mocytes with TL antiserum and complement (more than 95 percent of the cells surviving) gave a similar MSI, 2.6 ± 0.20; treatment of C57/TL$^+$ thymocytes (H-2^b) with H-2^b antiserum plus complement (which killed over 95 percent of them) gave no GVHR (Fig. 3). Thus the minority population of TL$^-$ cells apparently contains most of the cells capable of producing GVHR (*10*).

The following consideration excludes the possibility that this minor population consists solely of blood lymphocytes contaminating the thymocyte suspensions. In control experiments, 1×10^5 blood leukocytes did not produce splenic enlargement whereas 2×10^5 blood leukocytes gave an MSI of 1.6 ± 0.41. The number 2×10^5 corresponds to about 0.02 ml of whole blood; since contamination of our thymocyte suspensions with erythrocytes never approached 5 percent, it is obvious that significant contamination with leukocyte-containing blood can be ruled out.

As for the possibility of some form of selective trapping of immunocompetent circulating cells by the thymus, the following data render this unlikely—15×10^6 C57/TL$^+$ thymocytes from neonatal donors (less than 24 hours old) gave an MSI of 2.7 ± 0.57 in six mice, indicating a GVH activity comparable with that of adult thymus [see also (*11*)]. But the spleens of neonatal mouse donors, and presumably their circulating blood, contain too few immunocompetent cells for demonstrable GVHR (*12*). Thus selective trapping of peripheral immunocompetent cells is scarcely a probable explanation of the GVH activity of the neonatal thymocyte population. We conclude that the immunocompetent (TL$^-$) fraction of both neonatal and adult thymocyte populations is derived in situ.

Immunoselection with cytotoxic TL antiserum has proved a feasible tool for isolating a discrete subpopulation of thymocytes, a subpopulation which carries most and possibly all of the GVH reactivity of thymus.

In normal mice, TL$^+$ cells are not found outside the thymus, which implies that peripheral migration of thymocytes entails preceding loss of TL antigens. Thus much evidence now points to the existence in thymus of a proportion of cells that have taken several maturative steps, including not only the loss of TL and the acquisition of immunocompetence but also major quantitative changes affecting surface antigens other than TL [see review (*3*)]. These maturation steps may not occur simultaneously, and this is perhaps the most likely reason why the GVH capacity of the subpopulation selected, while considerably greater than that of the unselected thymocyte population, is still only about one-tenth of that possessed by lymph node lymphocytes.

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References and Notes

1. R. E. Billingham and W. K. Silvers, in *The Thymus*, V. Defendi and D. Metcalf, Eds. (Wistar Inst. Press, Philadelphia, 1960), p. 41.
2. L. J. Old, E. A. Boyse, E. Stockert, *J. Nat. Cancer Inst.* **31**, 977 (1963).
3. E. A. Boyse and L. J. Old, *Annu. Rev. Genet.* **3**, 269 (1969).
4. E. A. Boyse, E. Stockert, L. J. Old, in *International Convocation on Immunology*, N. R. Rose and F. Milgrom, Eds. (Karger, Basel, 1968), p. 353. The stock used in the present experiments was derived from the 18th generation of backcrossing to C57BL/6.
5. J. N. Blau and J. M. Gaugas, *Immunology* **14**, 763 (1968).
6. Pelikan (Gunther Wanger, C11/1431 a). The Pelikan was centrifuged at 2000 rev/min for 15 minutes. The supernatant was diluted one-half in medium 199, and 0.2 ml was injected intraperitoneally into a mouse. The mouse was killed ½ hour later by cervical dislocation, care being taken to avoid bleeding near the thymus.
7. R. E. Billingham, in *Transplantation of Tissues and Cells*, R. E. Billingham and W. K. Silvers, Eds. (Wistar Inst. Press, Philadelphia, 1961), p. 90. We used medium 199 with $NaHCO_3$ and glutamine (Grand Island Biological, Chagrin Falls, Ohio).
8. M. Simonsen, *Progr. Allergy* **6**, 349 (1962).
9. The antiserum was C57BL/6 antiserum to A strain leukemia ASL1 [see (*2*)]. The conditions of exposure to antiserum and complement were similar to those used in cytotoxic tests [E. A. Boyse, L. J. Old, I. Chouroulinkov, *Method. Med. Res.* **10**, 373 (1964)]. Washed C57/TL$^+$ thymocytes (500×10^6), in 25 ml of medium 199, were incubated for 50 minutes at 37°C with 25 ml of TL antiserum (1:50; cytotoxic titer, 1:4000 on C57/TL$^+$ thymocytes) and 25 ml of guinea pig serum (1:4; selected for low toxicity to thymocytes). The cells were recovered by slow centrifugation, viability was checked with trypan blue, and cell concentration was adjusted to give the number of cells required for injection in 0.06 ml.
10. This conclusion is consistent with a report that systemic administration of cortisone caused the disappearance of TL$^+$ cells from thymus [M. Schlesinger and V. K. Golakai, *Science* **155**, 1114 (1967)], and an independent report that cortisone treatment combined with sublethal total-body irradiation gave rise 2 days later to a thymus cell population with tenfold increased GVH reactivity [H. Blomgren and B. Andersson, *Exp. Cell Res.* **57**, 187 (1969)].
11. G. J. Thorbecke and M. W. Cohen, in *The Thymus*, V. Defendi and D. Metcalf, Eds. (Wistar Inst. Press, Philadelphia, 1964), p. 33.
12. In this study and in previous experiments (E. Leckband and L. W. Law, unpublished), spleen cells from donors less than 24 hours old did not produce GVHR.
13. Supported in part by National Cancer Institute grant CA 08748 and a grant from the John A. Hartford Foundation, Inc.

