Table 1. Minimum concentrations (percent) of folic acid derivatives inhibiting reproductive capacity of screw-worm flies (two replicates).

Compound	Daily treatments (♂ and ♀)	One treatment (♂ and ♀)	One treatment (♀)
Inhibiting production of eggs			
Aminopterin	0.01	0.1	5.0
Methotrexate	0.001	0.05	0.5
Chloromethotrexate	0.001	0.5	0.1
Dichloromethotrexate	1.0	*	†
Inhibiting production of viable larvae			
Aminopterin	0.001	*	1.0
Methotrexate	*	*	0.1

* No inhibition. † Not tested.

Table 2. Oviposition by nonparous (two replicates) and parous (three replicates) screw-worm flies treated orally at different ages with 0.5 percent chloromethotrexate for 24 hours.

Age (days)	Females ovipositing (%)	
	Nonparous	Parous
1	0	*
3	75	*
7	50	10
10	*	0
14	45	3

* Not tested.

(*7*). This affinity accounts for the resistance of treated flies to restoration of normal fertility with large doses of folic acid. Mitlin *et al.* were unable to reverse the effect of aminopterin in house flies (*5*) because they used inadequate amounts of substrate.

Sterility induced by chloromethotrexate was not permanent. Virgin females, fed 0.5 percent chloromethotrexate for 24 hours after emergence, were given the opportunity to lay eggs without mating when 7 days old and every 3 or 4 days thereafter. A different group was allowed to oviposit for the first time on each of these occasions and again at 3- to 4-day intervals. (Maturation of normal ovarioles occurs synchronously; a gravid female may deposit 200 to 250 eggs in a shingle-like mass every 3 or 4 days.) No eggs were laid at the first two opportunities for oviposition, whether the first opportunity was at 7 or 23 days. From the time of the third opportunity for oviposition onward, production of eggs was considerably reduced (about 5 to 15 percent of controls). In a similar experiment, females treated after emergence and mated with normal males did not lay eggs when first permitted to oviposit 6 or 9 days later. Oviposition (less than 15 percent of controls) occurred on and after the 13th day; fewer than 10 percent of the eggs hatched. Moreover, some fertility was restored by the time second egg masses matured at any age. Perhaps insufficient unbound chemical remained to inactivate completely the second and third egg masses of mated and unmated flies, respectively. Spontaneous recovery from inhibitory effects of folic acid antagonists apparently also occurs in *Drosophila* (*4*), *Bracon* (*6*), and *Musca* (*8*).

Thus, mating evidently influenced egg deposition. Unmated females never oviposited at the first or second opportunity, but mated females always produced a first egg mass from the 13th day onward and usually a second mass irrespective of age. Eggs were not laid by 80 percent of unmated and 30 percent of mated flies (among 595 and 277 oviposition opportunities, respectively, of 197 unmated and 167 mated flies).

Table 2 shows the relation of oviposition by treated 7- and 14-day-old females to mating and parity. Only the youngest virgin (nonparous) females did not lay eggs 7 days after treatment and without mating; about half to three-quarters of older flies oviposited normally. Production of viable eggs by normally fertile, mated (parous) females at the three ages was less than 1 percent of controls 7 days after treatment. (Prior to treatment, 87 percent laid eggs, of which 84 percent hatched.) Not more than 20 percent as many parous as nonparous females laid eggs. Hence, chloromethotrexate retained more than 99 percent activity during the maturation of the egg mass of flies that had been treated at 7 or 14 days of age. Although comparison of nonparous and parous females is made between first and second egg masses, respectively, virgin females treated upon emergence in earlier tests were equally infecund at the first two opportunities of oviposition. Chloromethotrexate no longer completely inhibited oviposition from the 3rd day on, at least in nonparous females.

