5. R. M. Rose, *Psychosom. Med.* **31**, 405 (1969).
6. ———, J. W. Holaday, I. S. Bernstein, *Nature (Lond.)* **231**, 366 (1971).
7. W. K. Whitten, *Adv. Reprod. Physiol.* **1**, 155 (1966).
8. F. H. Bronson and C. Desjardins, *J. Endocrinol.* **44**, 293 (1969); V. Chapman, C. Desjardins, W. K. Whitten, *J. Reprod. Fertil.* **21**, 333 (1970); B. E. Eleftheriou, C. M. Christenson, M. X. Zarrow, *J. Endocrinol.* **57**, 363 (1973); P. C. Hoppe and W. K. Whitten, *Biol. Reprod.* **7**, 254 (1972).
9. M. Bowers and B. K. Alexander, *Science* **158**, 1208 (1967).
10. E. L. Bliss, A. Frischat, L. Samuels, *Life Sci.* **11**, 231 (1972).
11. In an initial study three male house mice each were paired with a receptive female for 2 hours. Following copulation to ejaculation, plasma T levels were 0.8, 0.4, and 0.7 ng/ml. This indicates that successful copulation does not lead to elevated T levels in the mouse, but a larger sample is needed to establish whether copulation results in a significant decrease as in the rat.
12. R. K. Chipman and F. H. Bronson, *Experientia* **15**, 199 (1968); H. M. Marsden and F. H. Bronson, *Nature (Lond.)* **207**, 878 (1965).
13. J. G. Vandenbergh, *Endocrinology* **84**, 658 (1969).
14. C. Desjardins, J. A. Maruniak, F. H. Bronson, *Science* **182**, 939 (1973).
15. Supported by NIH research career development award 5 K04 HD70369 to A.B., NIH grant 5 RO1 HD06867, and NSF grant GB-36070. We thank B. V. Caldwell for antiserum to testosterone, and M. L. Lincourt and S. Schneider for assistance.

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Dystrophic Spinal Cord Transplants Induce Abnormal Thymidine Kinase Activity in Normal Muscles

Abstract. *The role of the neural tube in the pathogenesis of muscular dystrophy was tested directly. Neural tubes from chicken embryos with hereditary muscular dystrophy and from genetically normal embryos were transplanted into normal recipient embryos. Dystrophic neural tissue induced in muscles of normal hosts high thymidine kinase activity characteristic of dystrophic muscle; normal neural tubes did not. We propose an early inductive effect of the neural tube on the presumptive myoblasts that sets their subsequent course of development, either normal or dystrophic.*

Hereditary muscular dystrophies in man and animals are characterized by progressive weakness accompanied by physiological, biochemical, and morphological changes in the affected muscles. Evidence from both human and animal studies has led to the suggestion that the primary lesion in the diseases is expressed in the nervous system, and that changes in the muscles are secondary to an abnormal neurotrophic influence (*1–4*). A general membrane defect has also been postulated (*5*). In most of the investigations on animals, genetically normal muscle has been innervated with genetically dystrophic nerves, and vice versa, the experiments involving either transplantation of muscle, (*2, 6*), parabiosis (*7*), or culture of muscle and nerve tissue (*3, 8*). Not all the results have agreed on the significance of a peripheral nerve influence (*2–4, 6–8*). We have found that neural tubes from dystrophic chicken embryos transplanted into normal chicken embryos induce a high level of thymidine kinase activity in the genetically normal host muscles. This high activity is characteristic of dystrophic muscle at that stage of development. Transplantation of normal neural tubes had no such effect. We propose that these results may be due to an early irreversible inductive effect of the neural tube on the developing musculature.

Experiments with muscle transplantation designed to test extramuscular influences on the progress of dystrophy have shown that, when muscle is transplanted from dystrophic embryos as young as 12 days in ovo to normal chicks, the phenotypic expression of dystrophy in the donor muscle is unaltered (*9*). However, these experiments have not taken into account the possibility that the defective gene might act extramuscularly at a much earlier state of embryonic development than 12 days in ovo. The direct test of the role of the nervous system in the pathogenesis of hereditary muscular dystrophy is to transplant the nervous system between dystrophic and normal animals before the muscles have been influenced by nerves of their own genotype. To this end we transplanted a neural tube from dystrophic to normal chickens at a time during ontogeny before the first known inductive neuronal influences are expressed on the development of the somites (*10*), and preceding muscle differentiation and innervation (*11*) [stage 13 in (*12*)]. We determined the effect of the transplanted neural tubes on an enzyme activity in the embryonic muscles that is characteristically higher in dystrophic than in normal muscles during development.

