

tion. The onset of POz:N220 occurred on the average 71.1 msec after the reproduced rhythm, and we assume that this interval included the verification of the 1 second that elapsed from the preceding visual stimulus. Therefore, the onset of POz:N220 might mark the beginning of the identification stage, and its offset either the end of template-matching or response selection (20). Thus the duration of POz:N220 may be considered an independent variable indicating on-line perceptual processing before P300 generation.

Although the duration of Cz:N265 did not vary with RT, its onset, peak, and offset latencies did ( $r = .58, .57$ , and  $.56$ , respectively). To show the effect of increasing RT on POz:N220 and Cz:N265 more clearly, the trials were averaged according to RT quartiles. The duration of POz:N220 and the peak latency of Cz:N265 were nearly twice as long in the fourth quartile as in the first (Fig. 2). It also appears that Cz:N265 (central sharp peak) occurred at the end of the parieto-occipital wave, which appeared between the reproduced rhythm and the subject's motor response.

The temporal relations between POz:N220 and Cz:N265 might aid in choosing between serial and parallel models of human information processing. Whatever underlying process is reflected by Cz:N265, probably orientation (21), it appears to be concurrent with the processes reflected by the last part of POz:N220 and sometimes with those responsible for the central initiation of the movement. However, these parallel processes are not independent since the onset and offset values of POz:N220 and Cz:N265 covary and are related to the motor response. Thus, from a theoretical point of view, the relationships between these cerebral and behavioral events support parallel contingent or cascade types of models (22). In the cascade model all processing stages operate continuously, and information, as it becomes available, is transferred from one to the next. In our view, a part of these information transfers and their subsequent processing could be reflected by the brain waves we have described.

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12. A number of maps did not show all four waves well; however, two negative foci followed by two positive foci appeared in the grand mean of 294 trials (free of artifact and eye movements), although they were not clearly differentiated. Because of the rapid potential changes between N200's and P300's, the offsets (zero crossing) of N200's were easily recognized. The parieto-occipital N200 onset was always the baseline zero crossing. The central N200 onset was more difficult to determine since this wave developed concurrently with the end of the parieto-occipital one. Thus both potentials added partially and the longer the parieto-occipital N200 lasted the more the onset of the central N200 was shifted away from the baseline. For this reason the beginning of the sharp central peak was not always a zero crossing.
13. These waves were labeled as recommended by E. Donchin, W. Ritter, and W. C. McCallum in *Event-Related Brain Potentials in Man*, E. Callaways, P. Tueting, S. H. Koslow, Eds. (Academic Press, New York, 1978), p. 349].
14. The POz:N220 showed a significant statistical difference from the Cz:N265 (correlated, paired  $t$ -tests,  $n - 1 = 106$ ) for onset latency ( $t = 12.9$ ,  $P < .001$ ), peak latency ( $t = 3.31$ ,  $P < .005$ ), peak location ( $t = 24.3$ ,  $P < .001$ ), and duration ( $t = 21.6$ ,  $P < .001$ ).
15. The correlation, number of trials, and two-tailed  $P$  values, when statistically significant, for each subject were: M.M., .81, 22,  $P < .001$ ; H.L., .36, 14; J.F., .39, 14; J.P.J., .69, 8; C.M., .26, 18; R.R., .21, 21; B.R., .25, 10; and all trials, .61, 107,  $P < .001$ .
16. Correlations between rhythm and Cz:N265 onset: M.M., .30; H.L., .39; J.F., .27; J.P.J., .68; C.M., -.19; R.R., .22; B.R., .55; and all trials, .33. Correlations between onsets of both Cz:N265 and POz:N220: M.M., .49 ( $P < .05$ ); H.L., .46; J.E., .56 ( $P < .05$ ); J.P.J., .82 ( $P < .05$ ); C.M., .52 ( $P < .05$ ); R.R., .34; B.R., .50; all trials, .57 ( $P < .001$ ). Correlations between offsets: .48, .46, .42, .89, .57, .41, .40, and .57 for all trials.
17. Correlations for POz:N220 and Cz:N265, respectively: M.M., .65 ( $P = .001$ ); .04; H.L., .57 ( $P < .05$ ); .06; J.F., .57 ( $P < .05$ ); .51; J.P.J., .63, -.81 ( $P < .05$ ); C.M., .48 ( $P < .05$ ); -.26; R.R., .77 ( $P < .001$ ); .38; B.R., .46, .09; and all trials, .57 ( $P < .001$ ), .09.
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## Dietary Restriction in Mice Beginning at 1 Year of Age: Effect on Life-Span and Spontaneous Cancer Incidence