Apparently, activity of folic acid and its antimetabolites is exerted during metabolic processes of egg maturation. Moreover, ovarian growth and egg production are inhibited by physical or chemical agents much more readily during the endomitotic phase of nurse cells than at 24 hours or later when this phase is completed (*9*). The present data reflect these relative susceptibilities of the ovary to inhibitory agents. The work of Grosch (*6*) similarly shows the relation of methotrexate inhibition of oviposition in *Bracon* to atrophy of nurse cells and degeneration of oocytes.

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Mutant Mice (Quaking and Jimpy) with Deficient Myelination in the Central Nervous System

Abstract. *Two mutant mice with deficient myelination are described. Quaking is a new autosomal recessive mutant mouse with marked tremor of the hindquarters. The mice eat, swim, breed, and nurse well even though the entire central nervous system is very deficient in myelin by histological and chemical criteria. Myelin formation is impaired; no destruction is seen. Peripheral nerves are myelinated. Jimpy, a known sex-linked mutation, has similar but more severe symptoms and similar pathology, with the additional feature of sudanophilic (nonpolar) lipid distributed in some white-matter tracts. Both mutants offer new opportunities for study of the formation and functions of myelin.*

Mammalian mutants are of great value for the study of development, structure, function, and disease of the nervous system. Yet few of the more than 30 different neurological mutants now available have been well characterized, and more mutants are rec-

Table 1. Numbers of quaking (*qkqk*) and normal (++ or +*qk*) offspring produced by several types of parental matings. The data demonstrate an autosomal recessive mode of inheritance. Obs., observed; Exp., expected.

Mating	Normal				Quaking			
	♀	♂	Total		♀	♂	Total	
			Obs.	Exp.			Obs.	Exp.
qkqk × ++	50	54	104	104	0	0	0	0
+*qk* × +*qk*	188	174	362	359.25	50	67	117	119.75
qkqk × +*qk*	53	68	121	119	66	51	117	119

ognized and saved each year. Among the mutant mice of current interest are: reeler (*rl*), with a developmental disorder affecting principally the hippocampus and cerebellum (*1*); staggerer (*sg*), with a hypoplastic cerebellum (*2*); dystonia musculorum (*dt*), with disease of the peripheral nervous system and spinal cord (*3*); spastic (*spa*), with locomotor symptoms partially relieved by drug therapy (*4*); and dilute lethal (d^1), with reduced phenylalanine hydroxylase activity as in human phenylketonuria (*5*). In this report we describe two separate mutant mice with deficient myelination throughout the central nervous system. One, named quaking (*qk*), has not been described before; the other, jimpy (*jp*), has been available since 1954 (*6*).

The first quaking mouse was observed in strain DBA/2J in December 1961. Since no siblings or parents were available, this female deviant was outcrossed to strain C3H/Di and one litter of six normal females was obtained. These were mated with the C3H/Di male and the male offspring were backcrossed to the six offspring of the original deviant. Additional quaking animals were recovered. These and subsequent matings showed the disorder to be inherited in an autosomal recessive pattern (Table 1). Linkage studies are in progress.

The name quaking describes the outstanding clinical feature. When an affected animal is at rest and its trunk is in contact with bedding, no abnormality is seen. When the bedding is cleared away the trunk begins to shake and as the mouse begins to explore, the amplitude of the tremor increases. The rate of tremor was estimated visually to be about two to three per second. The tremor is most marked in the caudal part of the trunk and proximal portions of the hind extremities, so that the mouse appears to be bouncing on its haunches. When the examiner's hand lightly touches the trunk the tremor diminishes in amplitude and may stop, only to increase on withdrawal of the stimulus. The abnormal motor behavior is recognized at 10 to 12 days of age and reaches its full expression by about 3 weeks. At 3 months or later, some animals have weak hind limbs and diminished quaking movements. Mature animals may have attacks in which the limbs adduct under the flexed trunk and the stiff, motionless posture is maintained for many seconds. After excessive swimming activity we have seen a mouse lie motionless on its side for 1 minute and then show tonic extension of hind limbs for several minutes, with no responses to pin prick during this period.