Muscular dystrophy in chickens is inherited as an autosomal codominant. The disease affects fast twitch glycolytic muscle fibers and particularly the superficial pectoral muscles that are innervated by the brachial plexus (*13*). The spinal cord region destined to give rise to the brachial plexus was located by reference to the adjacent somites and was transplanted isotopically to the host embryo (Fig. 1). The operation was performed as early as was anatomically possible: that is, as soon as the 21st somite was clearly demarcated from the unsegmented somitic tissue. At this stage of development the differentiation of muscle from the host somites has not occurred and innervation has not been established.

Initial experiments were done to confirm the integrity and location of this type of graft, by transplanting quail neural tubes into chicken embryos. Quail cells can readily be distinguished from chicken cells by their large conspicuous nucleoli (*14*). The brachial motor neurons in the resultant embryos (quail–White Leghorn) were clearly of quail origin. In both quail-chicken and chicken-chicken chimeras no macroscopic evidence of discontinuity between host and donor was observed, and histological examination of the spinal cord at the junction of the transplant and host tissue showed that the graft was anatomically continuous with the host tissue.

Of 65 chicken-chicken transplants, 14 embryos survived to 18 days in ovo. Of these eight had received a neural tube from normal embryos (N → N) and six from dystrophic embryos (am → N). Most embryonic deaths were attributed to surgical trauma and occurred within 3 days of the operation. A second peak in embryonic mortality occurred at 12 to 13 days in ovo and may have resulted from the mechanical stresses on the embryos (*15*).

Thymidine kinase activity in the pectoral muscles of dystrophic chicken embryos at 18 days in ovo is significantly higher than in the pectoral muscles of normal em-

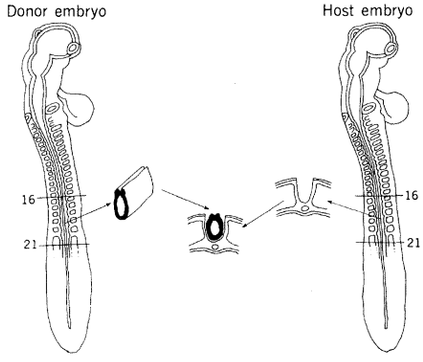

Fig. 1. Diagram of procedure used for transplanting neural tubes. The donor embryo was removed from the egg and washed in saline; the number of somites was then determined. The spinal cord between somites 16 to 21 was removed, washed in 0.25 percent trypsin to remove adherent mesenchyme, and subsequently washed in soybean trypsin inhibitor. It was then stored in Eagle's minimal essential medium buffered with *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) (25 m*M*) while the host animal was being prepared. The host embryo was exposed by drilling a small window in the shell directly over the embryo, the vitelline membrane above the brachial region was removed, and the spinal cord cut out with a fine steel knife. Care was taken not injure the somites or the underlying notochord. The donor cord was dropped in place from a Pasteur pipette and was oriented by means of a fine tungsten needle under visual control both anteroposteriorly and dorsoventrally. The egg was sealed with sterile Parafilm and incubated with the window side up for a further 16 days.

bryos at this stage of development (*16*). Because of the very high mortality at hatching after this type of operation, we examined the pectoral muscles of embryos at 18 days in ovo for the expression of this difference in thymidine kinase activity. Control muscles from unoperated normal and dystrophic 18-day embryos were also assayed. The thymidine kinase activity of the muscles was determined by a modification (*16, 17*) of the methods of Weissman *et al.* (*18*) and Brietman (*19*). Our results (Fig. 2) show that genetically dystrophic neural tubes induce a significantly higher thymidine kinase activity in normal muscles (am → N) compared with normal unoperated embryos (N), or with normal embryos that had received a normal neural tube (N → N) ($P < .01$). The high thymidine kinase activity of normal muscles innervated by dystrophic neural tissue is the same as that in unoperated dystrophic embryos (am) ($P > .2$). Thus, the nervous system is providing an abnormal influence that results in this feature of muscle pathology being expressed. Although this elevation of thymidine kinase activity is the earliest enzyme change reported in the muscles of chickens with muscular dystrophy, its precise significance is unknown. However, thymidine kinase is reported (*17*) to be a rate-limiting enzyme that regulates the synthesis of thymidine phosphate precursors of DNA. The high activity of this enzyme in dystrophic muscles may, therefore, be related to the increased density of nuclei that has been described in these muscles (*20*) and so be directly related to the pathological changes.

