

Effect of Dietary Factors on Incidence of "Spontaneous" and Induced Convulsions in C₃H Male Mice.* (23168)

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This report concerns the results of observations made in connection with life span studies in mice as affected by various modifications of a basal diet. The data on convulsive seizures are being described separately because it is believed that the results may have special interest to other investigators of convulsion mechanisms in rodents. The results relating to the incidence of other specific diseases upon length of life will be reported elsewhere.

It was observed in studying singly housed C₃H male mice that convulsive seizures occur with significant frequency from 6 months of age, without intentional stimulation. The seizures are more frequent at the time of/or after handling for physical checking, weighing and cage changing. The seizures may be fatal. The frequency of seizure or the incidence of convulsive death is related to the diet used. Caloric restriction or vit. B₁₂ supplementation was effective in preventing or delaying convulsive deaths while a common hydrogenated vegetable fat diet was associated with a high incidence of fatal convulsions.

Methods. *Exp. 1.* Two hundred and twenty C₃H male mice were selected and separated into 11 groups matched as evenly as possible for age and weight at weaning. Each group contained 20 mice which were maintained under conditions described elsewhere (1). All animals were observed until natural death or sacrifice in terminal illness. *Exp. 2.* To test effects of certain dietary factors upon induced and/or "spontaneous" convulsions, 120 weanling C₃H male mice were distributed into 6 groups. Three groups were placed on lard-containing diets: lard (17%), lard plus extra vit. E (30 mg % alpha tocopherol acetate) and low lard (5%). The other 3 groups were fed the corresponding hydrogenated vege-

table fat (HVF)-containing diet: HVF (17%), HVF plus extra vit. E (30 mg % alpha tocopherol acetate) and low HVF (5%). The animals were treated as in Exp. 1, except they were housed 2 per cage. For auditory stimulation the procedure described by Hall (2) was adopted with slight modification. The stimulus was applied bi-weekly from the 15th week to the 30th week on experiment. For the administration of electric shock the method of Gellhorn and Ballin(3) was employed, modified by giving the shocks (60 cycle/sec) at regularly spaced intervals. Survivors were sacrificed at 370 to 390 days of age.

Results. The results of Exp. 1, with C₃H male mice tested on 11 different diets, are presented in Table I. It may be noted that restriction in calories alone (with normal intake of protein, minerals and vitamins) was associated with significant reduction in frequency of convulsive seizures and the incidence of convulsive deaths. The group of mice restricted both in calories and vit. E was similarly affected. The youngest animal to show a convulsion was in the group on a diet containing HVF instead of lard, the mean age at first convulsion noted was 204 days in contrast to 279 days for the control. Ninety per cent of mice fed HVF died from convulsions as compared to 55% of those on lard. Statistically the difference from control is significant at the 5% level. The average age at death in the group on the HVF diet was significantly lower than in the control group ($p < .01$), being 302 days for the former and 382 days for the latter. The earliest death in the group on HVF was at 6 months while no death was noted before 8 months in any other group. The percentage of animals showing convulsions as well as incidence of convulsive deaths decreased and the age at first seizure was increased by a supplement of vit. B₁₂ in the diet, the mean age

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TABLE I. Some Dietary Effects on Incidence of Convulsive Seizure and Convulsive Death.

Diets	% of mice showing convulsions	Avg age at 1st convulsion noted (days)	% of mice dying from convulsions	Avg age at convulsive death (days)
Basal*	75	278.7 (184-457)	55	382.3 (267-503)
H-M-W salt†	80	289.5 (185-641)	60	393.9 (270-661)
Hydrogenated vegetable fat (Criseo)	95	203.8 (118-272)	90	302.2 (185-409)
Low fat (3% lard)	75	284.0 (193-427)	55	344.5 (236-439)
Amigel (replaced glucose)	65	249.2 (180-375)	40	334.0 (243-432)
Fox chow	55	335.3 (185-499)	45	456.1 (318-612)
1/3 caloric restriction	15	319.3 (237-397)	15	372.6 (304-457)
0.5% animal protein factor‡	50	387.4 (241-476)	25	399.0 (246-477)
6 γ % vit. B ₁₂ §	35	410.2 (260-556)	10	459.0 (432-486)
Without wheat germ oil	55	299.2 (210-442)	30	371.7 (313-442)
<i>Idem</i> , and 1/3 caloric restriction	5	653.0	0	

* Basal diet: Casein 28.87, glucose 34.02, lard 17.53, Brewer's yeast 8.25, alfalfa 4.12, salt 4.12 (modified Jones Foster formula), cod liver oil 2.06, wheat germ oil 1.03.