**Abstract.** Lifelong dietary restriction beginning at 3 to 6 weeks of age in rodents is known to decelerate the rate of aging, increase mean and maximum life-spans, and inhibit the occurrence of many spontaneous cancers. Little is known about the effects of dietary restriction started in middle age. In the experiments now reported the food intake of 12- to 13-month-old mice of two long-lived strains was restricted by using nutrient-enriched diets in accordance with the concept of "undernutrition without malnutrition." The mice on the restricted diet averaged 10 to 20 percent increases in mean and maximum survival times compared to the control mice. Spontaneous lymphoma was inhibited by the food restriction.

Rats and mice given restricted diets from about the age of weaning (3 to 6 weeks) show extended mean and maximum survival times (1) and a decreased incidence or delayed onset of several diseases of old age (2). Other strategies for delaying aging in rodents (for example, administration of antioxidants or hormones) differ from weaning-initiated dietary restriction in that they do not cause clear-cut increases in maximum

longevities (3) or inhibit age-related increases in mortality rates (4). Old rodents that have been subjected to restricted diets since weaning show more youthful physiologic (5) and immunologic (6) responses than do age-matched controls. Although underfed rodents consume fewer calories than control animals (25 to 50 percent less in most studies), intakes of other essential nutrients (such as vitamins, salts, and protein)

may be maintained by diet enrichment. "Undernutrition without malnutrition" is the key concept in increasing life-span by dietary restriction.

Little is known about aging processes in rodents subjected to dietary restriction from midway through their usual life-span or later. Such adult-initiated

dietary restriction (considered here as starting at or beyond 10 months of age) increases mean survival times (7-11), but the findings on maximum life-spans are less convincing (12). Shortened survival has also been observed in rodents on restricted diets since adulthood (13). We decided that the influences on survival of

dietary restriction initiated in adults warranted further study because in previous studies (i) the dietary restriction was imposed abruptly rather than gradually; (ii) the diets were not enriched, so that some of the animals may have been subjected to malnutrition; and (iii) obese rats were often used (14), in which case the effects of the restricted diet may have been due to the inhibition of obesity. We conducted experiments with male mice from two long-lived strains, (C57BL/10Sn × C3H/HeDiSn)<sub>F</sub><sub>1</sub>, here referred to as B10C3F<sub>1</sub>, and C57BL/6J (referred to as B6). At 12 to 13 months of age the mice were subjected to gradual dietary restriction on nutrient-enriched diets, on which they were maintained until they died. The mean and maximum life-spans of these animals increased by an average of 10 to 20 percent. Also, spontaneous lymphoma was inhibited by the under-

feeding. The B10C3F<sub>1</sub> mice were bred in our colony and weaned at 3 to 4 weeks of age. The males were housed in plastic cages (four to six animals per cage) and given free access to Purina Lab Chow. At 12 to 13 months of age, 135 males were individually caged and fed semipurified diets (15). Control mice (*N* = 68) were fed enough of diet 1 (≅ 160 kcal/week) to maintain their initial body weights throughout much of their subsequent life-spans. The mice to be restricted in their diet (*N* = 67) received diet 2 (≅ 115 kcal/week) for 1 month and diet 3 (≅ 90 kcal/week) thereafter (15). Except for the cages being changed and body weights being recorded, the animals were undisturbed. Carcasses were frozen and later inspected grossly for signs of cancer.