If limb buds or muscles are transplanted between normal and dystrophic chickens, the nervous system appears to have no influence on the subsequent development of the muscle pathology (*21*). However, these transplant studies were not done as early in development as the neural tube transplants reported here. Cosmos and Butler (*22*) have suggested the possibility that nerves "imprint" dystrophic features on the muscle when they are first innervated. We suggest a modification of their "imprint" hypothesis, and propose that the neural tube itself plays an important but as yet undefined inductive role in differentiation of muscle tissue and that dystrophic neural tube is abnormal in this respect.

An inductive role for the neural tube is already described for differentiation of the mesonephric tubules and the vertebral column (*23*). In each case, the induction is independent of innervation. The inductive activity of the neural tube is expressed between 2 and 4 days in ovo during which time a number of potential inductors such as collagen (*10*) and glycosaminoglycans (*24*) are elaborated by the neural tube. This critical inductive period occurs after the stage at which the neural tube transplants are done, but prior to limb bud formation. If dystrophic features can be irreversibly induced at a very early stage in cells that will give rise to muscles, experiments done after this critical period would support a primary myogenic origin of muscular dystrophy, and would not reveal the influence of the nervous system. This could explain why limb bud transplants between normal and dystrophic chicken embryos retained the characteristics of the donor animals (*21*). These experiments were done 36 to 48 hours later than the neural tube transplants reported here and after the critical time during which the neural tube exerts its inductive influence (*10, 24*).

The role of the nervous system in the pathogenesis of murine muscular dystrophy is also controversial. Many workers using mice in which muscles were already innervated have found evidence of a myogenic defect (*7–9*). However, experiments with mouse embryos have indicated that here too the disease has an extramuscular pathogenesis (*25*). Our hypothesis of an early irreversible inductive effect of the neural tube on the developing musculature could also explain the discrepancy in the mouse results.

Our results demonstrate an early induction in genetically normal chicken muscles of an abnormally high thymidine kinase activity by neural tubes from dystrophic chickens. A high thymidine kinase activity has been shown by Weinstock and Dju (*16, 17*) to be characteristic of dystrophic chicken muscles and is the earliest enzyme change reported in this disease. We propose that an early interaction between neural tube and somites sets the presumptive myoblasts on a specific course of differentiation. Once this interaction is complete, the muscle that develops retains its induced characteristics even when placed in a different environment.

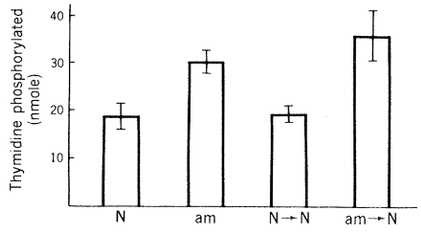

Fig. 2. Thymidine kinase activity of experimental and control pectoral muscles of 18-day chicken embryos expressed as nanomoles of thymidine phosphorylated per gram (wet weight) of superficial pectoral muscle per 120 minutes. Mean values ± standard error of the mean. The values for N and N → N are not different ($P > .2$) (Student's t-test), and the values for am and am → N are also not different ($P > .2$). However, both am and am → N are significantly different from N and N → N ($P < .01$). Abbreviations: N, normal muscle from unoperated embryos ($n = 8$); am, dystrophic muscles from unoperated embryos ($n = 10$); N → N, normal muscles that had been innervated by neural tubes from normal embryos ($n = 8$); am → N, normal muscles that had been innervated by neural tubes from dystrophic embryos ($n = 6$).

