tivity was shown to be due to a small amount of contaminating DNA in the FeLV 70*S* RNA preparation. When this DNA was removed by Cs_2SO_4 density-gradient centrifugation, no activity of NHL polymerases with the 70*S* RNA was observed.

In order to show that the failure of cellular DNA polymerases to utilize a 70*S* RNA template was not due to an inhibitor in the purified enzyme preparations, we assayed each of the cellular DNA polymerases in combination with the AMV polymerase. No inhibition of AMV polymerase activity with 70*S* RNA was observed, nor was a decrease in stimulation by oligo$(dT)_{10}$ seen. In fact, when the AMV enzyme was mixed with the cellular enzymes, slightly greater activity was detected. This small stimulation perhaps reflects increased stability of the AMV polymerase during the course of the reaction due to the presence of additional protein. The 70*S* RNA was used in these mixing experiments because of its great sensitivity to ribonuclease. Deoxyribonucleases were not a concern because the enzymes all accept natural DNA or poly[d(A-T)] as template (data not shown).

These experiments confirm and extend other reports that viral and cellular DNA polymerases can be distinguished by their activity with certain oligomer-homopolymer templates, and by the ability of viral reverse transcriptases, but not the cellular DNA polymerases, to react with purified, single-stranded 70*S* RNA templates (*6*). Based on these criteria, the two purified NHL DNA polymerases did not resemble viral reverse transcriptase, but reacted in a manner similar to that of *E. coli* DNA polymerase I.

That poly(rA) • poly(dT) is a nonspecific template which is equally effective with viral and cellular DNA polymerases must be emphasized. Other synthetic templates, as poly(rA) • oligo$(dT)_{12-18}$ and poly(dA)•oligo$(dT)_{12-18}$, used in combination, can provide a sensitive indicator for viral reverse transcriptase activity. However, even these templates only serve as effective indicators when purified DNA polymerases are assayed in the presence of magnesium. For example, at least one cellular DNA polymerase will accept poly(rA) • oligo$(dT)_{12-18}$ more effectively than poly(dA) • oligo$(dT)_{12-18}$ in the presence of manganese, though there is no indication that it is a true reverse transcriptase (*18*). Purified 70*S* RNA, free of contaminating DNA, is a less sensitive, although a biologically more important indicator of reverse transcriptase activity. Acceptable evidence that a DNA polymerase resembles or is identical to a viral reverse transcriptase can be achieved when the product of a DNA polymerase reaction templated by 70*S* RNA is shown to specifically hybridize to the RNA template (*6*). All of these distinguishing criteria will enable more critical determinations to be made as to whether a viral-like reverse transcriptase is associated with neoplastic disease. The RNA-dependent DNA polymerase from human acute leukemic cells satisfies all these criteria for a reverse transcriptase (*15, 19*).

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Cribriform Degeneration (cri): A New Recessive Neurological Mutation in the Mouse

Abstract. *The mutation cribriform degeneration* (cri) *occurred in the DBA/2J strain; it is in linkage group VIII, 31 recombination units from* b. *Homozygotes show severe vacuolar degeneration in white and gray matter of the spinal cord and brainstem, normocytic anemia at birth which decreases in severity with age, and abnormalities of electrolyte distribution.*

Mutant genes with multiple or pleiotropic effects may be useful for revealing relations between apparently diverse developmental processes. Such mutations in laboratory animals can be subjected to experimental analysis, and if they resemble, even in part, human inherited diseases, they may serve as models to aid in the analysis of basic mechanisms. We report here a new pleiotropic mutation in the laboratory mouse, which causes neurological, hematological, and electrolyte abnormalities.

The new autosomal recessive mutation was discovered by the late M. M. Dickie in the DBA/2J strain of mice in

May 1967. Homozygotes were first recognized by their small size, weakness, and ataxic behavior beginning at about 2½ to 3 weeks of age. They were later found to have an anemia that was often easily visible as pallor at birth, and an electrolyte imbalance that was first suspected by Dickie because of a tendency of the mice to lick the sweat of her hands. We have called the mutation cribriform degeneration (*cri*) because of its characteristic neuropathological feature.

