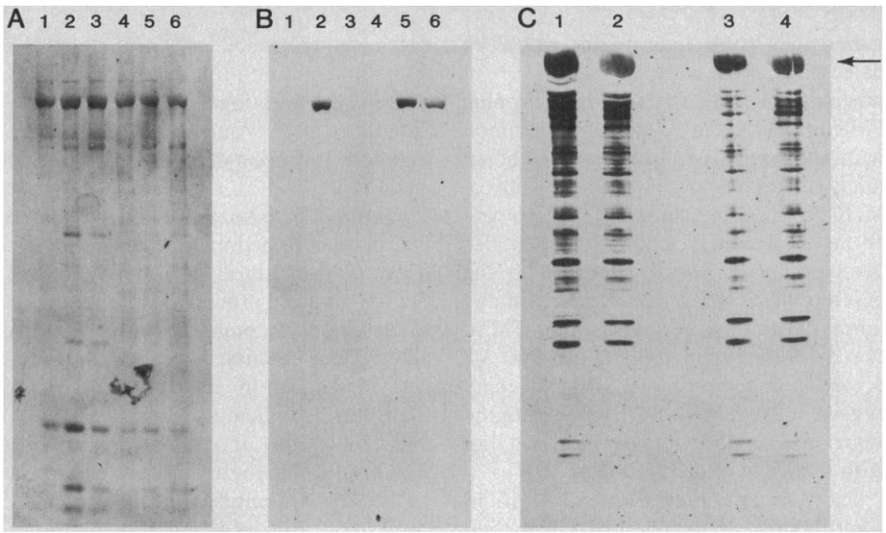


Fig. 2. Western blot analysis of myosins with 2E9A antibody. Myosins were subjected to electrophoresis on 5 percent sodium dodecyl sulfate–polyacrylamide gels. Replicate gels were electrophoretically transferred to nitrocellulose (*10*) and one was stained with amido black (A) while the other was reacted with 2E9A antibody (B). Bound antibody was detected with horseradish peroxidase–conjugated rabbit antibody to mouse immunoglobulin G (Dako Laboratories). (A and B) Lane 1, myosin from 12-day normal embryo PM; lane 2, myosin from 20-day normal chick PM; lane 3, myosin from 1-year normal chicken PM; lane 4, myosin from 12-day dystrophic embryo PM; lane 5, myosin from 20-day dystrophic chick PM; and lane 6, myosin from 1-year dystrophic chicken PM. (C) MHC peptide maps were prepared from myosins in the PM of 20-day normal chicks and from myosins of the PM of 1-year dystrophic chicks, transferred to nitrocellulose, and reacted with 2E9A antibody as in (B). Lane 1, MHC peptide map of myosin from 20-day normal chick PM cleaved with 25 ng of *Staphylococcus aureus* V8 protease; lane 2, MHC peptide map of myosin from the PM of 1-year dystrophic chicken cleaved with 25 ng of *S. aureus* V8 protease; lane 3, MHC peptide map of myosin from the PM of 20-day normal chick cleaved with 50 ng of *S. aureus* V8 protease; and lane 4, MHC peptide map of myosin from the PM of 1-year dystrophic chicken cleaved with 50 ng of *S. aureus* V8 protease. The arrow indicates the position of undigested MHC. The fact that the 2E9A determinant is on identical cleavage fragments in both samples suggests that the same MHC is present in the 20-day normal chicken and the 1-year adult dystrophic chicken.

ELISA at 4 months. These results are consistent with previous observations on the appearance of neonatal MHC isoform (*1*), and they demonstrate a monoclonal antibody specific to the neonatal myosin.