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References and Notes

1. A. J. McComas and R. E. P. Sica, *Lancet* **1970-I**, 1119 (1970); _____, M. J. Campbell, *ibid.* **1971-I**, 321 (1971); R. E. P. Sica and A. J. McComas, *J. Neurol. Neurosurg. Psychiatry* **34**, 469 (1971); *ibid.*, p. 461; J. Harris and P. Wilson, *Nature (Lond.)* **229**, 61 (1971); J. B. Harris, *Dev. Med. Child Neurol.* **13**, 669 (1971); E. X. Albuquerque and J. E. Warnick, *Science* **172**, 1260 (1971); J. J. Gilbert, N. C. Steinberg, B. Q. Banker, *J. Neuropathol. Exp. Neurol.* **32**, 345 (1973).
2. B. Salafsky, *Nature (Lond.)* **229**, 270 (1971).
3. B. Gallup and V. Dubowitz, *ibid.* **243**, 287 (1973).
4. V. Dubowitz, *J. Physiol. (Lond.)* **231**, 59P (1973).
5. A. D. Roses and S. H. Appel, *Nature (Lond.)* **250**, 245 (1974).
6. T. Hironaka and Y. Miyata, *Nat. New Biol.* **244**, 221 (1973).
7. W. B. Douglas and E. Cosmos, *Fed. Proc.* **33**, 402 (1974).
8. M. Hamburgh, M. B. Bornsteine, E. R. Peterson, S. M. Crain, E. B. Masurovsky, C. Kirk, *Prog. Brain Res.* **40**, 497 (1971).
9. E. Cosmos, *Physiologists* **16**(2), 167 (1973).
10. A. M. Cohen and E. D. Hay, *Dev. Biol.* **26**, 578 (1971).
11. R. J. Przybylski and J. M. Blumberg, *Lab. Invest.* **15**, 836 (1966).
12. V. Hamburger and H. L. Hamilton, *J. Morphol.* **88**, 49 (1951).
13. The brachial plexus in chickens is composed of spinal nerves C13-16 and occasionally T1 [C. H. Narayanan, *J. Exp. Zool.* **156**, 49 (1964); K. Strazznicky, *Acta Biol. Acad. Sci. Hung.* **18**(4), 437 (1967)]. Their locations correspond to somite levels 17 to 20 [G. DeO. Castro, *J. Exp. Zool.* **152**, 279 (1963)].
14. N. LeDouarin, *Dev. Biol.* **30**, 217 (1973).
15. H. R. Moseley and W. Landauer, in *Fertility and Hatchability of Chicken and Turkey Eggs*, L. W. Taylor, Ed. (Wiley, New York, 1949).
16. I. M. Weinstock and M. Y. Dju, *Life Sci.* **6**, 797 (1967).
17. _____, *Biochim. Biophys. Acta* **232**, 5 (1971).
18. J. A. Weissman, R. M. S. Smellie, J. Paul, *ibid.* **45**, 101 (1960).
19. T. R. Brietman, *ibid.* **67**, 153 (1963).
20. I. M. Weinstock and M. Lukas, *Enzymol. Biol. Clin.* **5**, 103 (1965).
21. A. Linkhart, G. W. Yee, B. W. Wilson, *Science* **187**, 549 (1975).
22. E. Cosmos and J. Butler, in *Research in Muscle Development and the Muscle Spindle*, B. Q. Banker, R. J. Przybylski, J. P. Van der Meulen, M. Victor, Eds. (Excerpta Medica, Amsterdam, 1972), p. 149.
23. J. Lash, S. Holtzer, H. Holtzer, *Exp. Cell Res.* **13**, 292 (1957); R. L. Watterson, I. Fowler, J. B. Fowler, *Am. J. Anat.* **95**, 337 (1954); C. Grobstein, *Exp. Cell Res.* **10**, 424 (1956).
24. E. D. Hay and S. Meier, *J. Cell Biol.* **62**, 889 (1974).
25. A. C. Peterson, *Nature (Lond.)* **248**, 561 (1974).

26. Eggs from dystrophic chickens were obtained through Dr. Louis Pierro, University of Connecticut, Storrs. Normal White Leghorn eggs were obtained from the Department of Poultry Husbandry, University of Guelph, Guelph, Ontario. Supported by a grant-in-aid to M.P.R. from the Muscular Dystrophy Association of Canada; an M.D.A.C. predoctoral fellowship to F.V.; and to P.A.S., an M.D.A.C. postdoctoral fellowship. We thank Drs. Ethel Cosmos, Jack Diamond, Alan McComas, and Alan Peterson for discussions, Dr. Michael Coughlin for assistance with photography, Dr. David McCallion for suggesting the use of the quail embryos, and Professor Nicole LeDouarin for the advice on the microsurgical procedure. M.P.R. is now a member of the M.R.C. group in developmental biology. Reprint requests should be addressed to M.P.R.

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