15. At the nomenclature conference, Cold Spring Harbor RNA Tumor Viruses Meeting (1987), adoption of the names "*tax*" for "transactivating gene of the *x* region" and "rex" for regulator of expression was recommended.
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17. To construct SV-HTLV-Sph I, SV-HTLV was digested with Eco RV (nt 1032) and Cla I (nt 7385), and the internal Eco RV–Cla I HTLV-II fragment was subcloned into the Pvu II and Cla I sites of pBR322. The subclone was digested with Sph I, the 4-bp overhang was removed by $S_1$ nuclease digestion (deleting the *rex* initiator methionine codon) and the fragment was religated. A 3.75-kb Xba I–Cla I fragment from the subclone was substituted for the corresponding Xba I–Cla fragment of SV-HTLV. To construct SV-HTLV-*rex*$^{term}$, a single-base substitution was introduced at the unique Cla I site of HTLV-II. SV-HTLV was digested with Cla I (nt 7385), followed by $S_1$ nuclease and Mlu I (nt 7392) digestions. The fragment was gel-purified and ligated to a linker with the sequences 5′-AGATGAA-3′, which substitutes a mutant A for the C at position 7386, introducing a stop codon specifically into *rex* coding sequences. The mutation was confirmed by Maxam-Gilbert sequencing.
18. To construct 91023-pX-b, a Nco I (nt 5179)–Sph I (nt 5123) HTLV-II fragment containing the *rex* initiator Met codon was ligated to the 5′ end of a Nco I (nt 5179)–Bam HI (nt 8550) fragment encoding p37$^{xII}$ (derived from the 91023*x*II construct (*20*) and subcloned into pUC18). The resulting subclone was digested with Eco RI (in pUC18 polylinker) and Sph I, and ligated to a 15-nt linker, 5′-AATTCCAACACCAGG-3′, in which the 5′ terminal four and 3′ terminal three nucleotides were not base-paired. This provided consensus Kozak sequences directly about the *rex* initiator Met. The final fragment was subcloned into the Eco RI site of the p91023-B expression vector, downstream from the adenovirus major late promoter. The p91023-B vector contains an SV40 origin of replication [G. G. Wong *et al.*, *Science* **228**, 810 (1985)].
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25. In separate experiments, parallel co-transfections were performed with SV-HTLV (3 μg), LTR-I-CAT (3 μg), and variable amounts of 91023-pX-b. Similar inhibition of transactivation was observed at high levels of *rex* expression.
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29. We thank L. Souza at Amgen for preparation of oligonucleotide linkers; K. Shimotohno at the National Cancer Center Research Institute, Tokyo, for antibody to the *rex* protein; M. Peterlin for the HIV constructs; N. Nameri, D. Keith, H. Koga, D. Aboulafia, E. Chin, and S. Quan for technical assistance; W. Aft for preparation of the manuscript; and J. Gasson, J. Lugo, and J. Zack for critical review of the manuscript. Supported by a grant from the California Institute for Cancer Research; National Institutes of Health grants 1-K11 CA 01314, CA 32737, and 1 R01 CA 43370; and the University-wide Task Force on AIDS award R87LA057. A.J.C. is a Leukemia Society of America Fellow.

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# Reduction of Naturally Occurring Motoneuron Death in Vivo by a Target-Derived Neurotrophic Factor

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**Treatment of chick embryos in ovo with crude and partially purified extracts from embryonic hindlimbs (days 8 to 9) during the normal cell death period (days 5 to 10) rescues a significant number of motoneurons from degeneration. The survival activity of partially purified extract was dose-dependent and developmentally regulated. The survival of sensory, sympathetic, parasympathetic, and a population of cholinergic sympathetic preganglionic neurons was unaffected by treatment with hindlimb extract. The massive motoneuron death that occurs after early target (hindlimb) removal was partially ameliorated by daily treatment with the hindlimb extract. These results indicate that a target-derived neurotrophic factor is involved in the regulation of motoneuron survival in vivo.**

