found between the 3 groups for tissue uptake and net portal transport (Table III).

Discussion. In the absence of pancreatic lipase, MCT undergo extensive hydrolysis by the intestinal mucosa[2]. However, because their maximum absorption rate is 4-fold less than for their constituent MCFA[9], it was elected to use the sodium salt of octanoic acid. Initial experiments showed that the probe molecule exhibited the transport characteristics of a passively absorbed substrate; as the concentration of Na octanoate was increased, the rate of absorption increased proportionately[10]. In order to minimize the detrimental effects of undernutrition[11] on absorption and more specifically on the activation of MCFA[12], adequate calories were provided during the 48 h period between the initial surgical procedure and the study of absorption. As a result, the average weight loss was negligible.

The disappearance rate of Na octanoate from the lumen was unchanged in the absence of bile for 48 h from the intestine of bile fistula and bile duct ligated animals. However, the fact that Na was better absorbed in the bile diverted than in the cholestatic group tends to support our previous observations[4,5]. Two major resistances to the flux of lipid into enterocytes are now recognized, a diffusion barrier due to an unstirred aqueous layer and the resistance to translocation through the cell membrane itself[13]. Since the resistance to diffusion of octanoic acid across the unstirred water layer does not represent a substantial fraction of the total resistance[14], the present results suggest that the permeability characteristics of the lipid membrane to octanoate were unaffected by the absence of bile. In contrast to LCFA which, on entering the mucosa from the lumen, are mainly esterified to triglycerides[15], a significant proportion of absorbed octanoic acid, once activated, is oxidized[16]. Therefore, the equally large net portal transport of $^{14}CO_2$ in the 3 groups of animals could suggest that mucosal metabolism of octanoate subsequent to its transport was unchanged. However, this interpretation may not be totally valid because oxidation may go on in the intestinal lumen and account for a small but significant % of the CO_2 in the portal vein[17]. The net portal transport of ^{14}C radioactivity in plasma and the counts in the tissue of the perfused segments were the same in the 3 groups. Since the absorption of octanoate in lymph is negligible[18], it can be concluded that extrusion from the enterocytes and subsequent transport were unaffected by biliary diversion and by experimental cholestasis.

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The (Na^+ + K^+)-ATPase Activity in Brain of Quaking Mice

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Summary. The (Na^+ + K^+)- and Mg^{2+}-dependent ATPase distribution in several brain areas has been investigated in Quaking mutant mice characterized by myelin deficiency. A marked decrease of (Na^+ + K^+)-ATPase activity has been found in limbic structures, hypothalamus and cerebellum. The Mg^{2+}-dependent activity did not change. A possible involvement of the impairment of the (Na^+ + K^+)-ATPase activity in the seizure susceptibility of this mice is discussed.

Sidman et al.[2] described Quaking mutation characterized by myelin deficiency on the central nervous system. The disease is autosomal recessive and animals can survive for several months. Mutant mice can be recognized at about the 12th day after birth and the disease reaches its full expression by about 3 weeks. Besides marked tremor of the hindquarters, epileptic fits can be induced in the adult by sensory stimulations.

There is a substantial amount of data indicating that the mutation could be related to a deficiency of myelination caused by a lowering of the rate of synthesis of several enzymes involved in biosynthesis of myelin constituents[3-7]. On the other hand, little has been done on the molecular basis of seizure susceptibility in mutant mice.

It was shown previously that slices of human epileptic brain tissue cannot take up potassium nor extrude sodium as well as normal human brain slices[8]. Moreover, sodium and potassium transport seems to be involved in the mechanism of the anticonvulsant action of diphenylhydantoin[9]. Due to the work of Skou et al.[10], an identity has been established between the sodium pump and an enzyme, the (Na^+ + K^+)-ATPase activity. Thus, in the present study we were concerned primarily with the

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Table I. $(Na^+ + K^+)$-activated and Mg^{2+}-dependent ATPase activities in different areas of control and mutant mice brain