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Electrophysiological Observations in Normal and Dystrophic Chicken Muscles

Abstract. *Intracellular recordings were made on the fast posterior latissimus dorsi muscles of normal and dystrophic chickens. In the dystrophic chickens, the rate of rise of the action potential was decreased. With repetitive indirect stimulation, the action potentials decreased in size and disappeared; only an end-plate potential remained. Membrane resistance, membrane capacitance, and duration of miniature end-plate potentials were increased. A decrease in sodium permeability may be in part responsible for the observed alterations in the electrical properties of the nerve terminal and postsynaptic muscle membrane.*

Inherited muscular dystrophy in chickens was first reported in 1956 (*1*) and was found to be similar to certain forms of the disease in man (*2*). The most consistent outward sign of the disease, which primarily affects the fast, white muscles, is a progressive inability of the chicken to right itself when placed on its back (*3*). The disease symptom first appeared in our chickens (*4*) 2 to 3 weeks ex ovo, and after 6 weeks the birds could not rise because of the limited range of wing movements (*5*). Although numerous studies have revealed biochemical abnormalities in dystrophic chicken muscles (*6*), none have dealt with the electrophysiological characteristics of the motor-nerve terminal and muscle membrane. We have therefore examined the fast posterior latissimus dorsi (PLD) muscles of normal and dystrophic chickens by standard electrophysiologic techniques for recording and stimulation (*7, 8*).

Our experiments briefly describe some alterations in the pre- and postsynaptic membranes of the dystrophic neuromuscular junction and in the extrajunctional muscle membrane. In dystrophic muscle of the chicken, there is a decrease in the frequency and amplitude of spontaneous transmitter release and an increase in the time course of the individual potentials. There was a two- to threefold increase

in membrane resistance and a decrease in the ability of the postsynaptic junctional membrane to generate an action potential in response to repetitive, indirect stimulation.

The PLD muscles and their motor nerve supply were removed from both sides of normal and dystrophic chickens of either sex. Their ages varied from 1 to 48 weeks ex ovo. The birds were killed by decapitation, and the muscles were exposed under a flow of physiological solution (*9*) and excised with part of the spinous processes. The adjacent connective tissue was then carefully removed, and the muscle was bisected at the tendons of origin in the midline.