EXPERIMENTS PERFORMED ALMOST 40 years ago by Hamburger and Levi-Montalcini (*1*) led to the idea that neurons within a population (for example, motoneurons or sensory neurons) are overproduced during development relative to the numbers present in the adult. Consequently, competition for a target-derived entity that is in limited supply is thought to result in the survival of only a portion of the original population. The experiments of Hamburger and Levi-Montalcini also paved the way for the discovery and characterization of nerve growth factor (NGF), the only known molecule that meets virtually all of the criteria for a target-derived neurotrophic factor that promotes the survival of developing neurons in vivo. Thus, NGF acts as a mechanism for regulating the population size of specific groups of neurons during periods of naturally occurring cell death (*2*). Although several other putative neurotrophic or survival agents (including motoneuron factors) have been isolated, characterized in vitro, and partially or completely purified (*3–5*), only one of these, brain-derived neurotrophic factor (BDNF), has been shown to regulate neuronal survival in vivo (*6*). Because putative neurotrophic factors characterized in vitro may promote neuronal survival by providing essential components that are missing or perturbed in the tissue culture environment, or by acting in a manner normally inoperative in vivo (*7*), such factors must be shown to be capable of affecting neuronal survival in vivo. In the chick embryo, 50% or more of the somatic motoneurons innervating skeletal muscle, at limb as well as nonlimb regions, degenerate between embryonic day (E) 5.5 and E12 (*8*). We found that hindlimb target tissues contain a putative neurotrophic factor that prevents the death of substantial numbers of motoneurons in vivo, in a manner consistent with its suspected role as a normal motoneuron survival factor.

Crude hindlimb or control tissue extracts were prepared from embryos at E8 to E9 when the hindlimb musculature is composed primarily, if not entirely, of primary myotubes and when normal motoneuron death is still occurring (*9*). Extracts were administered daily through a small window in the shell onto the vascularized chorioallantoic membrane beginning either on E5 or on E6. Control embryos received equal volumes of a physiological saline solution. Because motoneuron survival depends on neuromuscular activity (*10*), we also monitored embryonic motility to determine whether these extracts alter neuromuscular activity. In all cases, significantly more motoneurons survived in the lumbar spinal cord of embryos treated with crude hindlimb extracts than in controls (Fig. 1). Kidney, lung, and liver extracts were ineffective in rescuing motoneurons. Heat inactivation (60°C for 45 minutes) of crude hindlimb extracts eliminated the motoneuron survival activity. Although the amount of motoneuron survival varied between experiments, treatment with hindlimb extracts always resulted in a significant increase in motoneuron numbers. Before cell death, approxi-

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mately 23,000 to 24,000 motoneurons innervated a single hindlimb. However, we could never maintain more than 18,000 of these by extract treatment. This could be because the doses were not high enough or because motoneuron survival may also depend on nonlimb-derived neurotrophic factors (*5, 11*) or afferent input (*12*). The morphology of the spinal cord and motoneurons appeared normal after treatment, and treated embryos displayed normal amounts of motility, indicating that increased motoneuron survival is not secondary to altered neuromuscular activity (*13*).

Ammonium sulfate ($AmSO_4$) fractionation of the crude extract indicated that the 25 to 75% $AmSO_4$ fraction contained the most activity, as well as a higher specific activity than the crude extract (Table 1). The survival effects mediated by this fraction were dose-related over the range of doses examined here (Table 1). Doses in excess of 250 μl were generally lethal to the embryo after only one or two injections.

**Table 1.** The number (mean ± SEM) of lumbar motoneurons in control (saline), crude muscle extract (CMX), and three $AmSO_4$ fraction groups on E8 to E9 (experiment A), and on E10 after treatment with 2.5, 25, or 250 μl of the 25 to 75% $AmSO_4$ CMX fraction (experiment B). Embryos were treated daily beginning on either E5 (experiment A) or E6 (experiment B). $AmSO_4$ fractionation was performed by dropwise addition of saturated $AmSO_4$ solution to achieve 25% and then 75% saturations. The mixtures were stirred for 2 hours, and precipitated protein was recovered by centrifugation for 30 minutes at 15,000*g*. Complete saturation was achieved by addition of $AmSO_4$ salt to the 75% $AmSO_4$ supernatant, equilibration for 18 hours, and centrifugation. The 0 to 25% $AmSO_4$ and 75 to 100% $AmSO_4$ fractions were resuspended in 0.9% NaCl, to final volumes equal to 25% of the original volume (1.5 to 2.0 mg of protein per milliliter), dialyzed, and frozen. The 25 to 75% $AmSO_4$ fraction was either resuspended in 0.9% saline to 50% of the original volume and applied to further purification steps, or was dialyzed, diluted to 3.0 to 3.5 mg of protein per milliliter, and frozen.