The B6 mice were purchased at 10 to 11 months of age from Charles River Laboratories, where they had been given free access to the Charles River 4RF diet since weaning. We housed the B6 mice in groups of four to six and gave them free access to Purina Lab Chow for 2 months. We then set up individually caged control mice (*N* = 24) and mice to be subjected to dietary restriction (*N* = 29). The B6 mice were fed the same diets as were the B10C3F<sub>1</sub> mice but they received lesser amounts (15). The enrichment of casein, vitamins, salts, brewer's yeast, and zinc oxide in diet 3 led to similar intakes of these ingredients for B10C3F<sub>1</sub> mice on diets 1 and 3; however, B6 mice on diet 3 consumed more (≅ 30 percent) of these ingredients than did B6 controls (15).

The B10C3F<sub>1</sub> mice entered the study averaging about 42 g (range 31 to 53 g). Most mice weighing more than 45 g

Table 1. Mean life-spans and incidence of spontaneous cancer in B10C3F<sub>1</sub> mice fed on control and restricted diets.

Type of tumor*	Incidence (%)		Mean age of death† (months)	
	Control	Restricted	Control	Restricted
Hepatoma	43	40	33.9 ± 0.8	35.1 ± 1.0
Lymphoma	47‡	31	31.9 ± 0.9§	36.2 ± 1.2
Lung	12	6	34.4 ± 1.7	38.6 ± 2.2
Multiple	16‡	6	34.1 ± 0.8§	40.3 ± 1.9
No tumor	13‡	25	33.7 ± 2.4§	41.3 ± 0.9

\*Data for 68 control mice and 67 underfed mice. Tumors were observed less frequently in other sites such as kidney (one restricted mouse), brain (one restricted mouse), and adrenal (one control mouse). Lymphomas usually involved either the spleen (57 percent of lymphomas in controls as opposed to 33 percent of the restricted mice) or lower gastrointestinal tract (38 percent controls; 57 percent restricted mice). Both the spleen and gastrointestinal tract were involved in three control mice. Also seen were omental lymphomas (two control mice) and thymomas (two restricted mice). Mice with multiple tumors bore two distinct tumor types at autopsy (mice with lymphoma involving several sites not included). †Mean ± standard error. These comparisons between restricted mice grouped according to type of tumor were significantly different (two-tailed *t*-test): no tumor > hepatoma (*P* < .001) and lymphoma (*P* < .01). Mean life-spans did not differ significantly between any of the five control subgroups. ‡These three intergroup comparisons failed to attain statistical significance (*P* < .08) based on testing for significance of difference between two proportions. §Significantly less than the value for mice on the restricted diet (*P* < .01). Mice with multiple tumors bore two distinct tumor types at autopsy (mice with lymphoma involving several sites not included).