Myosin from the PM of 6-month and 1-year dystrophic chickens (*6*) reacted with 2E9A, while myosin from age-matched control birds did not. Figure 1B shows that myosins from the PM of 6-month and 1-year dystrophic birds reacted identically with 2E9A at different antibody dilutions. At antibody saturation, myosin from dystrophic muscle was 60 percent as reactive as myosin from neonatal normal muscle. As before, myosin from 6-month and 1-year normal muscle did not react significantly with 2E9A. Similar results were obtained when the concentration of antigen was varied (Fig. 1C). These results suggest that myosin from adult dystrophic muscle contains the antigenic determinant detected by 2E9A, which is present on the neonatal MHC. The fact that myosin from dystrophic muscle is not as reactive as myosin from neonatal muscle may mean that other nonreactive myosins are present in dystrophic muscle or that a different MHC is present in dystrophic PM that contains a similar but not identical determinant.

The reactivity of 2E9A with Western blots of myosin samples from normal and dystrophic chickens of different ages is shown in Fig. 2, A and B. Antibody reacted with MHC of 20-day normal chicks but not with MHC of embryonic or adult normal chickens. As expected, 2E9A reacted with MHC from the PM of adult dystrophic chickens in addition to reacting with myosin from 20-day neonatal dystrophic chicks. No reaction was observed with MHC from embryonic dystrophic chickens. While equal amounts of MHC were transferred to nitrocellulose, it appeared that the reaction with adult dystrophic myosin was less than the reaction with MHC from 20-day normal or 20-day dystrophic PM.

Many monoclonal antibodies have been shown to react with more than one myosin isoform (*7*). Thus the same antigenic determinant may be found in closely related isozymes. To determine whether 2E9A was reacting with the same myosin in 20-day normal PM and in 1-year dystrophic PM, I prepared peptide maps of the MHC from the 20-day normal PM and the 1-year dystrophic PM by a peptide mapping procedure that I have previously used to characterize many MHC isoforms. The maps were transferred to nitrocellulose by Western blotting and subsequently reacted with 2E9A antibody. By this procedure only those cleavage products that still con-

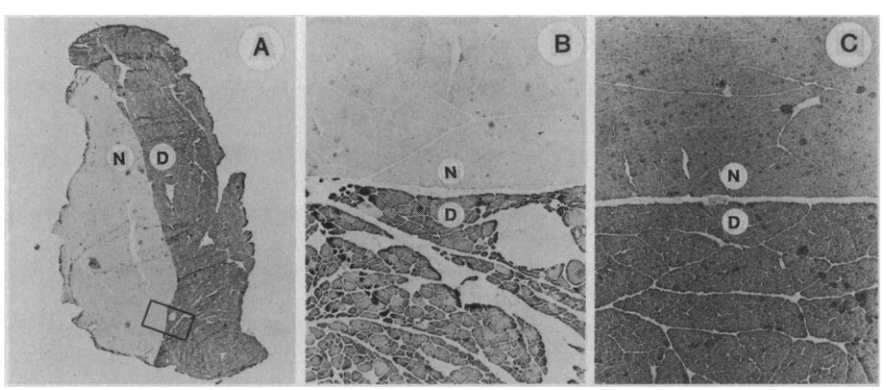


Fig. 3. Immunocytochemical analysis of the PM of normal and dystrophic chickens with 2E9A antibody. PM's from dystrophic and aged-matched normal chickens were frozen side by side in liquid Freon and 10-μm sections were cut and air-dried for 30 minutes. Sections were reacted with 2E9A antibody for 1 hour at 37°C after blocking with 2 percent horse serum. Bound antibody was reacted with Vectastain ABC reagents as described in the legend to Fig. 1. Bound antibody was visualized with diaminobenzidine. (A) Low-power magnification of PM from 6-month normal (N) and 6-month dystrophic (D) chickens. (B) Higher power view (×200) of the area in the rectangle in (A). (C) PM from 20-day normal (N) and 20-day dystrophic (D) chicks (×200). In the muscle from 6-month dystrophic chickens all the fibers were stained with 2E9A, while no fibers were stained in the muscle from 6-month normal chickens. The small dark spots in the normal muscle are due to contraction artifacts during staining. All fibers in 20-day muscle of 20-day normal and dystrophic chicks reacted with 2E9A antibody.

tained the 2E9A determinant would be detected. I previously showed that peptide maps of MHC from adult dystrophic PM contained many peptides in common with neonatal MHC but were not absolutely identical to the peptide map of MHC from 20-day chick PM (*8*). However, as shown in Fig. 2C, the peptides detected by 2E9A are identical in the peptide maps of MHC's from 20-day normal PM and 1-year dystrophic PM at two different *Staphylococcus aureus* V8 protease concentrations. This strongly suggests that 2E9A was reacting with the same myosin in both samples and not with a closely related isoform.