| Treatment | *n* | Motoneurons |
|---|---|---|
| *Experiment A (AmSO₄ fractions)* | | |
| Saline (E8 to E9) | 9 | 13,600 ± 237 |
| CMX | 8 | 16,120 ± 226* |
| $AmSO_4$ | | |
| 0 to 25% | 6 | 14,827 ± 353† |
| 25 to 75% | 6 | 17,364 ± 185‡ |
| 75 to 100% | 5 | 11,070 ± 168† |
| *Experiment B (dose response)* | | |
| Saline (E10) | 5 | 13,387 ± 221 |
| CMX | | |
| 2.5 μl | 6 | 13,017 ± 255 |
| 25 μl | 7 | 16,108 ± 309‖ |
| 250 μl | 8 | 17,650 ± 462‖¶ |

*$P < 0.002$ compared to saline. †$P < 0.05$ compared to saline. ‡$P < 0.02$ compared to saline. §$P < 0.05$ compared to CMX. ‖$P < 0.005$ compared to saline. ¶$P < 0.05$ compared to 25 μl of CMX (*t* tests with Bonferroni correction).

The partially purified hindlimb extract acts only on a subpopulation of spinal cord neurons. Neurons in the dorsal root ganglia, sympathetic ganglia, parasympathetic (ciliary) ganglia, and sympathetic preganglionic column were unaffected by the hindlimb extract (Fig. 2). By contrast, NGF treatment altered the survival of some of these neuronal populations but had no effect on motoneuron survival. The hindlimb extract can rescue motoneurons throughout the lumbar spinal cord (Fig. 3) as well as motoneurons in the thoracic (nonlimb) region (*14*). The increase in number of motoneurons after extract treatment is associated with a significant decrease in degenerating motoneurons (*15, 16*). Thus, the partially purified hindlimb extract appears to be acting by maintaining or promoting the survival of motoneurons that would otherwise die. Partially purified hindlimb extract from E16 embryos was ineffective in promoting motoneuron survival or in reducing the number of degenerating motoneurons (*17*).

We next determined whether treatment with partially purified hindlimb extract could rescue motoneurons destined to die because of early target removal. Limb-bud removals were performed on E2, and treatment was begun on E4. Although embryos lacking limb buds treated with extract still showed increased cell loss, the extent of the loss was decreased (Table 2). The failure to rescue more of the target-deprived cells could be due to (i) insufficient dose of extract, (ii) relatively inefficient delivery of the factor (that is, trophic factors may be

**Table 2.** The number (mean ± SEM) of surviving lumbar motoneurons on E6 to E6.5 and E8 to E9 on the side of the spinal cord ipsilateral (operated) and contralateral (control) to hindlimb removal after daily treatment with saline or partially purified extract (CMX) beginning on E4 (experiment A); and the number (mean ± SEM) of surviving lumbar motoneurons on E9 to E10 in embryos treated daily from E6 with saline, 25 to 75% $AmSO_4$, or three molecular size fractions (experiment B). Hindlimb bud removals were carried out on E2.5 (*18*). Only embryos lacking all hindlimb musculature were retained for analysis. For molecular size characterization, the 25 to 75% $AmSO_4$ fraction was applied in aliquots of 15 ml to a 2.6 × 100 cm column of Sephadex G-100, equilibrated in 10 m*M* phosphate and 50 m*M* NaCl, *p*H 6.8. Fractions were combined to estimated molecular size ranges of >75 kD, 30 to 75 kD, and <30 kD, based on column calibration with blue dextran, bovine serum albumin, ovalbumin, chymotrypsinogen, and ribonuclease A. Pooled fractions were each concentrated by lyophilization to the originally applied volumes, dialyzed, and frozen.

