shared environment to these familial factors and the possible mechanisms of inheritance (single gene or polygenic) will have to be determined by family and twin studies. Familial factors might alter serum DBH activity in many ways, including changes in the quantity of releasable DBH, the rate of release, the access of DBH to the circulation, or the clearance of the enzyme from the blood. The data also suggest the possible existence of a separate subgroup of normal subjects with very low serum activity. The fact that this group includes approximately the same percentage of the adult population and of a population of children older than 6 years of age makes it less likely that the existence of such a subgroup among children represents only delayed maturation of the sympathetic nervous system. All of these data raise the possibility of significant familial differences in catecholamine biosynthesis in man.

The results of this study are also important in the interpretation of both previous and future clinical studies of serum DBH activity. The wide range of normal human serum DBH values, the previous demonstration that changes in these values in response to stress are relatively small (*5*), and the finding that familial factors significantly influence baseline levels of circulating enzyme activity all make it less likely that an isolated determination of serum DBH activity will be useful in establishing the level of sympathetic nervous system function in a particular individual. However, serial determinations of serum DBH activity in an individual or the comparison of single determinations in members of one population with determinations performed on blood from an appropriate control population might be useful clinically. The finding of very low serum DBH activity in blood from normal adults and children suggests that previous reports of very low enzyme activity in patients with diseases such as familial dysautonomia and Down's syndrome (*7*) must be interpreted with caution.

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12. The percentages of recovery of exogenously added purified bovine adrenal DBH activity from serum samples containing very low, low normal, and high normal activity, respectively, were 105 ± 5 percent ($N = 6$), 107 ± 4 percent ($N = 5$), and 108 ± 6 percent ($N = 6$).
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Isolation of a Neurotropic Type C Virus

Abstract. *A neurogenic paralysis of the lower limb can be induced and serially transmitted in mice by a nontransforming type C virus strain that originated in an embryo of a wild mouse. The virus exerted a neurotropic effect on the anterior horn neurons.*

Stansly (*1*) encountered in BALB/c mice a lower limb paralytic disease associated with virus-induced reticular neoplasms and suggested that the paralytic agent and neoplastic virus could be the same. Apart from this report (*1*), pathogenic effects in vivo, other than cancer and possibly an autoimmune type kidney disease (*2*), have not been causatively associated with type C virus activity in mice or any other vertebrate species.

We isolated a type C virus (1504E) from the embryo culture of a wild mouse (*Mus musculus*) (*3*). Inoculation in vivo of purified concentrated 1504E virus from cells passaged in tissue culture into newborn wild mice and NIH Swiss laboratory mice led, after many months, to several instances of lymphoma, as might be expected. More surprising was the apparent induction of a neurogenic type of lower limb paralysis in many of the recipient wild and Swiss mice. We have described (*4*) a natural population of lymphoma-prone wild mice in which an identical lower limb paralysis, also experimentally transmissible, occurs spontaneously in association with elevated activity of indigenous type C virus (*5*).

The 1504E embryo culture was established 30 months ago from a single, whole, wild mouse embryo of about 18 to 19 days' gestation. The mother mouse was extensively infected with type C virus, as was evidenced by the recovery in vitro of infectious virus from many extracts of various organs, including uterus (*3*). Type C virus, which appeared spontaneously in two separate laboratories at the second subpassage of the 1504E embryo cell cultures, fulfilled necessary morphologic, immunologic, and biologic criteria for characterization as a nontransforming murine type C virus (*3*). It was infectious for NIH Swiss embryo cells in vitro but differed from the established strains of murine type C virus in that it produced unusually small syncytia in the XC plaque test (*6*) and was not neutralized by antiserums to Gross passage A, AKR, or FMR viruses. The 1504E culture, now in its 57th passage, is free of pleuropneumonia-like organisms and grows well with a doubling time of 3 to 4 days. The growth reaches saturation at a density of 7.5×10^5 cell/cm^2, and the cells continue to produce a type C virus without any diminution in titer. The cells were diploid in early culture but in later passage became heteroploid and showed the telo-

Table 1. In vivo transmission of 1504E cells and virus. Abbreviations: S.C., subcutaneous; I.P., intraperitoneal; I.V., intravenous; and I.C., intracerebral.