The mean value of the resting membrane potential in the PLD muscle fibers of chickens with inherited muscular dystrophy was -71.3 ± 6.0 mv ($N = 736$), which is similar to that of the normal muscle fibers (-70.3 ± 4.6 mv, $N = 338$). The mean values for the amplitude of the action potentials, its rate of rise, and the threshold for its generation in normal and dystrophic chickens are shown in Table 1 (*7, 8, 10*). Six weeks ex ovo, there was a reduction in the rate of rise of the action potential recorded in dystrophic muscle fibers; the rate of rise of the spike in the dystrophic muscle was 58 percent of the normal value at 28 weeks (Table 1 and Fig. 1A). This reduction suggests that there is some inactivation of the passive processes involved in the generation of the action potential (*11*).

Stimulation of the motor nerve to either normal or dystrophic muscles resulted in the generation of an action potential recorded focally at the end-plate region (Fig. 1B). The twitch induced after-depolarization which succeeds an indirect (or direct) elicited action potential as the result of a dislodgement of the microelectrode, was present in normal, but not in dystrophic, chicken muscle. The dystrophic muscle could be stimulated indirectly at a frequency of 5 to 10 per second; the regenerative activity first decreased and subsequently disappeared, and only end-plate potentials remained (Fig. 2, A–C). After the appearance of end-plate potentials in the dystrophic chicken muscle, cessation of repetitive stimulation for 5 seconds and subsequent stimulation resulted in the reappearance of spike potentials which then decreased in amplitude; the spike potentials were followed by the appearance of end-plate potentials (Fig. 2A). This procedure could be repeated numerous times in the same fiber, and the presence of action potentials could be excluded by decreasing the interval between trains of stimuli. The number of action potentials that appeared before subthreshold end-plate potentials were recorded was increased by increasing the interval time between trains of stimuli. During repetitive stimulation there was a hyperpolarization of the muscle membrane of 5 to 7 mv, and the resting membrane potential returned to control values a few seconds after stimulation had subsided. When a second microelectrode was inserted into the same fiber near the recording electrode and in the presence of these end-plate potentials, a cathodal current applied across the muscle membrane elicited an action potential and a local contraction. In the majority of cases only a local contraction occurred during the generation of spike activity whether elicited by direct or indirect stimulation.

Table 1. Action potential amplitude, threshold, and rate of rise of normal (N) and dystrophic (D) posterior latissimus dorsi muscle of the chicken.

Subjects	Age (wk)	Fibers studied (No.)	Action potential		
			Threshold (mv)	Amplitude (mv)	Rate of rise (volt/sec)
28 (N)	6	5	39.8 ± 6.1*	65.4 ± 5.2	158 ± 56
18 (D)	6	6	39.5 ± 4.8	64.2 ± 8.0	53 ± 23
33 (N)	13	7	45.2 ± 4.4	85.5 ± 8.6	208 ± 89
34 (D)	13	5	41.8 ± 4.1	78.0 ± 16.0	132 ± 50
38 (N)	28	17	47.6 ± 4.0	71.4 ± 6.3	164 ± 61
44 (D)	29	7	44.1 ± 6.7	68.6 ± 4.2	95 ± 13

* Values presented are the mean ± S.D.

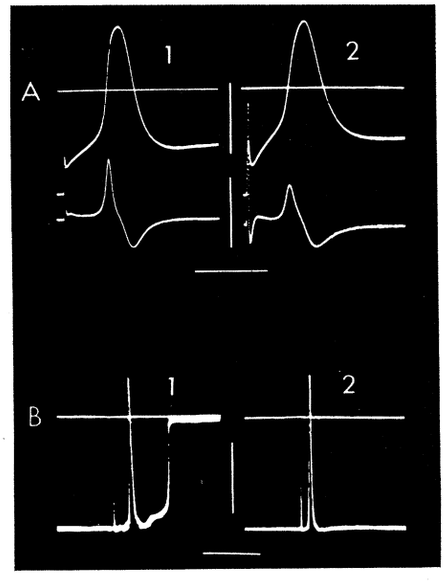

Fig. 1. Action potentials recorded intracellularly from surface fibers of normal and dystrophic posterior latissimus dorsi muscles of chicken. A1 and A2 are, respectively, the direct elicited action potentials and their first derivatives from normal (resting membrane potential, —70 mv) and dystrophic (resting membrane potential, —76 mv) muscle fibers. The horizontal line in both A and B is the zero potential (upper vertical calibration, 50 mv; lower vertical calibration, 200 volt/sec; horizontal calibration, 5 msec). The animals were 28 weeks old. B1 and B2 are the indirectly elicited action potentials from normal (resting membrane potential, —74 mv) and from dystrophic (resting membrane potential, —75 mv) muscle fibers, respectively (vertical calibration, 50 mv; horizontal calibration, 50 msec). The animals were 28 weeks old.