The distribution of neonatal MHC in adult dystrophic muscle fibers was then determined by immunocytochemistry. As shown in Fig. 3, 2E9A reacted with virtually all fibers in adult dystrophic muscle but with no fibers in adult normal muscle. All fibers of normal and dystrophic PM from 20-day chicks reacted with 2E9A. Thus all fibers of the dystrophic PM continued to express neonatal MHC. In a previous report I suggested from my analysis of MHC peptide maps that adult dystrophic PM contained predominantly neonatal MHC in addition to other myosins. Now, having developed a monoclonal antibody specific to the neonatal MHC, I have been able to confirm the presence of this isoform in all fibers of dystrophic PM. The present results also suggest that other myosins are present in dystrophic PM. However, since other monoclonal antibodies that will react specifically with embryonic or adult MHC's are not available, these myosins cannot now be identified.

While it is widely assumed that degeneration and regeneration is occurring in dystrophic muscle, there is little direct evidence of this. Since the amount of atrophy or hypertrophy varies in different dystrophic lines depending on background genes (*6*), regenerative processes are probably secondary effects of muscular dystrophy. In my study, virtually all muscle cells of the dystrophic PM, irrespective of fiber size, reacted with 2E9A. Thus dystrophic muscle fibers continue to accumulate neonatal MHC and fail to undergo the isoform transition from neonate to adult that is characteristic of normal muscle. This makes it unlikely that regeneration per se is responsible for the continued expression of immature isoforms, although the occasional cell that does not react with 2E9A may represent a regenerating fiber that has been shown to contain embryonic myosin (*9*). It should be pointed out that neonatal MHC has not been found in regenerating muscle (*9*), and if all the fibers in a 6-month-old dystrophic chicken are regenerating fibers (an unlikely situation), this would probably be the first demonstration of neonatal myosin in such fibers.

While other isozyme changes have been shown to be inhibited in dystrophic fibers (*3*), myosin is, to my knowledge, the only muscle protein that undergoes a transition from embryo to neonate to adult. Since the first isoform change occurs normally in dystrophic muscle, these results indicate a subsequent block in maturation 1 month after hatching. The product of the dystrophic gene has not yet been identified, but its action is consistent with its being a regulator of the expression of many gene families. One would predict that such a regulator would be present at very low levels, which may explain its elusive nature. Our knowledge of the regulation of multigene families in which different genes are expressed at different ages is very limited. It remains possible that the processes involved in turning genes on and off during normal development are impaired in dystrophic muscle, resulting in the variety of isozyme abnormalities that have thus far been identified.

*Note added in proof*: I have now produced a monoclonal antibody specific for the adult MHC and found a small amount of this isoform to be present in adult dystrophic PM.

EVERETT BANDMAN
*Department of Food Science and Technology, University of California, Davis 95616*