† 7% of a modified Hubbell, Mendel & Wakeman salt mixture.

‡ B-Con APF-3+ Pfizer.

§ General Biochemical, Inc., crystal, U.S.P.

at first convulsive seizure noted being 410 days in contrast to 279 days for the control. Only 10% of the mice had died from convulsions at a mean age of 459 days in the B₁₂ group contrasted to 55% at a mean age of 382 days in the control. This difference is highly significant ($p < .01$). Furthermore, the increased age at first attack and the lowering of incidence in the group on a diet with Animal Protein Factor could be due to the presence of vit. B₁₂ in that factor. As seen in Table I, the incidence of convulsive deaths in the group on Fox Chow was also high though the age at death was delayed about 2 months as compared with the control.

It should be pointed out that the difference in number of mice showing seizures or dying from convulsion between groups on a diet without wheat germ oil and the control is not statistically significant. The lower incidence of convulsive death in the group on that diet is probably due to early death of mice from a "heart failure syndrome," details of which on the diets here used have been reported (4). No difference has been noted between the groups on 2 different salt mixtures, or the group on a low fat diet.

The results of Exp. 2 designed to test susceptibility of mice on the different dietary fats by use of sound and electrical stimulation are summarized in Table II, where the number of animals found convulsing upon bi-weekly auditory stimulation (from 15th to

30th week on experiment) is small on all diets. However, the incidence of convulsions induced by auditory stimulation of mice fed on diets containing HVF is higher than in those fed diets containing lard. Only one out of 60 mice fed diets containing lard while 9 out of 60 mice fed diets containing HVF show audiogenic convulsions. This difference between the two groups may be significant ($p < .05$). The data do not permit a definite conclusion as to whether the early response to auditory stimulation is related to "spontaneous" seizures at a later age. Six out of 10 mice responding to auditory stimulation show "spontaneous" convulsions later. The remaining 4 mice died at an age much earlier than that at which any of the "spontaneous" convulsions appeared. On the other hand, there were 5 mice in the groups on lard diets and 11 in the groups on HVF diets showing "spontaneous" convulsions, without having responded to auditory stimulation at an earlier age. As shown in Table II, when audiogenic and "spontaneous" convulsions are considered together, the difference between the lard-fed and HVF-fed groups appears less likely to be due to chance ($p < .01$).

The results obtained from the measurement of the electroshock convulsion threshold at the age of 10 months again indicated a lower threshold in the HVF-fed groups than in the lard-fed groups. The convulsive seizures were produced by the electroshock in the

TABLE II. Mice Manifesting "Spontaneous" and/or Audiogenic Convulsions.

Type	Diets (20 mice/group)						Total No. showing convulsions in 3 groups on:	
	Lard (17%)	HVF (17%)	Lard + extra vit. E*	HVF + extra vit. E*	Low lard (5%)	Low HVF (5%)	Lard diets	HVF diets
Audiogenic	1	3	0	4	0	2	1	9
"Spontaneous"	1	5	3	4	2	7	6	16
Both or either type of seizure†	1	6	3	6	2	8	6	20

* 30 mg % alpha-tocopherol acetate.

† In some instances the same mouse displayed "spontaneous" and audiogenic convulsions, therefore numbers in horizontal column are not the sums of the 2 lines of numbers above them.

majority of mice on HVF-diets with 20 to 30 cycles (4/12 - 6/12 sec) while 25 to 35 cycles (5/12 - 7/12 sec) were necessary for the groups on lard-diets. The difference of the mean electroshock thresholds required to induce convulsive seizures from the groups on HVF-diets (24.2 cycles) and the groups on lard-diets (28.5 cycles) is statistically significant ($p < .01$). The mortality in the former is slightly higher (37%) than in the latter (22%). All the results suggest that the mice on a HVF-diet are more susceptible to intentional stimulation of the types used.

Discussion. These results indicate that the dietary regimen exerts an effect on the "spontaneous" convulsions in C_3H male mice, although environmental conditions, such as caging, handling and others are determining or precipitating factors(1). There is no explanation as to why the incidence of convulsions is higher on a diet containing hydrogenated vegetable fat. Davenport and Davenport(5) described a reduction of electroshock threshold in normal rats fed a diet supplemented with extra fat (Crisco). In the studies herein reported a quantitative difference in fat intake did not influence the frequency of "spontaneous" convulsive seizures. However, when animals were fed a diet containing HVF instead of lard their electroshock threshold was reduced.