Action potentials with no overshoot were also frequently observed in the dystrophic muscle in response to indirect stimulation, although the resting membrane potential was normal or slightly hyperpolarized (Fig. 2, B–C). Whereas action potentials could be observed in many of the dystrophic muscle fibers in response to repetitive, indirect stimulation, other fibers generated only end-plate potentials. At frequencies up to 40 per second, the end-plate potentials decayed in amplitude and no failure of the single potentials was observed.

A decrease in the frequency and amplitude of the miniature end-plate potentials (MEPP's) occurred after the onset of dystrophic symptoms. The mean frequency and modal amplitude of MEPP's of normal PLD muscle were, respectively, 0.30 per second and 0.20 mv. The MEPP frequency of the dystrophic PLD muscle was about 0.35 per second at 1 week ex ovo and declined slightly to 0.30 per second after 6 weeks, whereas the modal MEPP amplitude remained at normal levels, that is, 0.20 mv; at 10 weeks the MEPP frequency and amplitude were greatly decreased, and it became increasingly difficult to find end plates with MEPP's. During the succeeding weeks, there was a large decrease in the amplitude of the single potentials such that most of the transmitter re-

Table 2. Values of various membrane characteristics of normal (N) and dystrophic (D) posterior latissimus dorsi muscle of the chicken.

Subjects	Age (wk)	V/I* (megohm)	λ (mm)	τ_m (msec)	ρ (calc) (μm)	R_m (ohm cm^2)	C_m (μF/cm^2)
28 (N)	6	1.68 ± 0.16†	0.26 ± 0.04	3.2 ± 1.57	6.2 ± 0.74	335 ± 17	9.8
18 (D)	6	1.77 ± 0.78	0.59 ± 0.25	4.9 ± 0.86	9.6 ± 3.25	1028 ± 382	4.7
33 (N)	13	0.49 ± 0.01	0.68 ± 0.18	2.9 ± 0.74	18.6 ± 2.42	767 ± 196	3.82
34 (D)	13	0.73 ± 0.08	1.01 ± 0.17	12.1 ± 0.14	18.8 ± 2.62	1687 ± 116	7.17
52 (N)	24	0.31 ± 0.08	0.68 ± 0.09	3.6 ± 0.24	24.1 ± 3.60	606 ± 154	5.96
40 (D)	24	0.27 ± 0.06	1.07 ± 0.30	8.6 ± 1.40	32.6 ± 8.88	1048 ± 195	8.21

* For an explanation of the symbols and methods used, see (*13*). † Values presented are mean ± S.D. for at least four fibers.

leased could not be reliably separated from the noise level of the recording system, although end-plate potentials were recorded in the PLD muscles of dystrophic birds upon stimulation of the motor nerve. After repetitive stimulation of the nerve at frequencies of 20 to 50 per second, MEPP's appeared briefly at a high frequency and with a mean amplitude of approximately 0.1 mv. That there was, in fact, transmitter available presynaptically was also demonstrated by the use of batrachotoxin (1.0×10^{-8} g/ml; 37°C), an agent that increases transmitter release by specifically increasing sodium permeability at the nerve terminal (*8*, *12*). Application of this toxin resulted in a large increase and subsequent cessation of the spontaneous and stimulus evoked transmitter release. The frequency and amplitude of MEPP's during exposure to batrachotoxin was similar for the dystrophic and for the normal PLD muscle. These effects of batrachotoxin could be fully antagonized by tetrodotoxin (1.0×10^{-6} g/ml).

The time course of the MEPP's also changed with the development of the dystrophic condition; the mean duration of the MEPP's increased from 20 msec in the normal muscle to 50 msec at 3 to 4 weeks ex ovo in the dystrophic muscles (Fig. 2C). Neither normal nor dystrophic fast PLD muscle fibers exhibited any extrajunctional sensitivity to microiontophoretically applied acetylcholine.