In view of the striking pathology to be described below, several relatively normal aspects of the mutant's behavior deserve emphasis. Affected animals find and consume food and water readily from the conventional dispensers in the cage lids. Swimming movements are rapid and well coordinated, and the tremor is absent while the mouse is in the water. Responses to rapid rotation, pin prick, and touch appear normal, as do placing, corneal, and pinna reflexes. Vision and hearing are probably present but are difficult to assess adequately. Both sexes are fertile, but the males seldom sire litters. The mutation is readily propagated by mating affected *qkqk* females and heterozygous +*qk* males. Affected females make nests and properly care for their young. Life span is regularly at least several months, and may be normal.

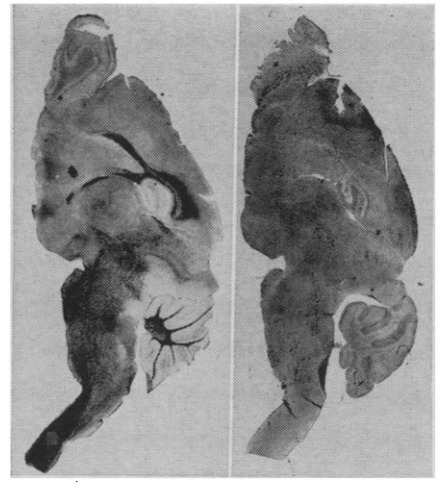

Fig. 1. (Left) Sagittal frozen section through the brain of a 48-day-old normal (+*qk*) mouse. The sudan black B dye colors myelinated fiber tracts. (Right) Comparable section through the brain of a quaking (*qkqk*) littermate. The fiber tracts are deficient in myelin and are visible as negative images. The architecture is normal; cells and fibers are present but have reduced or absent myelin sheaths. (× 4)

The first gross observations of the brain in weanling and 4-month-old quaking mice were made by Richard H. Swigart (*7*). He noted that the optic chiasm, spinal cord, and ventral surface of brain appeared translucent gray instead of the normal shiny white, and concluded correctly that myelin was deficient. Subsequently we prepared serial sections of frozen or wax-embedded brains in the three cardinal planes and stained them to show myelin, cells, and axons. Affected mice and heterozygous littermate controls from 12 days to 4 months of age were studied.

The major neuropathological findings, to be documented elsewhere more fully, were these. The entire central nervous system, from olfactory bulbs to sacral spinal cord, was very deficient in myelin in all ages studied. The white matter tracts were present, but were seen as negative images (Fig. 1). However, some fragments of myelin were formed in almost all tracts and had normal staining properties. Fibers which appeared unmyelinated may have had sheaths too small to be resolved with the light microscope. The white matter tracts contained intact axons, the normal types of neuroglial cells, and an increased concentration of pleomorphic cells. There was no evidence of destruction, no globoid cells or metachromatic lipids, and no inflammation. Cells in gray matter appeared normal. Except for the optic nerve, the cranial and spinal nerves were well myelinated. Along a given root fiber, poor myelination began abruptly at the junction of the peripheral and central nervous systems.

Jimpy, the second mutant, is a sex-

linked recessive lethal gene (*6, 8*). The only kind of affected animals available are the hemizygous males, *jpY*. The tremor is very similar in jimpy and quaking mice, and appears at about the same age. Jimpy mice have a more severe disease. Some have weak hind limbs by weaning age, and a few develop complete paralysis of hind limbs. Most jimpy mice respond to sound and swim well. By the 4th week of life they have generalized tonic-clonic seizures without focal onset. The head is arched back, while the extremities are extended and abducted at the distal joints. Most seizures last less than 1 minute, and are followed by total cessation of activity and then by resumption of prior behavior. The animals usually die by 30 days of age, commonly after a seizure.