Inoculum	Recipient	Age	Route	Paralysis	Lymphoma	Latent period (months)
		P1				
Cultured cells shedding virus *	Wild mice	Newborn	S.C.	5/12 †	1/10	10–12
Virus from carrier cell culture ‡	Wild mice	Newborn	S.C. + I.P.	9/18	1/18 §	9–11
Virus from carrier cell culture ||	NIH Swiss	Newborn	I.V. + I.C.	21/21	1/21	2–5
		P2				
Virus from brain of paralyzed NIH Swiss mouse grown in NIH embryo cells ¶	NIH Swiss	4 days	I.C.	10/14	0/14	3–5
	NIH Swiss	3 weeks	I.C.	0/13	0/10	3–5

* 10^5 cells from P15 and P24 in 0.1 ml subcutaneously. † Number positive per number of surviving mice. ‡ Culture fluids (banded 1.16), nonfiltered, 100- to 300-fold concentrated, P17 to 40; 0.01 ml inoculated both subcutaneously and intraperitoneally. § This lymphomatous mouse was also paralyzed. || Culture fluids (banded 1.16), filtered (0.45 μm), 25-fold concentrated, P47; 0.1 ml inoculated intravenously and 0.03 ml inoculated intracerebrally. ¶ Culture fluids (banded 1.16), filtered (0.45 μm), 50-fold concentrated, P1; 0.03 ml inoculated intracerebrally.

centric chromosomes characteristic of the mouse. Mouse antibody production tests (*7*) of the banded virus have confirmed the lack of other indigenous murine virus contaminants.

Cultures were maintained as monolayers in Falcon flasks, fed with Earle's minimum essential medium containing inactivated calf serum (5 percent) and incubated at 37°C in a CO_2 incubator. The cultures were subdivided twice weekly or when confluent. The culture fluids with virus were collected at 24- to 48-hour intervals and stored at 4°C until 500 to 1000 ml had been collected. The virus was then concentrated by the polyethylene glycol (6 percent) precipitation method with the sodium chloride being adjusted to 2.2 percent (*8*). After storage overnight at 4°C the virus-containing precipitate was centrifuged at 2000 rev/min for 15 minutes at 4°C (Sorvall). The sedimented virus, resuspended in TNE buffer (0.01*M* tris, 0.1*M* NaCl, 0.01*M* EDTA), was overlaid on a sucrose gradient (15 to 60 percent) and centrifuged in the SW27 rotor for 4 hours at 24,000 rev/min. The band having a density of 1.16 was resuspended in minimum essential medium buffered with tris (*p*H 7.4 to 7.6) to the desired concentration (25- to 300-fold) and filtered through a Millipore filter (0.45 μm). Freshly banded virus was usually used immediately for animal inoculation; however, some virus preparations were stored at 4°C until inoculation. The newborn wild mice were obtained from our laboratory-adapted breeding colonies, which were initially derived from wild mice trapped in Los Angeles County and in Maryland.

Results of the transmission in vivo of 1504E cell and virus preparations are shown in Table 1. When 10^5 cultured cells were inoculated subcutaneously into newborn wild mice, 5 of 12 came down with lower limb paralysis in 10 to 12 months, and one of the surviving mice without paralysis developed lymphoma after 11 months. When purified, concentrated banded virus was inoculated subcutaneously and intraperitoneally into newborn wild mice, 9 of 18 developed paralysis after 9 to 11 months. One of these paralyzed mice also had lymphoma. Intravenous and intracerebral inoculation of concentrated banded virus into newborn NIH Swiss mice led to paralysis without lymphoma in all 21 surviving mice after 2 to 5 months. One of the paralyzed Swiss mice which survived 5 months also had lymphoma.