The values for the electrical constants of the membrane of the PLD muscles are represented in Table 2 (*13*). In comparison with normal muscle, the values for input resistance, membrane resistance of a unit area, time constant, and membrane capacitance were increased in dystrophic muscle. Because there was no significant change in fiber diameter in this type of chicken dystrophy, we assume that the increase in membrane electrical constants is correlated with a decrease in ionic permeability and possible alterations in the sarcotubular system.

These observations together with the decrease in the rate of rise of the direct and indirect elicited action potentials in dystrophic muscle and the alterations in spontaneous transmitter release suggest that a decrease in sodium permeability may be in part responsible for the observed alteration in the electrical properties of the motor nerve terminal and muscle membrane in chicken muscular dystrophy. We further suggest that these changes in the electrical properties of the fast twitch muscles of the dystrophic chicken may be correlated with the lack of neurotrophic factor or factors that regulate ionic mechanisms in the excitable membranes of the chicken.

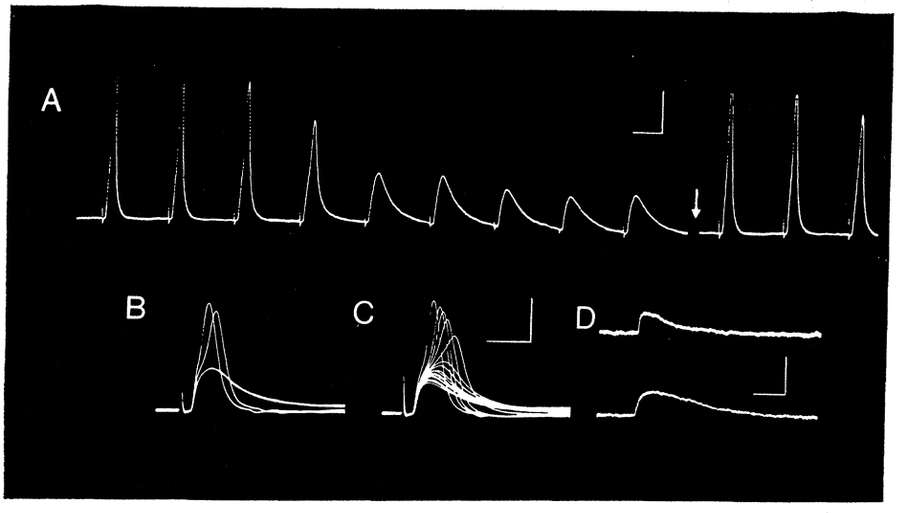

Fig. 2. (A) Action potentials and end-plate potentials recorded intracellularly at the end-plate region of a surface fiber in a dystrophic posterior latissimus dorsi muscle of a 12-week-old chicken. The arrow indicates an interval of 5 seconds between cessation and resumption of indirect stimulation (frequency of stimulation, 10 per second; resting membrane potential, —76 mv; vertical calibration, 25 mv; horizontal calibration, 50 msec). (B and C) Superimposed sweeps of indirectly elicited action potentials and end-plate potentials. Intracellular recordings of the posterior latissimus dorsi muscle from two dystrophic chickens whose ages were (B) 25 and (C) 40 weeks (frequency of stimulation, 5 per second; vertical calibration for B and C is 25 mv and the horizonal calibration is 25 msec). (D) Miniature end-plate potentials recorded intracellularly from surface fibers of normal (upper) and dystrophic (lower) posterior latissimus dorsi muscle of the chicken. The resting membrane potential of the normal muscle fiber was —72 mv and that of the dystrophic fiber was —75 mv. Both animals were 3 weeks old (vertical calibration, 1.0 mv; horizontal calibration, 10 msec).