Area of brain	$(Na^+ + K^+)$-activated ATPase		Mg^{2+}-dependent ATPase	
	Control	Mutant	Control	Mutant
Cortex	15.67 ± 0.29	14.40 ± 0.32 [b]	13.52 ± 0.33	12.70 ± 0.34
Limbic structures	15.35 ± 0.40	11.84 ± 0.58 [c]	7.65 ± 0.36	7.94 ± 0.58
Olfactory zones	17.54 ± 0.37	17.49 ± 0.25	11.31 ± 0.11	11.61 ± 0.10
Cerebellum	16.17 ± 0.54	12.86 ± 0.30 [c]	14.40 ± 0.19	14.16 ± 0.34
Hypothalamus	22.93 ± 0.73	19.10 ± 0.64 [c]	12.39 ± 0.29	12.78 ± 0.28
Pons	25.47 ± 0.36	23.29 ± 0.71 [a]	12.36 ± 0.23	12.99 ± 0.28
Medulla	23.45 ± 0.66	24.57 ± 0.68	9.55 ± 0.20	10.73 ± 0.53

Values are expressed as μmoles Pi liberated/mg protein/h. Each value is the mean of 8 experiments ± standard error. [a] $p < 0.02$; [b] $p < 0.01$; [c] $p < 0.001$.

possible involvement of the $(Na^+ + K^+)$-ATPase activity (ouabain sensitive, ATP phosphohydrolase, EC 3.6.1.3) in the marked tremor and seizure susceptibility in Quaking mice induced by sensory stimulations.

Methods. Animals used in this study were male, 6-week-old Quaking mice (*Qk/Qk*) with corresponding apparently normal controls (*Qk/+* or +/+) purchased from the Centre de Sélection et d'Elevage d'Animaux de Laboratoire, Orléans-La Source, France.

The animals were killed by cervical dislocation. Dorsal cortex was peeled off down to the temporal ridge and the ventral cortex separated; the limbic structures from the pericallous area, olfactory structures (bulb, peduncle and tuberculum), hypothalamus, cerebellum, pons and medulla, were also dissected. Immediately after dissection the samples were frozen on dry ice and then homogenized in twice distilled water (1 μl/100 μg wet tissue) using Potter glass homogenizers. Homogenates were stored at −20 °C and processed after 24 h.

In some experiments, microsomes isolated from brain of normal and mutant mice were used. Brains were pooled and homogenized in 10 vol. of 0.25 *M* sucrose containing 30 m*M* histidine, 2 m*M* Na_2 EDTA, pH 7.2. The supernatant obtained after centrifugation at 18,000 ×*g* for 15 min was centrifuged at 105,000 ×*g* for 60 min. The pellet obtained was resuspended in twice distilled water, and stored at −20 °C till used.

The incubation medium for ATPase activity contained about 80–100 μg protein, 30 m*M* *Tris*-HCl buffer (pH 7.4), 3 m*M* ATP sodium salt (Sigma), 3 m*M* $MgCl_2$, 100 m*M* NaCl and 20 m*M* KCl. One set of triplicates contained 1 m*M* ouabain (Sigma). The reaction mixture was preincubated at 37 °C for 5 min before starting the reaction with ATP, and stopped after 15 min by the addition of the same volume (1 ml) of TCA 10% (w/v). Liberated inorganic phosphate was estimated in the supernatant fluid according to the method of GOMORI[11]. Enzymatic activity was linear for the 15 min incubation interval. Values were corrected with appropriate blanks for spontaneous hydrolysis of ATP and endogenous Pi in brain homogenates. Protein was measured by the ATPase activity, which is inhibited by the addition of 1 m*M* ouabain. Specific activity of ATPase is defined as μmoles of Pi liberated/mg protein/h[12].

Results and discussion. As a first step we studied the $(Na^+ + K^+)$-ATPase and Mg^{2+}-ATPase distribution in 7 areas of brain of the Quaking mice. Results presented in Table I show that Mg^{2+}-dependent activity is similar in all investigated areas of control and mutant mice. In contrast, in limbic structures, hypothalamus and cerebellum of mutant mice, we noted a highly significant decrease of the $(Na^+ + K^+)$-ATPase activity; a less pronounced decrease of sodium pump enzyme system activity in cortex and pons is found.

In order to gather some information about the nature of the observed reduction of $(Na^+ + K^+)$-ATPase activity, we studied some properties of this enzyme. Microsomes isolated from brain of Quaking mice and their apparently normal littermate controls were used.

The $(Na^+ + K^+)$-ATPase activity showed essentially the same pH optimum (pH 7.4–8.2) in both, control and mutant brain microsomal fraction. The same Km (1.4 m*M*) for ATP of both control and Quaking brain enzyme has been found.