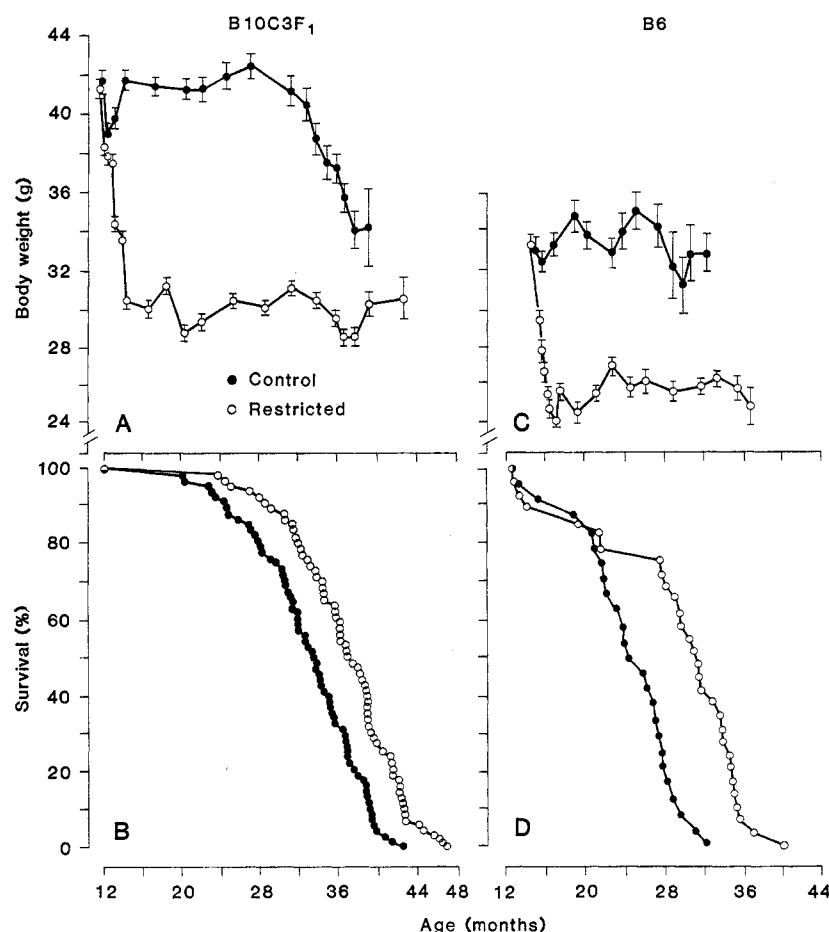


Fig. 1. Body weights and survival of B10C3F<sub>1</sub> mice (A and B) and B6 mice (C and D) fed on control and restricted diets. Weights are plotted as means ± standard error for all mice alive at the indicated ages. Each point in the survival curves represents one mouse.

appeared obese (about 25 percent of the initial population). The controls maintained their weights until they were about 28 months of age but tended to lose weight thereafter (Fig. 1A). The mice on the restricted diet stabilized at 29 to 31 g after about 2½ months of restriction; they also tended to maintain a healthy appearance longer than controls and showed longer life-spans (Fig. 1B). The first deaths occurred at about 20 months of age for controls but not until 25 months for the underfed mice. The mean survival time for all of the B10C3F<sub>1</sub> controls was 33.0 ± 0.7 months (± standard error) as opposed to 36.9 ± 0.7 months for all the restricted mice (12 percent increase). The mean survival time for the longest lived 10 percent of each population (N = 7) was 40.6 ± 0.5 months for controls and 45.1 ± 0.6 months for the underfed mice (11 percent increase). These two indices of longevity were greater for the underfed mice (P < .001; two-tailed t-test for two means). The survival curves appeared similar in shape for the two groups but separated by a 3- to 4-month interval.

The B6 mice averaged about 32 g at the beginning of the study (range 22 to 41 g, with the 22-g mouse dying within 2 weeks). Only one B6 mouse looked obese. Mature body weights (Fig. 1C) for controls were maintained over the life-span. The B6 mice on the restricted diet stabilized at about 25 g after 2 months of underfeeding and survived longer than the B6 controls (Fig. 1D). In contrast to the B10C3F<sub>1</sub> mice, a few B6 males (two controls and three on the restricted diet) died early (before they were 16 months old). However, 12 controls died between the ages of 22 and 28 months, whereas none of the underfed mice died in this period. Mean survival was 24.9 ± 0.9 months for all of the B6 controls as opposed to 29.9 ± 1.4 months for all the restricted B6 mice (20 percent increase). Mean survival for the longest lived 10 percent of each B6 group (N = 3) was 31.5 ± 0.5 months for controls, and 38.2 ± 1.4 months for the underfed mice (21 percent increase). Again, both survival parameters were greater for the underfed mice (P < .02). The survival curves appeared similar in shape for the two groups but separated by a 5- to 8-month interval.

Spontaneous cancer patterns for B10C3F<sub>1</sub> mice are given in Table 1. The overall incidence of cancer was 87 percent for the controls and 75 percent for the underfed mice. This lower incidence of cancer in underfed mice approached statistical significance (z = 1.91, P <

.06, two-sided) as did the lower incidences of lymphoma (z = 1.80, P < .08, two-sided) and multiple tumors (z = 1.85, P < .07, two-sided). Neither the hepatoma incidence nor the mean age of death for hepatoma-bearing mice was influenced by the diet. The mean longevity for the underfed mice with lymphoma, multiple tumors, or no tumor significantly exceeded that of controls in each category. No tumor was found in ten of the 14 longest lived B10C3F<sub>1</sub> mice on the restricted diet. Tumor status did not influence control longevity.