From matings between heterozygotes (*cri*/+) on the DBA/2J background, 113 (17.1 percent) of the 660 offspring, classified at birth, were pale. Five of these showed normal behavior at 3 weeks and may have been anemic at birth because of hemorrhage or other reasons not associated with *cri*. Only 520 offspring survived to weaning; 73 (14.0 percent), including 24 not recognized as anemic at birth, were classified as *cri*/*cri* by their abnormal behavior. Thus, a minimum of 18.2 percent [24/(113 − 5 + 24)] of the homozygotes escaped detection at birth. The significant deficiency of *cri*/*cri* offspring at weaning (expected, 25 percent) is probably due entirely to their reduced viability.

Data from a coupling intercross (*cri b*/++ × *cri b*/++) that produced 203 offspring showed that the new gene is linked to brown (*b*) in linkage group VIII with 31.4 ± 4.0 percent recombination.

Affected mice from the linkage cross have the same array of defects as those on the inbred DBA/2J background, although possibly less severe. The following description is based on mutant and normal mice on the DBA/2J genetic background. Since no affected mice have reproduced, the mutation is propagated by matings between heterozygotes, and the normal littermates consist of both heterozygotes (+/*cri*) and homozygous normals (+/+).

Brain, retina, spinal cord, nerve roots, dorsal root ganglia, and sciatic nerve trunks of affected and littermate control mice were examined at 15 to 68 days postnatal. The most obvious abnormalities were symmetrical, cribriform (vacuolated, sievelike) lesions in the white matter of the spinal cord (Fig. 1B). The vacuoles were uncolored in frozen or wax sections stained for lipids (Sudan dyes), or for carbohydrate polymers (periodic acid–Schiff method). Vacuolation was preceded by an increased density, increased surface area, and fragmentation of myelin sheaths; these changes were already evident at the periphery of the white matter of the cord at 15 days, and progressed toward the center during the ensuing weeks (Fig. 1D). In addition to the lesions in the myelin sheaths, the axons themselves were intermittently swollen (Fig. 1D) and were stained for long distances in spinal cord and brainstem by the Nauta and Fink-Heimer methods for degenerating axoplasm. These observations indicate focal damage of myelin sheaths and axons, as well as secondary Wallerian degeneration.

Electron microscopic examination confirmed these abnormalities in the myelin sheaths. Many sheaths were distorted although the axons within them still appeared normal; it was not possible to establish which of these two tissue components was affected first. The electron micrographs added the additional point that the vacuolation was almost entirely intracellular. The smallest vacuoles were seen in that part of the oligodendroglial cell cytoplasm closest to the axon surface, and also between the membranes of the inner turns of the myelin. Although comparable vacuoles were present in the control material, they were consistently and markedly increased in number in the mutant. The mutant also showed numerous larger vacuoles up to several micrometers in diameter; these vacuoles commonly contained membrane fragments or a sparse, irregular, membranous web that suggested the coalescence of small membrane-bound vacuoles into larger ones.

The cribriform lesions in the spinal cord were seen in ventral, lateral, and dorsal funiculi, being least severe in the fasciculus proprius, and in the descending tracts in the ventral part of the dorsal funiculus (Fig. 1B). Similar but less severe lesions were found in the brainstem up to the midbrain (Fig. 2A). Relatively few vacuoles were encountered more rostrally although there was some myelin condensation and fragmentation. Selected tracts of the brainstem were consistently affected while others were spared (Fig. 2A), even in the oldest animals examined.

Large numbers of vacuoles of various sizes were found also in the gray matter of spinal cord and brainstem and gave a spongy appearance to the affected areas (Fig. 2A). These vacuoles, like the ones in white matter, were almost entirely intracellular (Fig. 2B) and were found in nerve cell bodies and dendrites, and, to a lesser extent, in axons, astrocytes, and oligodendroglial cells. Many neurons were destroyed, and in response there was a moderate astroglial proliferation.