| Treatment | *n* | Motoneurons |
|---|---|---|
| *Experiment A* | | |
| Control (E6 to E6.5) | | |
| CMX | 8 | 20,018 ± 963 |
| Saline | 11 | 21,145 ± 957 |
| Operated (E6 to E6.5) | | |
| CMX | 8 | 15,682 ± 1,088*‡ |
| Saline | 11 | 10,932 ± 1,321‡ |
| Control (E8 to E9) | | |
| CMX | 5 | 18,109 ± 857* |
| Saline | 10 | 14,719 ± 494 |
| Operated (E8 to E9) | | |
| CMX | 5 | 5,176 ± 233†‡ |
| Saline | 10 | 3,015 ± 212‡ |
| *Experiment B* | | |
| Saline (E9 to E10) | 12 | 13,587 ± 309 |
| $AmSO_4$ (25 to 75%) | 5 | 16,346 ± 584§ |
| <30 kD | 8 | 16,297 ± 571¶ |
| 30 to 75 kD | 4 | 13,751 ± 612 |
| >75 kD | 4 | 12,222 ± 680 |

*$P < 0.01$ compared to saline. †$P < 0.05$ compared to saline. ‡$P < 0.001$ compared to control. §$P < 0.002$ compared to saline. ¶$P < 0.0004$ compared to saline (where appropriate, *t* tests with Bonferroni correction).

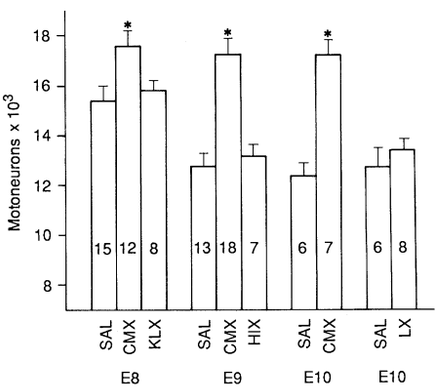


**Fig. 1.** The number (mean ± SEM) of surviving lumbar motoneurons on E8, E9, and E10 after daily treatment from E5 or E6 with 250 μl of saline (SAL), chick muscle extract (CMX), adult kidney and lung extract (KLX), embryonic liver extract (LX), or heat-inactivated muscle extract (HIX). Numbers in bars are the sample sizes. Embryos were killed, staged (*22*), processed, and motoneurons counted (*15*). Cell counts were made blind as to treatment (control versus experimental) in this and in all other experiments reported here. The CMX, unless otherwise indicated, was prepared from the legs of E8 to E9 white leghorns. Lung and kidney extracts were prepared from adult chickens. Liver extracts were from E8 to E9 white leghorns. The washed tissue was homogenized in two volumes of phosphate-buffered saline (137 m*M* NaCl, 2.7 m*M* KCl, 8.1 m*M* $Na_2HPO_4$, and 1.5 m*M* $KH_2PO_4$, *p*H 7.4) containing 1 m*M* EDTA, 1 m*M* EGTA, 0.1 m*M* phenylmethylsulfonyl fluoride, 1 m*M* *N*-ethyl maleimide, and 1 m*M* benzamidine, and centrifuged at 23,000*g* for 1 hour. The resulting supernatant was either applied to further purification steps or dialyzed. As the final step before use, all applied extracts were dialyzed through 1000 MWCO Spectropor 1 membranes for 36 to 48 hours, against two changes of 0.9% NaCl. After dialysis, samples were centrifuged for 15 minutes at 1000*g*, and any pellet that formed was discarded. Protein concentrations were determined with bicinchoninic acid (*23*) or the flourescamine method (*24*). All dilutions were made by addition of 0.9% NaCl to attain 3 to 3.5 mg of protein per milliliter. The *p*H of applied solutions was adjusted to 7.4. All procedures were carried out at 4°C, unless otherwise noted, and all fractions were stored at −70°C. *$P < 0.01$, *t* test.

most effective after terminal uptake; those neurons that would have innervated the missing limb lack terminals), or (iii) another mechanism that may operate after limb removal (*18*, *19*).