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References and Notes

1. V. S. Asmundson and L. M. Julian, *J. Hered.* **47**, 248 (1956).
2. L. M. Julian and V. S. Asmundson, in *Muscular Dystrophy in Man and Animals*, G. H. Bourne and M. N. Golarz, Eds. (Hafner, New York, 1963), pp. 457–498.
3. P. M. Farrell and L. L. Tureen, *Proc. Soc. Exp. Biol. Med.* **127**, 678 (1968).
4. We thank Dr. L. J. Pierro, University of Connecticut, for the dystrophic chickens used in our study. The dystrophic mutation originated in a New Hampshire stock but was twice outcrossed to a White Leghorn stock before having been established in Dr. Pierro's laboratory. Similar results to those presented here were obtained by us in normal (line 200) and dystrophic (line 304) chickens obtained from Dr. B. W. Wilson, University of California at Davis.
5. V. S. Asmundson, F. H. Kratzer, L. M. Julian, *Ann. N.Y. Acad. Sci.* **138**, 49 (1956).
6. E. Cosmos and J. Butler, in *Exploratory Concepts in Muscular Dystrophy and Related Disorders*, A. T. Milhorat, Ed. (Excerpta Medica Foundation, Amsterdam, 1967), pp. 197–204; E. Cosmos, *J. Cell Biol.* **23**, 241 (1964); *Develop. Biol.* **13**, 163 (1963); D. Baieve and J. R. Florini, *Arch. Biochem. Biophys.* **139**, 393 (1970); B. W. Wilson, M. A. Montgomery, R. V. Asmundson, *Proc. Soc. Exp. Biol. Med.* **129**, 199 (1968).

7. P. Fatt and B. Katz, *J. Physiol.* (*London*) **115**, 320 (1951); B. Katz and R. Miledi, *ibid.* **170**, 379 (1964); E. X. Albuquerque and S. Thesleff, *Acta Physiol. Scand.* **73**, 471 (1968); E. X. Albuquerque and R. J. McIsaac, *Exp. Neurol.* **26**, 183 (1970).
8. E. X. Albuquerque, J. E. Warnick, F. M. Sansone, *J. Pharmacol. Exp. Ther.* **176**, 511 (1971).
9. B. L. Ginsborg, *J. Physiol.* (*London*) **150**, 707 (1960); *ibid.* **154**, 581 (1960).
10. Two microelectrodes were inserted into the same surface fiber 100 to 150 microns apart, one to pass a current and the other to record the action potential. The resting membrane potential was set at a value of about −75 mv by passing a constant anodal current across the membrane through the stimulating microelectrode. A first derivative resistance-capacitance circuit was used to display the rate of rise of the action potential. The time constant of the recording circuit was about 40 μsec. All experiments except those involving the use of batrachotoxin were performed at 23°C.
11. A. L. Hodgkin and B. Katz [*J. Physiol.* (*London*) **108**, 37 (1949)] observed that a decrease in the rate of rise of the action potential occurred when the extracellular sodium concentration of the giant axon of *Loligo* was reduced.
12. J. E. Warnick, E. X. Albuquerque, F. M. Sansone. *J. Pharmacol. Exp. Ther.*, **176**, 497 (1971).
13. The electrical constants of the membrane were measured by inserting two microelectrodes into a surface fiber of the muscle. For details of this technique and an explanation of the cable equations used in this study, see Albuquerque and McIsaac (*7*). For each interelectrode distance the average value of the ratio V/I was calculated from three different current pulses producing a membrane potential change of 5 to 30 mv. The $\log_{10}$ of V/I was plotted against the distance in millimeters between microelectrodes, and the data was further analyzed when a linear relationship was found. The symbol λ is the space constant and refers to the negative of the inverse of the slope of the line, R_m refers to the transverse resistance of a unit area, and ρ indicates the calculated fiber radius. The symbols τ_m and C_m refer to the time constant and capacitance of the membrane, respectively.
14. Supported by PHS grant NB-08233. J.E.W. is a recipient of a special fellowship award from the NINDS (NIH) 1 F 10 NS-02375-01 NSRA. We thank D. Timmer and M. Zelle for technical assistance.

