Liver Esterase Abnormalities in Hereditary Neurological Diseases of Mice

Abnormalities of esterase enzymes occur in diseases of man, mice, and probably other species. For example, the composition of esterase isozymes is altered in the brain of patients with multiple sclerosis and Schilder's disease[1]. Similarly, aberrant patterns of non-specific esterases as well as cholinesterases occur in the brain of neuromuscular mutants of mice[2]. Furthermore, deficiences of esterase isozymes were observed in livers of mice with hereditary muscular dystrophy[3].

In this report we describe our findings of esterase activities in the liver of mice with hereditary neurological disorders. We studied the effects of mutant genes in congenic strains or lines of mice which differ only with respect to allelic substitutions at the locus under investigation. In each strain or line, two kinds of genetically similar mice, mutant and control, are available.

Materials and methods. We studied liver esterase activities of 7 different mutant mice each homozygous for a recessive mutation; the mutations are ducky (gene symbol, *du*)[4], jolting (*jo*)[5], myelin synthesis deficiency (*msd*)[6], reeler (*rl*)[7], shambling (*shm*)[8], teetering (*tn*)[9], and tottering (*tg*)[10]. Each of these mutant syndromes causes a specific neurological disease; some have abnormalities resembling neurological disorders of man[11].

We used 3- to 8-week-old mice, usually of both sexes. At least 20 pairs of mice from each mutant type were studied. The mice had been given free access to water and food. The food was Old Guilford laboratory chow, a diet containing 19% protein, 11% fat, and 2% fiber. We used liver homogenates for the determination of total non-specific esterase activity according to the method of NACHLAS and SELIGMAN[12]. For isozyme analyses, livers were first perfused with isotonic saline and homogenized with an equal volume of saline; the homogenates were then centrifuged at $12{,}000 \times g$ for 40 min at 10 °C, and the supernate electrophoresed directly or stored for a few weeks at −20 °C. We employed thin-layer agar gel electrophoresis according to a previous description[13]. In inhibition experiments we preincubated gel plates for 30 min at 37 °C with 0.1 m*M* eserine sulfate, 0.1 m*M* *p*-chloromercuribenzoate, or 10 m*M* sodium fluoride (NaF).

Results and discussion. Compared with normal like-sexed littermates, mutant mice had less liver esterase activity (Table). The degree of decrease corresponded closely with the severity of clinical signs exhibited by each mutant syndrome.

The normal adult pattern of control mice consists of 20 isozymes to which we have assigned a number representing a percentage of the distance from the anodal to cathodal bands. Isozyme alterations were observed in the liver of all mutants, and certain are apparent even before their respective control littermates reach the adult pattern. Thus, ducky mice lack bands II-16, III-56, and V-83, and II-21 is weak compared with controls; jolting mice are deficient in II-6, II-21, II-25, III-56, and V-100; mice with myelin synthesis deficiency have lost bands III-56 and IV-74, and bands II-21, II-25, and V-83 are weak; in reeler mice bands II-21, III-56, and IV-74 are missing; shambling mice lack bands II-16, II-21, and II-25, and V-87 is present but faint; in teetering mice bands II-16, II-21, and III-56 are missing; and tottering mice lack band I-5[a] (Figure).

Thus, most deficiencies involve isozymes of zones II and III. For example, band II-21 is absent in all but 2 mutants, and band III-56 is deficient in 4 of 6 mutants, although each mutation is characterized by a number of other specific isozyme deletions. Clearly, the tottering mutation is distinct from all others. We have previously reported that the *tg* locus is closely linked with the *Es*-1 locus in linkage group XVIII[14]. In our own stock of mice, C57BL/10 Gn-*tg*, the *tg*/*tg* mice are *Es*-1[b]/*Es*-1[b], the +/+ mice are *Es*-1[a]/*Es*-1[a], and the *tg*/+ mice are *Es*-1[a]/*Es*-1[a]. Since we have used *tg*/+ mice rather than +/+ as controls for tottering mutants, two bands appear in zymograms because of the codominance of *Es*-1[a] and *Es*-1[b]. Also, the presence of *Es*-1[a] in control mice gives rise to higher total esterase activity compared with tottering mutants (Table).

The occurrence of major deficiencies in liver esterase isozymes common to a number of different neurological mutations is of considerable interest because it may reflect their essential metabolic importance, e.g., they