**References and Notes**

1. R. G. Whalen *et al.*, *Nature (London)* **292**, 805 (1981); E. Bandman, R. Matsuda, R. C. Strohman, *Dev. Biol.* **93**, 508 (1982); S. Lowey, P. A. Benfield, D. D. LeBlanc, G. S. Waller, *J. Muscle Res. Cell Motil.* **4**, 695 (1983).
2. V. S. Asmundson and L. M. Julian, *J. Hered.* **47**, 248 (1956); D. W. Peterson, W. H. Hamilton, A. L. Lillyblade, *Proc. Soc. Exp. Biol. Med.* **127**, 300 (1968).
3. C. R. Ashmore and L. Doerr, *Exp. Neurol.* **30**, 431 (1971); E. Cosmos and J. Butler, in *Exploratory Concepts in Muscular Dystrophy and Related Disorders*, A. T. Milhorat, Ed. (Excerpta Medica, Amsterdam, 1967), p. 197; T. Gordon and G. Vrbova, *Pfluegers Arch.* **360**, 199 (1975); B. W. Wilson, W. R. Randall, G. T. Patterson, R. K. Entriken, *Annu. Rev. N.Y. Acad. Sci.* **317**, 224 (1979); J. Sketelj, M. G. McNamee, B. W. Wilson, *Exp. Neurol.* **60**, 624 (1978); T. Obinata, H. Takano-Ohmuro, R. Matsuda, *FEBS Lett.* **120**, 195 (1980); T. Mikawa, S. Takeda, T. Shimizu, T. J. Kitaura, *J. Biochem.* **89**, 1951 (1981); S. Takeda and Y. Nonomura, *Biochem Res.* **1**, 176 (1980).
4. Myosin was prepared as described by E. Bandman, R. Matsuda and R. C. Strohman [*Dev. Biol.* **93**, 508 (1982)]. After electrophoresis on sodium dodecyl sulfate–polyacrylamide gel, MHC was eluted from the gel in an Isco electrophoretic concentrator.
5. M. L. Gefter, D. H. Margulies, M. D. Scharff, *Somat. Cell Genet.* **3**, 231 (1977).
6. In this study we used inbred White Leghorn dystrophic line 433 and its isogenic White Leghorn control line 03, which are maintained at the Department of Avian Science, University of California, Davis. These lines have more than 95 percent of their genes in common, minimizing any genetic differences that may affect expression of the disease. For a comparison of the various normal and dystrophic chicken lines, see B. W. Wilson, W. R. Randall, G. T. Patterson, R. K. Entriken, *Annu. Rev. N.Y. Acad. Sci.* **317**, 224 (1979).
7. D. Bader, T. Masaki, D. A. Fischman, *J. Cell Biol.* **95**, 763 (1982); D. A. Winkelmann, S. Lowey, V. L. Press, *Cell* **34**, 295 (1983).
8. E. Bandman, *Muscle Nerve* **7**, 312 (1984).
9. S. Sartore, L. Gorza, S. Schiaffino, *Nature (London)* **298**, 294 (1982); R. Matsuda, D. H. Spector, R. C. Strohman, *Dev. Biol.* **100**, 478 (1983).
10. H. Towbin, T. Stahelin, J. Gordon, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350 (1979).
11. I acknowledge the expert technical assistance of S. Matoba and D. Fraser. This research was supported by a grant from the Muscular Dystrophy Association of America and by NIH grant AM31731.

2 July 1984; accepted 19 November 1984

## Prolactin Stimulation of Maternal Behavior in Female Rats

Abstract. *Inexperienced, hypophysectomized female rats treated with steroids were used in experiments to investigate the roles of the pituitary gland and prolactin in the expression of maternal behavior. Administration of ovine prolactin or treatment with ectopic pituitary grafts, which release prolactin into the circulation, stimulated maternal care in these females toward rat young. Steroid treatment alone, while stimulating maternal behavior in rats with intact pituitary glands, did not facilitate maternal responsiveness in hypophysectomized females. These findings indicate a stimulatory behavioral role for pituitary prolactin in the establishment of maternal care and suggest that exposure to prolactin during pregnancy helps to stimulate the immediate onset of maternal behavior at parturition.*

Studies of the relation between endocrines and behavior have indicated that changes in hormonal status during pregnancy contribute to the expression of maternal care at parturition in mammals (*1*). While the steroids estradiol and progesterone have been shown to stimulate maternal responsiveness in behaviorally inexperienced females, evidence for a role of pituitary hormones—most notably prolactin, which is secreted in large amounts during early and late stages of pregnancy (*2*)—in the induction of maternal behavior in mammals is equivocal. The early finding of Riddle *et al.* (*3*) that repeated injections of prolactin stimulated maternal behavior in female rats led to the suggestion that prolactin was a