We used gel filtration chromatography to estimate the molecular size range of the active factor contained in the 25 to 75% $AmSO_4$ fraction. Virtually all of the survival activity can be recovered in a fraction composed of agents <30,000 daltons (Table 2). The amount of survival activity in this fraction is roughly equivalent to that contained in the 25 to 75% $AmSO_4$ starting material.

The fact that the motoneuron survival activity in crude hindlimb extracts is heatlabile (Fig. 1) suggests that the active factor may be a protein. Further support for this idea comes from experiments in which the in vivo survival activity in the 25 to 75% $AmSO_4$ fraction was shown to be trypsin-sensitive.

Our putative factor does not appear to be a previously identified neurotrophic factor, such as NGF, BDNF, ciliary neurotrophic factor, fibroblast growth factor, or other nontarget tissue–derived factors. The putative motoneuron survival factor described here is also unlikely to be the putative neurotrophic factor neuroleukin (*20*), which promotes the in vitro survival of an unidentified population of spinal cord neurons as well as the survival of a subpopulation of spinal sensory ganglion neurons.

In accord with our results, Dohrmann *et al.* (*3*) reported that maximum motoneuron survival activity in vitro is retained in a 25 to 75% $AmSO_4$ fraction of muscle extract. In vivo studies of motoneurons and other neuronal types indicate that, in addition to target-derived factors, afferent or central nervous system (CNS)–derived influences are important for neuronal survival (*12*). In vitro studies also indicate that nontarget, CNS-derived factors are important for motoneuron survival (*5, 11*). Finally, both in vivo and in vitro studies suggest that muscle-derived factors affecting motoneuron survival are influenced by activity or innervation (*10*, *21*). Thus, results from both in vitro and in vivo approaches agree on several essential points regarding the presence of a target-derived survival factor involved in the regulation of motoneuron numbers.

It is conceivable that the factor we have identified is acting directly on the muscle by increasing myotube numbers, size, or type, all of which are characteristics that could conceivably indirectly influence motoneuron survival (*9*). However, preliminary studies of the medial adductor muscle from E9 to E10 extract-treated embryos indicate that this is unlikely to be the case.

We have identified a putative neurotrophic factor derived from normal target tissue that acts in vivo to rescue motoneurons in a manner consistent with its proposed role in the regulation of naturally occurring neuronal death in the developing chick embryo. Thus an NGF-insensitive population of neurons may also depend on target-derived neurotrophic factors for their normal development and survival in vivo.

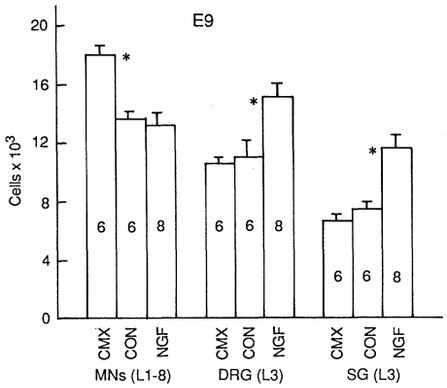


**Fig. 2.** The number (mean ± SEM) of surviving lumbar motoneurons (MNs) in segments L1–8, dorsal root ganglion cells (DRG), and sympathetic ganglion cells (SG) in L3 on E9 after treatment with CMX, saline (control, CON), or NGF (20 μg per day). The numbers in the bars are sample sizes. DRG and SG cells were counted in every fifth section (*25*). *$P < 0.01$, $t$ test with Bonferroni correction. Cholinergic sympathetic preganglionic neurons were also counted in thoracic segments 5 and 6 (T5 to T6). Controls had 4610 ± 435, and the CMX group had 4663 ± 484. This difference was not statistically significant. Ciliary ganglion neurons were counted (*12*) in a separate group of E14 embryos that were treated daily (E9 to E13) with 250 μl of the 25 to 75% $AmSO_4$ fraction from E9 hindlimb extract. Controls had 2947 ± 221 ($n = 6$), and the CMX group had 2917 ± 333 ($n = 6$). This difference was not statistically significant.