Spontaneous cancer incidence was evaluated in the B6 mice that died after 22.5 months of age. Tumors were found in six of 23 underfed B6 mice (26 percent) as opposed to nine of 17 controls (53 percent) (z = 1.73, P < .09, two-sided). Lymphoma was the most common tumor for controls (eight of nine tumors). The underfed mice bore hepatomas (N = 3), lung tumors (N = 2), and lymphoma (N = 1).

These results indicate that restricted feeding beginning in adulthood can increase mean and maximum longevity in mice, even in the more long-lived B10C3F<sub>1</sub> strain (see controls in Fig. 1). The mean survival of the B6 mice on the restricted diet (about 25 months) was close to 2 months longer than the average mean survival of the normally fed B6 males from the seven colonies reviewed by Goodrick (16); however, survival times similar to those of our B6 mice on the restricted diet have occasionally been reported for B6 males on normal diets (17). Stresses associated with shipment or changed cagemates (some of them aggressive) could possibly have reduced B6 longevity. The improved survival observed in the underfed mice of both strains in our experiments occurred by way of a delayed onset of age-dependent increases in mortality.

Recently, we reported (18) that male (C3H.SW/Sn × C57BL10.RIII/Sn)F<sub>1</sub> mice maintained on restricted diets from 12 months of age showed, relative to controls, (i) a lessening of certain age decrements in immune responses and (ii) reduced tumor incidence in those killed between 19 and 25 months of age. These results, together with those reported here, indicate that appropriate restriction of the diet, even when started in middle-aged mice, can inhibit cancer and extend the average and maximum life-span.

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15. Three semipurified diets were prepared monthly and stored at 4°C. Diet 1 was fed to control animals; diet 2 was fed for only the first month of restriction followed thereafter by reduced amounts of diet 3. Levels of casein, salts, vita-

mins, brewer's yeast, and zinc oxide ranked the diets as  $3 > 2 > 1$ . These nearly isocaloric diets ( $\approx 4$  kcal/g) were composed of the following (grams of ingredient per kilogram of diet 1, 2, and 3, respectively): casein, vitamin-free test, 200.0, 280.0, and 350.0; cornstarch, 260.8, 192.6, and 157.6; sucrose, 260.8, 192.6, and 157.6; corn oil (Mazola), 135.0, 135.0, and 135.0; nonnutritive fiber, 56.4, 51.9, and 40.0; salt mixture (U.S. Pharmacopoeia XIV), 60.0, 102.0, and 110.0; vitamin mixture, 23.0, 39.0, and 42.2; brewer's yeast, 4.0, 6.9, and 7.4; zinc oxide, 0.05, 0.08, and 0.10. The B10C3F<sub>1</sub> control mice were fed diet 1,  $\approx 40$  g/week [7 g daily on Monday through Thursday and 12 g on Friday ( $\approx 160$  kcal/week)]. For the first month of restriction the B10C3F<sub>1</sub> mice received diet 2, about 29 g/week [6 g daily on Monday through Wednesday and 11 g on Friday (115 kcal/week)]; thereafter they received diet 3, about 22 g/week [6 g on Mondays and Wednesdays and 10 g on Fridays (90 kcal/week)]. The B6 control mice were fed diet 1, about 27 g/week [4 g daily on Monday through Thursday and 11 g on Friday

(110 kcal/week)]. For the first month of restriction the B6 mice received diet 2, about 23 g/week [5 g daily on Monday through Wednesday and 8 g on Friday (90 kcal/week)]; thereafter they received diet 3, about 20 g/week [6 g on Monday and Wednesday and 8 g on Friday (80 kcal/week)]. Casein, fiber, salt mixture, vitamin mixture, and brewer's yeast were purchased from ICN Pharmaceuticals (Cleveland, Ohio); the other ingredients were bought locally.

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## Alterations in Precision of the Crossed Retinotectal Projection During Chick Development

**Abstract.** Chick embryos received partial eye lesions. Examination of the embryos early in development showed the partial retina projecting across the entire tectum. At later stages the partial retina projected only to the appropriate portion of the tectum. The results suggest that the retina initially projects diffusely to the tectum and that the topographically aberrant projections are eliminated during subsequent development.

