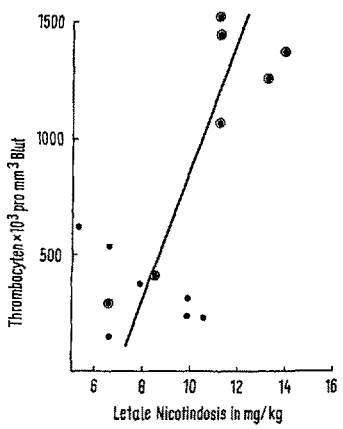
Ergebnisse. In der Figur ist die letale Gesamtdosis von Nicotin in Beziehung gesetzt zur Zahl der Thrombocyten.



Beziehung zwischen der Thrombocytenzahl und der Toxizität von Nicotin bei Kaninchen. Korrelationskoeffizient $r = 0{,}685$, Signifikanz $P = 0{,}05$. ⊙ = Tiere, denen eine Thrombocytensuspension infundiert wurde. ● = Kontrolltiere.

Zwischen der tödlichen Gesamtdosis Nicotin und der Zahl der Thrombocyten im Blut besteht eine positive Korrelation.

Die Summe der in den einzelnen Fraktionen des Blutes, Erythrocyten, Leukocyten, Thrombocyten und Plasma nach dem Tod der Tiere gemessenen Nicotinmenge betrug nur rund 10% des infundierten Nicotins, wovon weniger als 1% auf die Thrombocyten entfielen.

Auf Grund unserer Versuche scheint es möglich, dass die akute Toxizität von Nicotin bei hohem Thrombocytengehalt des Blutes deshalb erniedrigt ist, weil die durch Nicotin freigesetzten biogenen Amine zum Teil in den Thrombocyten gespeichert werden können.

Summary. The toxicity of intravenously injected nicotine in rabbits is decreased when the number of thrombocytes in the blood is augmented by administration of platelet suspensions.

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Esterase Alterations in Neurological Mutants of the Mouse[1]

We have used vertical starch and agar gel electrophoresis for the preparation of zymograms for a variety of hydrolytic enzymes contained in mouse brains[2,3]. The procedure of Smithies was used for both starch gel preparation and separation[4]; a gradient of 5 volts/cm was maintained for 16 h. For agar gel electrophoresis (1% Ionoagar-Difco dissolved in 0.05 *M* barbital, pH 8.6) a voltage drop of 2.5–5 volts/cm was imposed for 3 h. Histochemical reactions were carried out by the azo-technique using β-naphthyl acetate, indoxyl acetate and indoxyl butyrate as substrates[3]; to distinguish cholinesterase activity gel strips were preincubated (30 min before addition of substrate) in 0.1 m*M* eserine sulfate. Brains were homogenized in barbital-veronal buffer (ionic strength 0.05, pH 8.5), centrifuged for 10 min at 200 g and aliquots of supernatant used for electrophoresis.

Differences were discernable in esterase patterns between all so-called neuromuscular mutants and their normal controls investigated. These differences were consistent despite the heterogeneity of some of the controls for the mutants, and always revealed themselves as deficiencies. The deletions of bands varied according to the neuromuscular disorder present and the substrate used; the following mutants were investigated[2,3]: dilute lethal (gene symbol, d^1), ducky (*du*), jittery (*ji*), reeler (*rl*), spastic (*spa*), teetering (*tn*), and tottering (*tg*). We have previously reported that (cholin-) esterase in reeler may be present in a cryptic form and tightly bound to a particulate fraction that is electrophoretically immobile[3].

Since deficiencies were observed independent of the mutant syndrome, it may be presumed that abnormalities in esterases could have basic importance in the genesis of neurologic disease, and that they are related to a defect in the normal metabolism of myelin[5]. This may apply particularly to demyelinating disorders; whether or not all of the neurologic diseases studied are characterized by demyelination is not as yet established although in certain mutants, e.g. the dilute lethal, degenerating myelin is observed in the vestibulo-spinal, spino-cerebellar and tecto-spinal system appearing within a day or two after the first signs of myelination[6]. It is of interest that in multiple sclerosis of man, as well, losses or diminuition of esterase bands occur in plaque tissues[7]. Because mutants at the dilute locus (alleles *d* and d^1) cause the carrier mice to be phenylketonuric, the neurologic abnormalities (seizures) were suggested to be related to an altered brain amine concentration resulting from decarboxylase inhibition by phenylalanine metabolites[8]. Comparison was made of amine levels (epinephrine, nonepinephrine, serotonin), 5-hydroxytryptophan decarboxylase and monoamine oxidase, between normal (*DD*) and Dd^1 animals, as well as between males and females[9]; the heterozygotes (Dd^1) have roughly a 25–50% phenylalanine hydroxylase inhibition[8]. No differences were observed either between

[1] This investigation was supported by Public Health Service research Grant CA 04691-06 from the National Cancer Institute.