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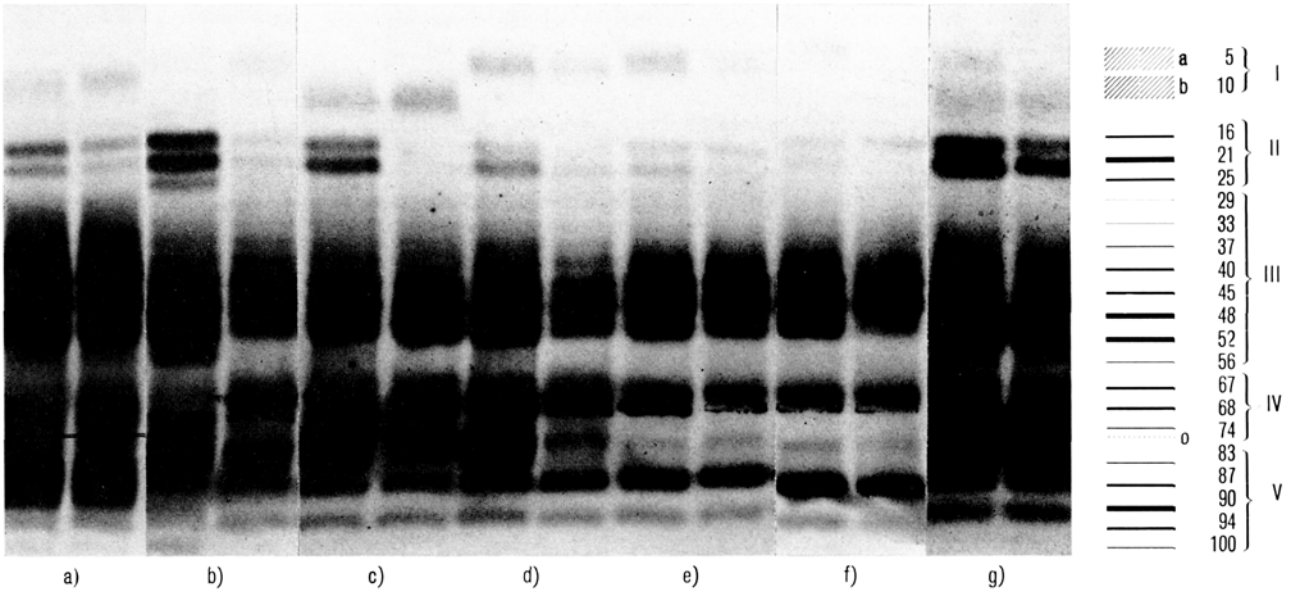
β-naphthyl acetate ester hydrolase activity in liver of several neurological mutations of the mouse

Name of mutation	Age	Mutant mice	Normal mice	Significance (p)
Ducky	5–7 week	4.60 ± 1.42[a]	7.68 ± 0.72[a]	<0.01
Jolting	8 week	6.48 ± 0.51	7.52 ± 0.72	<0.01
Myelin synthesis deficiency	3 week	4.35 ± 0.72	7.01 ± 1.44	<0.01
Reeler	3 week	3.26 ± 0.38	9.72 ± 0.60	<0.01
Shambling	3–5 week	7.60 ± 1.06	10.76 ± 1.14	<0.01
Teetering	5 week	4.98 ± 1.38	8.44 ± 1.44	<0.01
Tottering	8 week	6.26 ± 0.68	8.26 ± 0.60	<0.01

[a] Mean values obtained from each of 8 mice ± standard error. Unit: mg naphthol liberated per mg tissue wet weight per min.



Esterase zymograms of liver extracts from 7 different neurological mutants of the mouse employing β-naphthyl acetate as substrate. a) Teetering; b) jolting; c) shambling; d) ducky; e) myelin synthesis deficiency; f) reeler; g) tottering. The mutant is on the right and the normal control on the left of each channel.

may function in protein metabolism since at least some hepatic aliesterases of the rat are capable of splitting amides and amino acid esters[15]. Indeed, bands of zone-II, including II-21 are ali-esterases. Bands III-29, III-52, and III-56 represent cholinesterases and are inhibited by eserine. The absence of either or both III-52 and III-56 in all but jolting, shambling and tottering mice, if true as well for the brain, may relate to the convulsive behavior and reduced lifespan of these mutants.

The sensitivity of bands III-52 and III-56 to inhibition by eserine sulfate and their relative electrophoretic mobilities suggest that they may correspond to esterase-3 bands controlled by *Es*-3, a locus whose linkage is not as yet known[16]. Our uncertainty stems from the fact that previous analyses of esterase isozymes were made with starch rather than thin-layer agar electrophoresis. Although *Es*-3 is not yet located, 4 other known esterase loci are mapped and belong to linkage group XVIII[17]; it seems unlikely that the deletions in zone III represent allelic differences at the *Es*-3 locus in these mutants.

Bands IV-74 and V-83, lacking singly or together in 3 of the mutants, represent lipolytic active esterases because of their ability to hydrolyse α-naphthyl stearate. Thus, they are important in lipid or lipoprotein metabolism.

The severe esterase isozyme deletions as well as deficiencies in total esterase activity occurring in livers of so-called neurological mutations may be considered as due either to a pleiotrophic gene effect or adaptive responses to the primary gene effect. Thus, in either case, hereditary neurological diseases represent primary or secondary systemic disorders[18,19].

Zusammenfassung. Alle von uns in Mäusen untersuchten vererblichen neurologischen Erkrankungen stellen entweder primäre oder sekundäre gesamtkörperliche Prozesse dar. Verschiedene allelische Substitutionen produzieren spezifische Leber-Esterase-Isozym-Defekte.

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[19] The principles of laboratory animal care as promulgated by the National Society for Medical Research are observed in this Laboratory.

Preparation of a Plasma Membrane Fraction from the Brown Adipose Tissue

The plasma membranes of brown and white adipose tissues are of great interest as the site of numerous metabolic controls. As a useful approach to the biochemical properties of the plasma membranes, it seemed of interest to isolate this fraction free of contaminants.

Preparation and characterization of plasma membranes from liver and ascites cells have been the subject of several investigations[1–9]. Two different methods have been described: in the first, the plasma membranes were separated from heavy fractions (nuclei, mitochondria),