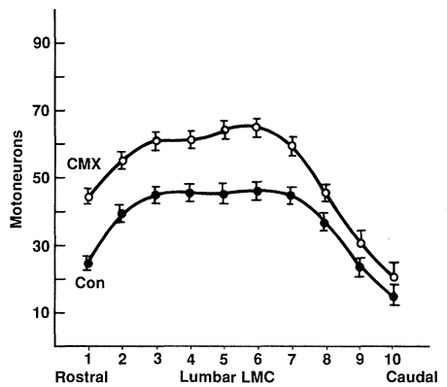


**Fig. 3.** The number (mean ± SEM) of surviving motoneurons in the lumar lateral motor column (LMC) along the rostral-caudal axis on E9. The eight lumbar segments were divided into ten equal lengths. Embryos were treated daily with partially purified CMX (250 μl of 25 to 75% $AmSO_4$ fraction) beginning on E6. $P < 0.01$ between CMX and control for all rostral-caudal points except 9 and 10 ($t$ tests).


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13. All somatic motility of selected embryos was recorded daily for 3 to 5 minutes [J. Reitzel, J. Maderdrut, R. W. Oppenheim, *Brain Res.* **172**, 487 (1979)]. Embryos treated with crude or partially purified extract (25 to 75% $AmSO_4$ fraction) exhibited 8.7 ± 2.1 ($n = 12$) movements per minute on E8; controls were 9.2 ± 2.6 ($n = 15$). On E10, extract-treated embryos exhibited 14.0 ± 3.3 ($n = 6$) movements per minute; controls were 15.6 ± 2.4 ($n = 16$). These differences are not statistically significant. By contrast, embryos treated daily (E6 to E9) with 2.0 mg of curare, which also rescues motoneurons from death (*10*), have 2.2 ± 1.4 ($n = 5$) movements per minute on E8 and 2.6 ± 1.7 ($n = 6$) on E10, both of which are significantly different from age-matched control values ($P < 0.01$, $t$ test).
14. Motoneurons were counted on E9 in every tenth section throughout thoracic segments 5 to 6. Ex-

tract-treated embryos had 3571 ± 456 ($n$ = 5); controls had 2567 ± 380 ($n$ = 6) ($P < 0.01$, $t$ test).
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16. E8 embryos treated with partially purified hindlimb extract (25 to 75% $AmSO_4$ fraction) beginning on E5 had 7.2 ± 1.3 ($n$ = 5) pyknotic motoneuron profiles per 1000 healthy neurons in the eight lumbar segments; controls had 18.0 ± 2.3 ($n$ = 5) ($P < 0.001$, $t$ test). Pyknotic motoneurons were identified by established criteria (*15*). Care was used to exclude degenerating glia and mitotic figures.
17. Embryos were treated daily beginning on E6 with partially purified hindlimb extract (25 to 75% $AmSO_4$ fraction, 3 mg of protein per milliliter) prepared from either E9 or E16 embryos. Motoneuron counts (mean ± SEM) on E10 were 12,256 ± 231 ($n$ = 10) for controls and 15,303 ± 256 ($n$ = 10) for E9 extract-treated, and 10,500 ± 187 ($n$ = 6) for E16 extract-treated embryos. The E16 group was significantly different from both control ($P < 0.05$, $t$ test) and E9 extract-treated ($P < 0.01$) groups. Control and E9 extract-treated groups were also significantly different ($P < 0.01$). Embryos treated with E9 extract had significantly fewer pyknotic motoneurons per 1000 healthy motoneurons on E10 than either the embryos treated with E16 extract or control embryos. Means ± SEM were 1.3 ± 0.5 for E9 extract, 3.3 ± 1.1 for E16 extract, and 3.5 ± 0.9 for controls ($P < 0.01$, $t$ test).
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# Theoretical Studies of DNA During Gel Electrophoresis

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**A numerical study of the motion of a long-chain macromolecule in a gel has shown unexpected features. The application of a field appears to induce the chain to contract on itself. This is followed by its "unwinding" into an extended configuration. For long chains, the mobility tends toward a constant, in accord with experiments. For the parameter range used, the observed molecular motion differs strongly from assumptions made in the present theory of electrophoresis.**

GEL ELECTROPHORESIS IS A WIDELY used technique for separating macromolecules according to size. There have been many substantive improvements in this technique recently (*1–3*) that allow the separation of much larger DNA molecules than was previously possible. Still, the basic underlying motion of the molecules has remained somewhat of a mystery.