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the genotypes or sexes, despite the fact that the urinary phenylketone excretion of *Dd*[1] females (14.5 ± 1.6 mg%) is almost twice that of *Dd*[1] (8.4 ± 2.1 mg%) males and therefore should have lower brain amines; it may be inferred from these results that a mechanism other than an altered brain amine content is responsible for the seizures. Analytical determination of pseudo(-cholin) esterases revealed, in confirmation of the results with the zymogram tests, a lower amount in *Dd*[1] (132.5 ± 19 mm³ CO_2 evolved/g brain tissue) than in *DD* (186 ± 13 mm³ CO_2)[10]; details of these findings and methodologies will be described elsewhere.

Abnormalities in esterases may correlate with myelin degradation which accompanies the disease; the fact that such abnormalities occurred in all of a number of clinically distinct neurologic disorders of *mice* and also multiple sclerosis of *man* is noteworthy and it may be assumed that non-specific esterases could have basic metabolic significance in normal myelin anabolism. Since it is unlikely that all mutations resulting in a neurologic disorder cause, among other disturbances, esterase deficiency, one may presume that it is a secondary (indirect) effect of the mutant gene.

Zusammenfassung. In Analysen von Gehirn-Zymogrammen verschiedener neurologischer Mäusemutanten wurden Abnormalitäten in Form von Esterasedefiziten gefunden. Da solche neulich auch für die multiple Sklerose des Menschen beschrieben wurden, wird vorgeschlagen, unspezifische Esterasen als signifikant für den normalen Myelinanabolismus zu bewerten.

H. MEIER, W. G. HOAG, and ESTHER M. JORDAN

The Jackson Laboratory, Bar Harbor (Maine USA), March 20, 1964.

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Effect of Feeding Pattern on Fatty Acid Oxidation by Rat Liver Slices

Intermittent starvation, i.e. the feeding pattern where periods of food intake alternate with periods of fasting, leads in rats to a series of adaptations which in many parameters differs from the sequelae of simple continuous caloric undernutrition[1]. Apart from a markedly enhanced lipogenesis[2] in intermittently starving rats the rate of oxidative tissue processes is increased, which manifests itself *in vitro* by a higher endogenous respiration of various organs[3] and a higher basal metabolism of the animal[4].

In the present work the *in vitro* oxidation of palmitate-1-C^{14} by liver slices of female Wister rats was investigated: (a) in intermittently starving animals fed during the first week on alternate days and for the rest of the experimental period three times a week; (b) in continuously underfed rats which received daily a reduced food ration, corresponding to $^1/_7$ of the amount ingested by the intermittently starving animals per week; (c) in *ad libitum* fed controls. All groups were given a standard laboratory diet[5] for a period of 7–8 weeks. In the subsequent experiments intermittently starving and *ad libitum* fed male rats were used, which received for a period of 5 weeks either a control diet[6] containing 20 cal.% protein, 20 cal.% fat and 60 cal.% carbohydrate (mainly starch), or a high-fat diet[6] containing 20 cal.% protein, 73 cal.% fat (mainly beef tallow) and 7 cal.% carbohydrate. In all experiments the total food intake in intermittently starving and continuously underfed rats was 30–40% lower than in the controls, which manifested itself by a lower body weight.

The animals were killed by decapitation either in a state of satiety, after having consumed a measured amount of food during the preceding night, or after a subsequent fasting. Liver slices were incubated for 2 h in Krebs-Ringer phosphate buffer with a complex of potassium palmitate (3 μC per sample), with albumin (2.5%); $C^{14}O_2$ was precipitated as $BaC^{14}O_3$ and its radioactivity was estimated. In parallel samples of the liver the protein, glycogen and fat content was estimated.

From the results summarized in the Table it is apparent that the liver slices of intermittently starving rats oxidized in all experimental series twice to three times as much palmitate as those of *ad libitum* fed controls or continuously underfed rats. This applied to the animals killed in a state of satiety as well as to those fasting for 16–48 h prior to sacrifice. The enhanced palmitate oxidation was also very marked in intermittently starving rats on the high fat diet, as compared with animals which ingested the same diet *ad libitum*. The oxidation values were in this case proportionally increased both in the intermittently and *ad libitum* fed groups, most probably as a manifestation of adaptation to a high fat intake.

Essentially the same increase in palmitate oxidation in intermittently starving rats was also found in subsequent experiments, where possible differences in the glycogen content (in fed animals) were compensated by the addition of glycogen to the incubation medium. As the protein and the fat content in the compared liver samples was practically equal, it is unlikely that the above results of palmitate oxidation were substantially influenced by the ratio of the main liver constituents.

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