The effect of some inhibitors and temperature-induced inactivation of enzyme activity are presented in Table II. *p*-Chloromercuribenzoate (PCMB), an inhibitor of SH- groups and $(Na^+ + K^+)$-ATPase[13] and fluoride, an

Table II. The effect of inhibitors and heat inactivation on ATPase activity of Quaking mice

Treatment		$(Na^+ + K^+)$-ATPase (%)		Mg^{2+}-dependent ATPase (%)	
		Control	Mutant	Control	Mutant
		100	100	100	100
PCMB	0.01 m*M*	8	8	56	54
	0.1 m*M*	0	0	43	44
NAF	3.0 m*M*	10	11	62	61
50 °C	15 min	65	68	63	60
	30 min	53	52	60	58
	60 min	42	39	45	44

The enzyme preparation was preincubated as indicated in the first column. Treatment with PCMB was done essentially as previously described[13].

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inhibitor of $(Na^+ + K^+)$-ATPase and active ion transport[14,15] inhibited to the same extent ATPase activity of both normal and mutant mice. The effect of heat inactivation by preincubation at 50 °C for 15–60 min does not differ in groups examined.

The rather small decrease of the $(Na^+ + K^+)$-ATPase activity in Quaking mouse is not sufficient enough to allow a detailed comparison of the properties of the enzymes. In spite of this, the properties of the $(Na^+ + K^+)$-ATPase seem to be unchanged, as shown by similar pH optimum, similar heat inactivation pattern, inhibition by PCMB and fluoride of both control and mutant mice enzyme, as well as by the results on Km presented in this work. Crossed incubations performed with normal mouse plus Jimpy (another myelin-deficient mutant) brain homogenate gave no evidence of activation or inhibition effects on ATPase activity (unpublished). Hence, decrease of the $(Na^+ + K^+)$-ATPase activity here observed is more probably related to the quantity of the enzyme present.

The most evident feature of mutants is tremor recognized at about the 12th day after birth, the exact nature of which has not been clarified. Previous studies which have been done on brain adrenergic transmitters content[16] and turnover[17] indicated an accelerated dopamine metabolism.

Impaired transport of sodium and potassium in epileptic brain tissue was previously demonstrated[8]. According to WOODBURY[18], the sodium pump enzyme is involved in the action of anticonvulsive drugs. We report a decrease of the $(Na^+ + K^+)$-ATPase activity in some areas of brain of Quaking mouse which presents seizures after slight stimulation. The inborn error of this myelin deficiency in mice concerns the synthesis of the myelin constituents. It seems difficult to include directly the deficiency of the sodium pump enzyme in the genetic determined inborn error. It seems rather likely that the reduction of $(Na^+ + K^+)$ activated, ouabain sensitive ATPase activity in some areas of the brain of mutant mice is a secondary phenomenon, like the changes in adrenergic system observed mainly in older mice[17]. The mechanism underlying the impairment of $(Na^+ + K^+)$-ATPase activity is under investigation[19].

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Morphine Suppression of Ethanol Withdrawal in Mice

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Summary. The acute administration of morphine, alcohol or dopamine results in a pronounced suppression of the convulsions produced by alcohol in mice. The suppressive action of morphine on alcohol withdrawal in the mouse apparently is not a product of morphine intoxication, but rather to some other specific interaction between alcohol and morphine in the central nervous system. The conclusion suggest that dopamine may play a significant role as a modulator in convulsions produced during alcohol withdrawal.

In spite of previous research[3–6] there is increasing evidence for a relationship between opiate and alcohol addiction[7–9]. There are reports of cross-tolerance between morphine and alcohol[10], blockade of ethanol dependence[9] and narcosis[11] in mice and there are reports of increased tolerance for morphine in mice[12] and in some human alcoholics that have been habituated to alcohol[13]. There is evidence for a connection between voluntary consumption of morphine and alcohol[8]. It has been found that a high % of opiate addicts that have graduated from drug free therapeutic communities become alcoholics five years later[14] and many opiate addicts have had earlier problems with alcohol drinking and often take large amounts of alcohol when narcotics are not available[15,16]. A strain of rats bred for high opiate consumption was found to drink more alcohol than a strain bred to ingest little morphine[17]. C 57 BL mice are known to

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