The lesions in the gray matter occupied relatively large circumscribed

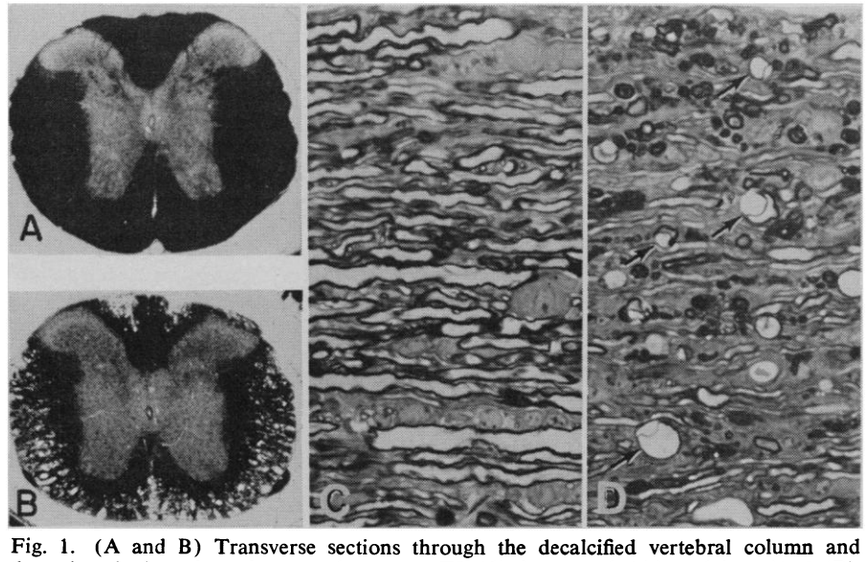

Fig. 1. (A and B) Transverse sections through the decalcified vertebral column and thoracic spinal cord at about cord segment T8 (*1*) in control (A) and in *cri*/*cri* (B) mice at 48 days of age. The cribriform lesions are present in the outer parts of the white matter of the spinal cord in the mutant; the gray matter looks virtually normal by light microscopy at this age. Celloidin, 20-μm sections, Loyez myelin stain (*1*), × 36. (C and D) Longitudinal sections through the ventromedial white matter of the cervical spinal cord in control (C) and *cri*/*cri* (D) mice at 25 days of age. Increased density, excessive folding, and fragmentation of myelin sheaths are present in the mutant, as is the early vacuolation (arrows). Epon, 1-μm sections, alkaline toluidine blue stain, × 435.

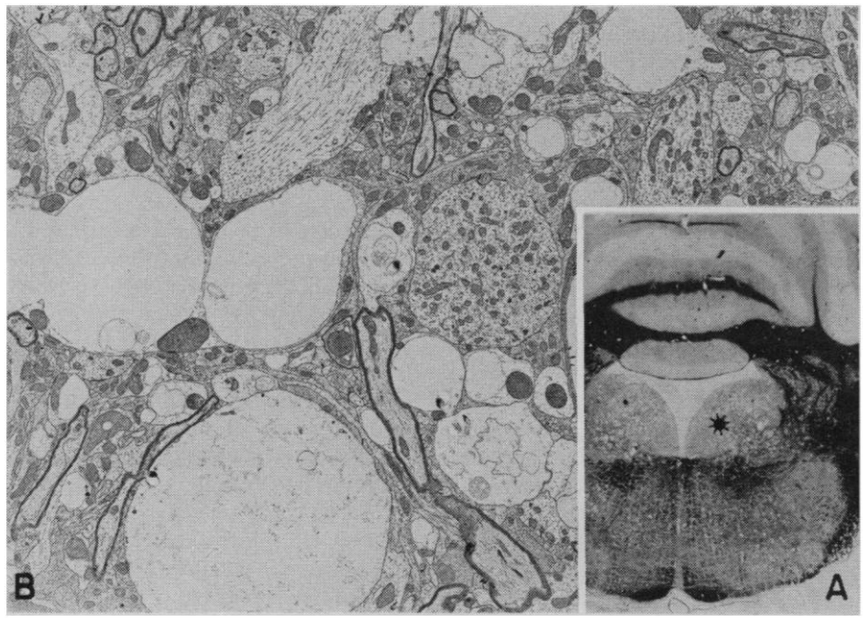

Fig. 2. (A) Transverse hemisection through pons and cerebellum of a 48-day-old *cri/cri* mouse. The spinal tract of the trigeminal nerve (right lower corner) is vacuolated, but the corticospinal tracts (bottom of picture) and white matter of the cerebellum (upper half of picture) are relatively spared. The gray matter has a spongy appearance that is secondary to vacuolation. Asterisk indicates the approximate position of Fig. 2B. Celloidin, 20-μm section, Loyez stain, $\times$ 25. (B) Electron micrograph in region indicated by asterisk in Fig. 2A. There is extensive intracellular vacuolation, mainly in dendrites. The mouse was perfused through its heart with glutaraldehyde-formaldehyde, and brain tissue was then postfixed in osmium tetroxide and embedded in Epon; ultrathin section stained with uranyl acetate and lead citrate, $\times$ 3700.

areas symmetrically disposed on left and right sides, but not conforming to anatomical groupings of nerve cell bodies (*1*). Among the most severely affected areas were the ventral gray matter and the substantia gelatinosa of the spinal cord in the lumbar region, the medial reticular formation in the lower brainstem (Fig. 2A), the dorsolateral gray matter of the upper medulla and pons (Fig. 2A), and the bipolar (inner nuclear) layer of the retina. The lesions in the retina and in the gray matter of the spinal cord appeared relatively late in the course of the disease.