The mechanism of caloric restriction alone, in protecting against convulsions remains unsolved. However, increased resistance to a number of diseases or stress has been noted in underfed or caloric restricted rats or mice(4, 6,7). Geyelin(8) reported that fasting reduced the incidence of seizures in epileptic

patients, and the effect has been ascribed to the resulting ketosis. The Davenports(9), on the other hand, found a lowering of electroshock threshold by dietary restriction in pyridoxin-deficient rats.

The low frequency of fatal convulsions or convulsive seizures in the group on a diet supplemented with extra vit. B_{12} indicated further the importance of nutritional factors in relation to the convulsive attacks.

The basal diet contained 25% crude casein. During the course of this life-span experiment many different batches of casein were used, none of which was assayed for B_{12} content. Lewis, *et al.*(10) found that the B_{12} content of crude casein was 3 $\mu\text{g}/100\text{ g}$. Excluding special materials such as liver, rumen contents, etc., this value for crude casein compares well with other natural products such as meats, egg yolk and cheese reported by these investigators. Collins, *et al.*(11) found little difference in the B_{12} content of milk from different pure breeds of cows. The values found for commercial cow's milk, raw and pasteurized, were somewhat lower than for the pure breeds studied (raw 3.4; pasteurized 4.0; pure breeds 6.6 $\mu\text{g}/\text{l}$).

All groups of mice fed the basal diet *ad libitum* were heavier after 9 weeks of age than the group fed commercial fox chow. This does not suggest that the basal diet was deficient in B_{12} . It is also known† that the basal diet is adequate for normal lactation in the A mouse. Nevertheless, the possibility remains that the B_{12} content of unsupplemented basal diet was sub-optimal for the C_3H mouse. In addition to the observed de-

† Unpublished data.

creased incidence of convulsions on B₁₂ supplement, this group showed the highest body weight of any group (after 15 weeks of age). Whether or not the unsupplemented basal diet contained the optimal amount of B₁₂ for our C₃H mice cannot be determined from the data available to us.

As seen in the Tables the incidence of "spontaneous" convulsive seizures in the first group of experimentals is higher than that obtained from the Exp. 2 regardless of diet. This difference may be attributed to the differences in caging. The first experimental group of mice was caged singly while the latter group was kept two per cage. It has been reported(1) that when C₃H male mice are housed 2 per cage, the incidence of "spontaneous" seizures was reduced and with twenty to a large cage the incidence was negligibly small. A factor of a "social" nature appears to be implicated.

Summary. 1. The incidence of "spontaneous" convulsions and of convulsive deaths in C₃H male mice is greatly reduced by caloric restriction or by supplement of vit. B₁₂ in the diet fed *ad libitum*. Substitution of hydrogenated vegetable fat for lard decreased

the age of onset of convulsions and increased the incidence of convulsive deaths. 2. Studies with auditory and electric stimulation appear to confirm the existence of a difference between the effect of HVF and of lard.

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Influence of Thrombin on Rate of Prothrombin Conversion. (23169)

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There appears to be general agreement among investigators of blood clotting that thrombin accelerates its own formation from prothrombin. However, the specific mode of this action of thrombin is, at present, not completely resolved. The present work is concerned with an attempt to show that traces of thrombin will accelerate the formation of a prothrombin-conversion factor. Inasmuch as it has been found that plasma accelerator-globulin*(1,2) and probably also other clot-

ting factors(2) may be associated with platelets, a phospholipid preparation from beef brain corresponding to the phosphatidyl ethanolamine fraction of Folch(3) has been used as a substitute for platelets.

Materials. Antihemophilic Factor (AHF) and Plasma Accelerator-Globulin (PACG): The method used for preparation of these 2 factors was a combination of the procedures described by Bidwell(4) and by Lewis and Ware(5). BaSO₄-treated oxalated bovine plasma was diluted 1:20 with distilled water and the pH adjusted to 5.5. Precipitate from 200 ml plasma was dissolved in 90 ml of 0.9% NaCl (pH 7.0) and the solution ad-

* For a different terminology of factors referred to in this paper reference is made to Appendix, The Coagulation of Blood. Methods of Study. Edited by L. M. Tocantins, Grune and Stratton, N. Y., 1955.