Retinal ganglion cells in the vertebrate eye project to visual nuclei in the brain, such as the optic tectum, in an orderly topographical fashion. A major problem in developmental neurobiology is to determine how these orderly projections develop. Since highly ordered optic pathways and terminations have been observed in a number of adult animals (1), it has been suggested that optic axons grow from the eye in an orderly fashion and maintain this order to the terminal nuclei (2). Another possibility, however, is that optic axons grow to the terminal nuclei in a diffuse manner and interactions in the terminal field somehow refine the projection into the ordered pattern of the adult. The two possibilities have not been adequately tested.

Crossland *et al.* (3) addressed this problem in the developing retinotectal pathway of the chick embryo. Partial ablation of an optic cup during the third day of incubation resulted in the absence of a retinal projection to a portion of the contralateral tectum at 18 days. The region of the tectum lacking innervation corresponded topographically to the ablated region of the eye. Since projections from the remaining portion of the eye were not found in inappropriate tectal regions, it was concluded that at least quadrantal topographic order was maintained in the developing retinotectal system. This experiment did not, however, rule out the possibility that transient projections of broad distribution or not conforming to the topographic order

were present before day 18 and subsequently disappeared. In the present experiment, chick embryos with partial retinal ablations were examined at earlier developmental stages to determine whether the projection pattern of retinal ganglion cell axons to the tectum is initially broad and subsequently refined to the mature pattern or whether the projection forms a tightly ordered map from the beginning.

Fertilized White Leghorn chicken eggs were incubated in a forced-draft incubator at 37°C. On the third day of incubation the eggs were removed from the shell and transferred to an egg culture chamber (4). At this time the superior or inferior nasal quadrant of the right eye of experimental embryos was burned to the point of bleaching with a fine-tipped electrocautery. Care was taken not to make a hole in the eye, since this results in developmental retardation of the entire retinotectal projection (5). The embryos were maintained in a forced-draft tissue culture incubator at 37°C, 95 percent humidity, and 1 percent CO<sub>2</sub> for the duration of the experiment.

Horseradish peroxidase (HRP) was used as an anterograde tracer to map the retinotectal projection. On day 10, 12, 14, or 16 of development the embryos were injected in the right eye with 0.5 to 2  $\mu$ l of 30 percent HRP (Boehringer Mannheim) in 2 percent dimethyl sulfoxide and saline. After 12 hours the embryos were perfused through the left ventricle with 0.5 percent glutaraldehyde in phosphate buffer followed by 2 percent buffered glutaraldehyde. The brains were removed and cut into 40- $\mu$ m serial sections. The sections were reacted with tetramethylbenzidine and hydrogen peroxide and counterstained with neutral red (6), and the tecta were reconstructed with a drawing tube attached to a microscope. The blue reaction product was plotted to show the distribution of the retinal projection. Normal embryos without lesions were treated in a similar manner to serve as controls. There were 52 experimental embryos and 43 control embryos.

The distribution of HRP reaction product revealed a heavy projection from the injected eye to the contralateral tectum. In 10-day control embryos this projection covered all but about the caudal quarter of the tectum (Figs. 1A and 2A). In 90 percent of the 12-day control embryos and all the 14- and 16-day control embryos the projection covered the entire tectal surface. This pattern is consistent with the results of previous developmental studies (5, 7).

In experimental embryos killed on day

Fig. 1. Diagrams showing retinal innervation of the tectum, as reconstructed from stained serial sections. Cross-hatching indicates normal retinal input; hatching, reduced retinal input; and white, no retinal input. (A) Innervation in a normal embryo on embryonic day 10. (B) Reduced retinal input to the caudal-inferior tectum in a 10-day embryo given a superior nasal retinal lesion on day 8. (C) Similarly reduced input resulting from a lesion on day 3. (D) Lack of retinal projection to the caudal-inferior tectum in a 16-day embryo given a superior nasal lesion on day 3.