Neuropathological expression in *cri/cri* mice appears to be influenced by environmental, perhaps nutritional, factors. Affected mice born and reared in Boston, Massachusetts, on a diet containing 26 percent protein and 7 percent fat rarely survived beyond 30 days of age, whereas animals raised in Bar Harbor, Maine, on a diet containing less protein and fat but supplemented with wheat germ, commonly survived to 90 days or longer, even if transferred to Boston after weaning.

The mode of inheritance and the neuropathologic features are similar in several respects to those of inherited spongy degeneration in humans (*2*). An intracellular vacuolation similar in electron microscopic appearance to that of *cri/cri* mice is seen also in certain human and experimental "slow virus" diseases (*3*).

The characteristics of the accompanying anemia in the mice were studied from the time the animals were born until they were about 50 days old; the anemia is essentially a normocytic one (Fig. 3). In normal mice, mean cell volume decreases about 50 percent during the first month of life. This process is slower in the mutants, so that their blood appears to be macrocytic when compared with normal littermates, but after 30 days cell volume is normal. The anemia decreases in severity with age but does not disappear completely. In 34 pairs of mice, 4 to 8 weeks old, hematocrits averaged 47.3 percent in *cri/cri* and 49.8 percent in +/?, with a mean difference of 2.5 $\pm$ 0.7 percent, which is highly significant. The data for the first 3 weeks of life probably give an exaggerated estimate of the average severity of anemia caused by *cri* because only mice that were obviously anemic at birth were studied.

Because the lesions of the central nervous system bear some resemblance to those of pernicious anemia, we studied the effect of vitamin B_{12} administration, even though the anemia is not a macrocytic one. Two different trials, one on adult mice and one on mice during the first month of life, indicated

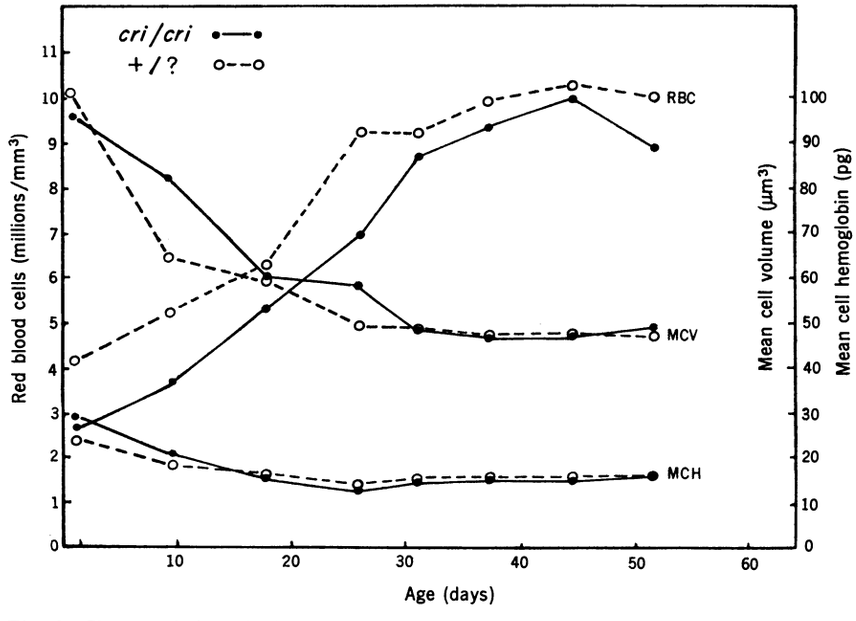

Fig. 3. Characteristics of blood (mean values) of *cri/cri* and +/? littermates on the DBA/2J background. Red cell counts are consistently lower in mutant than in normal animals, and the differences are significant except at 45 and 52 days.

no curative effect of the vitamin. Humans with pernicious anemia respond to treatment with B_{12} by a marked increase in reticulocytes within 3 to 5 days (*4*). We found no increase in percentage of reticulocytes in either affected or control mice, 48 and 58 days old, at 5 days after the first of three daily injections of 3.75 μg of B_{12}. In affected growing mice, daily injections of 1 μg of B_{12} for 5 days per week, beginning at 2 or 4 days of age, failed to alleviate the anemia or the weight deficit. The treatment also failed to prevent the onset of behavioral symptoms at the usual time or to prevent early death.