Type C virus, which has a titer of 2×10^3 XC plaque-forming units (PFU) (*6*) in NIH Swiss embryo cells, was isolated from the brain extracts of one of the paralyzed Swiss mice. Concentrated banded virus from the infected NIH Swiss culture fluids was inoculated intracerebrally into 4-day-old NIH Swiss mice and led to lower limb paralysis in 10 of 14 surviving mice after 3 to 5 months. The same concentrated virus preparation has not given rise to paralysis or lymphoma in 13 NIH Swiss mice inoculated intracerebrally at 3 weeks of age and followed for 3 to 5 months.

We looked for type C virus expression in the form of complement-fixing (CF) group-specific (gs) antigen in several tissues from experimentally paralyzed wild mice and NIH Swiss mice (Table 2). In the paralyzed wild mice, CF gs antigen was detected (titer 1 : 4 or greater) in each of the 4 skeletal muscle and 2 spinal cord extracts tested and in 6 of 13 brain extracts. In uninoculated wild mice the brain, spinal cord, and muscle were always free of detectable CF antigen. The prevalence (11 of 15) and titer (1 : 4 or greater) of CF antigen in the spleens of experimentally paralyzed wild mice was significantly greater than the prevalence (6 of 30) and titer (1 : 2) of CF antigen found in spleens of uninoculated control wild mice.

In the two generations of paralyzed NIH Swiss (P1 and P2) almost all of the skeletal muscle (20 of 21), brain (22 of 28), spinal cord (6 of 6), and spleen (25 of 26) extracts were positive for CF antigen at a titer of 1 : 4 or greater. No CF antigen was detected in the brain or spinal cord of nine clinically normal NIH Swiss mice inoculated intracerebrally with concentrated virus at 3 weeks of age. In the uninoculated age-matched NIH Swiss controls, the brain, spinal cord, and skeletal muscle were entirely negative for CF antigen and

Table 2. Type C virus CF gs antigen in experimentally paralyzed and control mice. Tissue extracts (20 percent) were tested with MSV 30 pooled rat serums. Representative positive reactions were confirmed in CF with MuLV gs-1 guinea pig serums. The wild mice controls were young adults from several trapping areas other than that in which mice develop the naturally occurring paralysis. The results are given as number of mice positive at 1 : 2 dilution or greater per number tested. The CF titers were generally higher (≥ 1 : 4) in the spleens of paralyzed wild and Swiss mice than in the spleens of their nonparalyzed controls (1 : 2).

Tissue	Paralyzed wild mice	Wild mice controls	Paralyzed NIH Swiss mice		NIH Swiss controls
			P1	P2	
Spleen	11/15	6/30	20/21	5/5	10/12
Skeletal muscle	4/4	0/22	20/21		0/22
Brain	6/13	0/50	19/22	3/6	0/12
Spinal cord	2/2	0/50		6/6	0/12

the positive spleen titers were seldom greater than 1 : 2.

We have further evidence that type C virus was responsible for these positive CF reactions. We found numerous type C virus particles by electron microscopy in the spinal cord, spleen, and skeletal musculature of one experimentally paralyzed wild mouse and in the spinal cord or skeletal musculature of four experimentally paralyzed (P1) NIH Swiss mice. We demonstrated by the XC plaque test (*6*) that type C virus from the brain, spinal cord, or skeletal musculature of two paralyzed wild mice, and from the brain, spinal cord, or spleen of seven paralyzed (P1 and P2) NIH Swiss mice was infectious for NIH Swiss embryo cells. Infectious virus could not be demonstrated by the XC plaque test in the brain or cord extracts of five NIH Swiss mice, 7 weeks old, that had been inoculated intracerebrally at age 3 weeks or in ten uninoculated NIH Swiss mice which were 6 to 8 weeks old.