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Correction of Abnormal Cerebroside Sulfate Metabolism in Cultured Metachromatic Leukodystrophy Fibroblasts

Abstract. *Cultured fibroblasts derived from patients with late infantile metachromatic leukodystrophy incorporated arylsulfatase A from the growth medium. Upon exposure to cerebroside sulfate, they exhibited patterns of uptake and hydrolysis indistinguishable from cells derived from control subjects. Furthermore, inclusion granules formed in the metachromatic leukodystrophy fibroblasts upon exposure to sulfatides were cleared by subsequent supplementation of the growth medium with arylsulfatase A.*

Metachromatic leukodystrophy or MLD is a hereditary neurological disorder of lipid metabolism in which there is progressive degeneration of the nervous system. Demyelination is accompanied by accumulation of cerebroside sulfates (sulfatides) in both the central and peripheral nervous systems (*1*). The biochemical lesion has been identified as a deficiency of the lysosomal enzyme arylsulfatase A (*2*). We have previously demonstrated that cultures of skin fibroblasts from patients with MLD have deficient arylsulfatase A activity and we have utilized these cultures as an in vitro model for the study of this disease (*3*).

Metachromatically staining inclusions do not normally occur in cultured MLD fibroblasts; however, when sulfatides are provided in the culture medium such inclusion granules are formed. Under similar conditions normal fibroblasts ingest the sulfatides and degrade them, as evidenced by release of inorganic sulfate into the medium (*4*). We now find that the manifestations of the genetic disorder in the tissue culture model can be corrected by an exogenous source of arylsulfatase A. When MLD cells are cultured in medium supplemented with a preparation of arylsulfatase A, the enzyme is incorporated by the cells and the functional activity of the enzyme is retained over an extended period. When these enzyme-supplemented cells are exposed to sulfatides, uptake and hydrolysis patterns characteristic of normal cells are observed. Conversely, if inclusion granules are formed by prior treatment with sulfatides, subsequent "enzyme replacement" clears the inclusions.

Procedures for initiation of primary cultures from skin biopsies, culture of outgrowths, enzyme assays, preparation of ^{35}S-labeled sulfatides, and determination of [^{35}S]sulfatide uptake and degradation have been described (*3*, *4*). Cultures for the present study were derived from two patients with late infantile MLD, an obligate heterozygous carrier (a parent of one of the patients), and a normal adult. Crude human arylsulfatase A was obtained by precipitation from pooled human urine with ammonium sulfate at 70 percent saturation as described by Austin (*5*). The precipitate was suspended in water and dialyzed against water until free of sulfate at 4°C. This material was lyophilized and stored in a vacuum at −20°C. Early studies were conducted with this material. Later studies were performed with an ammonium sulfate (40 to 50 percent saturation) fraction of the lyophilized material. Preparations of arylsulfatase A were added to culture medium to provide 2 units of enzyme per milliliter (a unit of enzyme catalyzes the hydrolysis of 1 μmole of *p*-nitrocatechol sulfate per hour) and the medium was sterilized by filtration.

When MLD fibroblasts were cultured in medium containing arylsulfatase A, they incorporated the enzyme and achieved intracellular specific activity of 0.3 to 0.4 unit per milligram of protein (Fig. 1). No decrease of this activity was observed over a 6-day period. In contrast, the enzyme activity in the growth medium had a half-life of less than 24 hours and no demonstrable activity remained at day 6. A similar precipitous loss of activity was observed in growth medium incubated without cells. The confluent enzyme-containing fibroblasts were harvested by trypsinization on day 7, replated at the same density, and incubated with medium unfortified with enzyme. The specific activity of the intracellular arylsulfatase A remained essentially unchanged for four more days. On day 11 the cells were again removed by trypsinization, but this time they were replated lightly to allow replication and growth. There was gradual loss of specific activity to day 16. The loss was presumably due to dilution by cell growth.

The MLD cells cultured in medium containing an arylsulfatase A preparation that had been inactivated by prior incubation for 6 days at 37°C contained no detectable arylsulfatase A. This finding makes it unlikely that the observed intracellular activity was due to some mechanism other than incorporation of active enzyme by the MLD cells.

When MLD cells were pretreated with arylsulfatase A, trypsinized, replated, and grown in the presence of [^{35}S]sulfatide, they metabolized the exogenous sulfatide in a manner indistinguishable from that of normal or heterozygous cells. They failed to accumulate excess sulfatide and excreted significant accounts of inorganic [^{35}S]-sulfate into the medium. In contrast,