As further evidence for lack of similarity between this syndrome and pernicious anemia, we have found that gastric acidity is normal (*p*H 1 to 2), and that the stomach is histologically normal in cribriform degeneration.

The distribution of Na^+, K^+, and Cl^- is abnormal in *cri/cri* mice. Observations (*5*) have shown that mutant mice drink physiological saline in preference to water, and that they have decreased concentration of K^+ in plasma, decreased concentrations of Na^+ and K^+ in urine, increased concentration of Na^+ in hair and feces, and increased concentration of Cl^- in the sweat on the plantar surface of the hind feet. With respect to electrolyte balance, *cri* has some similarity to cystic fibrosis, a recessively inherited disease in humans.

Other effects of *cri/cri* include a reduction in size of some of the endocrine glands. In particular, the anterior lobe of the hypophysis is disproportionately reduced in comparison with the other two lobes, the testes are small with fewer mature sperm and fewer interstitial cells than normal controls, and the thyroid has small follicles lined with epithelium that is lower in height than that of normal controls. These effects may be nonspecific consequences of the general debility of the affected animals. Electron microscopic examination of the anterior hypophysis of *cri/cri* mice shows growth hormone cells of normal appearance, as well as the other cell types seen in controls. In addition, although the vaginas of most *cri/cri* females do not open even if they survive 20 days or more beyond the normal time of vaginal opening (about 30 days in DBA/2J controls), vaginal opening was found in one fairly healthy affected female at 54 days, and was followed by a normal estrous cycle 10 days later.

The fur and vibrissae of most *cri/cri* mice are slightly wavy. This is true both on the DBA/2J background and in affected mice from the linkage cross. This characteristic is often helpful in identifying affected mice before the abnormal behavior becomes severe enough to be diagnostic.

The basic defect caused by *cri* is as yet unknown. The abnormal distribution of electrolytes suggests that an abnormality of electrolyte transport may underlie the other defects, but the mechanism remains to be discovered.

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Primate Phylogeny and Immunological Distance

Abstract. *Recalculation of the time of divergence of the Pongidae and Hominidae after correction of immunological distance by inclusion of generation length yields minimum dates of approximately 14 million years ago.*

Estimates of the temporal separation of the Hominidae and Pongidae have been the source of heated debate for over a century. Calculations based upon immunological distances of homologous serum proteins have been used to challenge more traditional approaches, which usually place the age of this division sometime in the Miocene (*1–4*). The strongest advocates of the "protein clock" approach have suggested dates as recent as 4 million to 6 million years ago (*5*, *6*). Their calculations are based upon two fundamental assumptions. The first is that some significant proportion of amino acid residues in the primary structure of a protein are subject to replacement by other residues without a significant alteration of the biological function of the protein. The second is that such replacements occur at rates that are subject to mensuration in units of geologic time (*7*, *8*). We believe that the latter may involve a fundamental error.

For either divergence times or "rates" of protein evolution to be determined, mutation rates and rates of amino acid substitutions must be calibrated in biologically meaningful units of time, such as nucleotide shifts (point mutations) per DNA replication per standard time unit. Mitosis provides an opportunity for nucleotide altera-

Table 1. Data for DNA hybridization and albumin immunological distances, ID, for various primates with calculated mutation rates, μ, in units of ID per generation per million years. The DNA hybridization distances were obtained by reassociation of radioactive nonrepeated DNA with total nonradioactive DNA, followed by assay of thermal stability (*10*). The albumin immunological distances were obtained by micro-complement fixation (*6*).

Primates	DNA		Serum albumin	
	Nucleotide change since divergence (%)	μ	ID	μ
Man–chimpanzee	2.6*		7*	
Man–prosimian†	54	1.19	123	2.71
Old World monkey–chimpanzee	10.8	0.98	30	2.72

* Used to calculate α. † The primate used for DNA study was the *Galago*. The albumin value is an average for *Lemur* and *Loris*.