The main neuropathologic findings were gliosis, a nonspecific vacuolar neuronal degeneration, spongiosis (edema), and neurogenic atrophy of the hind leg musculature. These changes were especially marked in the anterior horns and adjacent white matter of the lower spinal cord. Although spongiosis and slight perivascular lymphoid cuffings were occasionally noted in the brainstem, inflammatory infiltrates, neuronophagia, and necrosis were conspicuously absent throughout the central nervous system. No obvious demyelination was noted in the brain, cord, or sciatic nerves.

Numerous type C virus particles were seen by electron microscopy in the lower spinal cord, budding from plasma membranes, and free in the extracellular space. An unusual feature was the finding of intracellular type C particles, sometimes in great number, replicating within the rough endoplasmic reticulum of anterior horn motor neurons. These intracellular replicating particles showed an aberrant cylindrical budding configuration. Virus particles were not identified within axons. In the sciatic nerves a few virus particles were noted in juxtaposition to the surface of normal appearing Schwann cells, but were not replicating at this site. Numerous type C particles were also found in the interstitium of the musculature of the paralyzed hind limb but virus was not seen deep within muscle cells and there was no evidence of virus replication or accompanying fiber degeneration. The same findings characterize the naturally paralyzed wild mice (*4*).

We have found that young adult wild mice obtained from the same trapping area as was the 1504E mouse embryo show a high incidence (about 70 percent) of gs antigen in the spleen detectable by CF. We observed an identical type of spontaneous lower limb paralysis in a wild mouse from this colony and we have now transmitted this lower limb paralysis to newborn NIH Swiss mice. The paralysis was observed 4 to 5 months after the newborn NIH Swiss mice were inoculated intracerebrally with brain and cord extracts from this paralyzed mouse. Thus, this colony of wild mice is similar to the colony of wild mice which was previously reported to be prone to lymphoma (*4*, *5*). Both are "switched-on" for type C virus activity and have the tendency to develop spontaneous lower limb paralysis.

The pathogenesis of the experimentally induced and naturally occurring neurologic disorders requires further definition. Based upon the morphologic, fine structural, and virologic findings, it appears that, although the type C virus is widespread in tissues of these paralyzed mice, it exerts its damaging influence primarily by a direct neurotropic effect on the anterior horn neurons of the lower spinal cord. The extensive replication of type C virus particles within the endoplasmic reticulum of some of these neurons, an atypical site for type C virus replication, could lead to disturbed neuronal function and eventual cell death.

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Growth Retardation in Young Mice Treated with dl-Methadone

Abstract. *Newborn mice injected daily for 6 weeks with* dl-*methadone in dosages of 2 to 8 milligrams per kilogram grew significantly more slowly than their saline-treated littermates. Litters given* d-*methadone, 4 milligrams per kilogram, grew normally. Concomitant treatment with naloxone, 10 milligrams per kilogram, prevented growth inhibition. A weight deficit persisted in mice observed 6 weeks after cessation of methadone treatment.*

Infants born of heroin-addicted mothers often are of low birth weight despite approximately full-term gestation (*1*). The cause for this immaturity is unknown.

Morphine and the pharmacologically related opioids can block the release of acetylcholine (ACh) from stimulated nervous tissues (*2*). Because of this property we wondered whether opioids such as methadone or levorphanol would affect limb regeneration rates and taste bud morphology in the salamander as did the inhibitors of ACh biosynthesis, hemicholinium or triethylcholine (*3*). We found virtually no limb growth occurring at the amputation site despite formation of a thick epidermal cap (*4*). Blastemal development which normally precedes differentiation appeared blocked for the entire 10-week treatment period. Opioid treatment also caused moderate taste bud atrophy. When daily treatment with the opioid was discontinued, growth rates rapidly increased and 5 weeks