The most successful theory of electrophoresis has made use of the concept of a "tube" (*4*) through which the chain passes. The resultant idealized motion, called "reptation," has been successfully applied to situations involving diffusion where no external electric field is present. Several groups (*5*) have extended this theory to the case where a uniform external field is applied. The extension is not entirely straightforward, and certain statistics for the tube must be postulated (*5*). It is further assumed that the overall tube length fluctuates little.

Experimental data for the constant field case (*6*) supports tube theories. On the other hand, a more recent technique of electrophoretic separation that periodically inverts the direction of the electric field (*2*) leads to very different mobilities than for the constant field case. This is not predicted by tube models, which give the same dependence of mobility versus chain length as in the constant field case.

There are qualitative differences between the predictions of the tube models and the results described here, which were obtained by direct numerical solution of equations describing the motion of the chain. These equations (defined below) contain far fewer assumptions than present tube theories, although various simplifications have still been made. Namely, intrachain repulsion and effects of gel heterogeneity have not been included. A uniform distribution of charge along the chain has also been assumed. To critically compare tube theories with the numerical results, it is only practical to start with a more basic but still rather idealized model.

Consider Fig. 1A. Here one sees a chain made up of $N$ beads on freely hinged links moving in a two-dimensional lattice of obstacles that represents the gel. There is a short-range repulsive force $\mathbf{F}_i$, between each obstacle and all beads on the chain. This force completely prohibits the chain crossing an obstacle. The effects of temperature are represented by a Gaussian random force that acts on each bead independently. More precisely, the following Langevin equation was solved numerically (*7*):

$$\nu \frac{\partial \mathbf{r}_i}{\partial_t} = \mathbf{T}_{i,i+1} - \mathbf{T}_{i,i-1} + q\mathbf{E} + \mathbf{F}_i + \mathbf{f}_i$$

Here $\nu$ is the friction coefficient between the $i$th bead and the solvent, $\mathbf{r}_i$ is the vector coordinate, $t$ is time, $\mathbf{T}_{i,j}$ is the tension between adjacent beads i and j, $q$ is the charge on a bead, $\mathbf{E}$ is the electric field, and $\mathbf{f}_i$ is the random force acting on bead i. The tensions are determined by the requirement that the distance between two adjacent beads remain constant and equal to $l$, that is, $(\mathbf{r}_{i+1} - \mathbf{r}_i)^2 = l^2$.

The case of experimental interest is where the pore size (that is, the lattice spacing) is

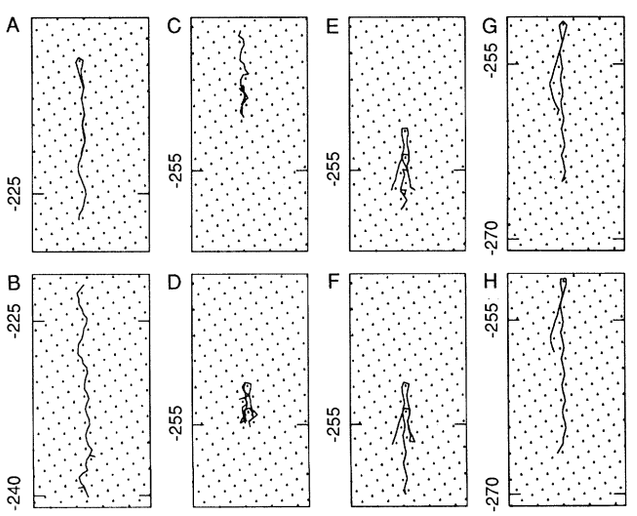


**Fig. 1.** The evolution of a DNA molecule in a gel. This sequence shows the different stages described in the text. The parameters in this run were $qEl/k_BT$ = 42.67. The relative times for each frame are (**A**) 100, (**B**) 105, (**C**) 110, (**D**) 112, (**E**) 114, (**F**) 116, (**G**) 118, and (**H**) 119. The direction of the electric field is vertically downward.

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