Brains of jimpy mice have been examined at 12 to 39 days of age. As with quaking, far less myelin was found in any given region of the central nervous system at any age than in the littermate controls. Again the peripheral nervous system was myelinated. The general architecture of gray and white matter was normal. Cell bodies and axons were normal or were affected to a slight degree compared with the myelin sheaths.

The pathology differs in jimpy and quaking in one major respect. In jimpy, cells of certain white matter tracts, particularly the basis pedunculi, optic tract, and white matter of cerebellum and spinal cord, contained nonpolar lipids in the form of strongly sudanophilic cytoplasmic droplets of various sizes. Most of these cells appear to be fatty macrophages. Some were present in the 12-day-old brain, when myelination in the controls was still at an early stage, and later the number of sudanophilic cells in the white matter increased.

Initial chemical analyses (*9*) confirmed the histological findings. Brains of adult quaking and jimpy mice were homogenized in chloroform-methanol. After washing, the lower phase was partitioned by differential elution from a Florisil column and each fraction was analyzed further by thin-layer chromatography. The brains of the mutant mice contained markedly reduced amounts of cerebroside and sulfatide, which are lipid constituents of myelin. Several phospholipid fractions were qualitatively normal. No cholesterol ester was found in the controls or in quaking; jimpy brains had traces of cholesterol ester, less than 1 percent of the total cholesterol.

These two mutants have several advantages for further study of myelin formation and its disorders. Both mutants can be propagated readily in numbers suitable for experimentation. The uniform distribution of the failure to form myelin throughout the central nervous system reduces the sampling problem which so often limits neurochemical studies. The apparent disparity between the relatively good neurological function, especially in quaking mice, and the paucity of myelin merits further attention.

The disease in quaking mice seems different from known human diseases and appears to be expressed almost purely as a severe (though not complete) failure of myelination in the central nervous system. Jimpy mice have a more complex disease, involving both impaired myelination and destruction of myelin. In terms of the onset early in life and the presence of sudanophilic breakdown products of myelin, the disease in jimpy mice resembles some of the human inherited sudanophilic leucodystrophies, and is the first readily available experimental model.

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Endogenous Metabolic Factor in Schizophrenic Behavior

Abstract. *Exacerbations of schizophrenic behavior are associated with elevations of urinary tryptophan metabolites. Free tryptophan and methionine possibly arising from the breakdown of muscle protein may act like an endogenous metabolic factor which enhances behavioral worsening. Some of the apparently spontaneous activations of psychotic symptoms may be intensified by the endogenous liberation of these two compounds.*

Pollin, Cardon, and Kety (*1*) have shown that the administration of either one of two essential amino acids, tryptophan and methionine, in the presence of a monoamine-oxidase inhibitor drug, evoked activations of psychotic behavior in some schizophrenic patients; these observations have been confirmed (*2, 3*). Unlike these studies on the effects of increased amounts of amino acids, ours was undertaken to determine whether or not reductions of the amino acids tryptophan and methionine in the diet could improve behavior and ameliorate psychotic symptoms. Other work from our laboratory (*4, 5*), based on an entirely different viewpoint, was concerned with urinary indole metabolism both during periods of spontaneous worsening of behavior and during the administration of drugs. In those studies the average outputs of urinary indole metabolites in patients with schizophrenia did not differ significantly from normal values when all data were considered. But a striking correlation was observed in some patients between increases in the urinary excretions of tryptophan derivatives, particularly tryptamine, and the exacerbations of schizophrenic activity in terms of aggravations of hallucinatory and delusional experiences as well as aggressiveness and hostility. This report concerns only those results from the current investigation which are directly applicable to a further analysis of the relationship between increases of urinary indoles and worsening of behavior.

Our observations were made under strictly controlled conditions including those of diet, nitrogen equilibrium, and intake of tryptophan and methionine.

Urine (24-hour and 5-day collections) was examined for three tryptophan derivatives—tryptamine (*6*), total 3-indoleacetic acid (*7*), 5-hydroxyindoleacetic acid (*8*)—as well as for creati-