DNA (Cell Number) in Neonatal Brain: Second Generation (F_2) Alteration by Maternal (F_0) Dietary Protein Restriction

Abstract. *Female rats were maintained on a protein-restricted diet 1 month prior to mating and throughout pregnancy. Their female offspring were maintained on a normal diet from birth or from weaning and were mated with normal males. The second generation offspring at birth still had significantly lower cerebral weight and total cerebral DNA (cerebral cell number).*

We have reported (*1*) that when female rats were maintained on a low (8 percent) protein diet 1 month prior to mating and throughout pregnancy, their offspring at birth (when the number of neurons becomes final) had significantly smaller body weights, cerebral weights, cerebral DNA (cell number), and cerebral protein. These results were confirmed in other laboratories (*2, 3*). It also has been reported that the effects of protein restriction (*4*) or stress (*5*) during pregnancy can influence the behavior of rats even in the second generation.

In the present work we have investigated the effect of maternal (F_0) dietary protein restriction on neonatal cerebral DNA (cell number) and protein of the second generation (F_2) offspring. To this aim, we have maintained female rats (F_0) on a low (8 percent), or normal (20.5 percent) protein diet, 1 month prior to mating and throughout pregnancy, as described previously (*1*). The offspring of animals maintained on an 8 percent protein diet will be referred to as "experimental F_1 newborns"; the offspring of animals maintained on a 20.5 percent protein diet, as "control F_1 newborns." Immediately after birth, the experimental F_1 newborns were divided into three groups (Table 1): group A, experimental F_1 newborns nursed by their own mothers maintained on the protein-restricted (8 percent) diet; group B, experimental F_1 newborns also nursed by their own mothers but changed to the normal protein diet; group C, experimental F_1 newborns fostered by control mothers maintained on the normal protein diet. In order to determine the effects of dietary protein restriction during weaning only, the following two groups were included: group D, control F_1 newborns fostered by experimental mothers maintained on the protein-restricted (8 percent) diet; group E, control F_1 newborns nursed by their own mothers (control F_0) but changed to the protein-restricted (8 percent) diet.

Table 1. The effect of restriction of maternal (F_0) dietary protein on newborns in second generation (F_2). V, average value, ± standard deviation; Δ, difference between experimental and control, in percentage of control; *P*, probability.

Group*	Number of		Item	F_2 (neonatal)			
				Weights (g)		Cerebral content	
	F_1♀	F_2		Body	Cerebral hemispheres	DNA (μg)	Protein (mg)
A	8	59	V	6.1 ± 0.75	0.1682 ± 0.0.119	562 ± 43,2	8.75 ± 0.866
			Δ	0	−1	−6	0
			P		.4 > *P* > .3	< .001	
B	10	63	V	5.7 ± 0.52	0.1578 ± 0.0146	565 ± 34.6	8.29 ± 0.782
			Δ	−7	−7	−6	−5
			P	< .001	< .001	< .001	.01 > *P* > .001
C	8	59	V	5.9 ± 0.45	0.1587 ± 0.0159	555 ± 32.3	8.19 ± 1.090
			Δ	−3	−7	−7	−6
			P	.05 > *P* > .02	< .001	< .001	.01 > *P* > .001
D	8	50	V	6.3 ± 0.80	0.1689 ± 0.0173	557 ± 37.5	8.73 ± 0.924
			Δ	+3	0	−7	0
			P	.2 > *P* > .1		< .001	
E	6	48	V	5.9 ± 0.656	0.1599 ± 0.0180	569 ± 41.3	8.30 ± 1.18
			Δ	−3	−6	−5	−5
			P	.17 > *P* > .05	.01 > *P* > .001	< .001	.05 > *P* > .02
Control	14	74	V	6.1 ± 0.52	0.1702 ± 0.0128	598 ± 32.6	8.75 ± 0.805

* See text for description of groups.

Animals that were maintained on the normal protein diet and bred in our colony for 22 generations served as controls. After weaning, all F_1 animals were maintained on the normal protein diet permanently, at which time all animals were coded to eliminate any possibility of bias. Upon reaching maturity, F_1 females were mated with normal males. In both F_1 and F_2, the mortality rate at birth was well within the normal range. Out of a total of 540 F_2 neonatal animals, 353 were weighed, and their cerebral hemispheres (without olfactory lobes) were dissected out, weighed, and homogenized for determination of DNA and protein content, as reported previously (*1*). The remaining newborns were allowed to reach maturity for further studies. From the DNA content, the total number of neonatal cerebral cells can be calculated as described previously (*1, 6*).

It can be seen (Table 1) that in all groups the total neonatal cerebral DNA (cell number) in F_2 animals was lower than that of the controls, and the difference was statistically highly significant. The decreases in cerebral weight and cerebral protein were significant in groups B, C, and E.

Malnutrition could not have caused a genetic effect. It may be of interest to add that when F_1 males from group A were mated with normal females, no effects on their offspring could be demonstrated.

In the groups previously discussed, one possible explanation could be that the effect on F_2 brains was due to poor lactation of F_0 nursing mothers that were protein-restricted before delivery. That this was not the case is apparent from group C, in which the effects on the brain in F_2 were essentially the same although the nursing mothers were never protein-restricted. Also, when the nursing mothers were protein-restricted both before and after delivery (group A), the effect on cerebral DNA (cell number) was not greater than in the case of protein restriction before delivery only (groups B and C).

Of other possible explanations of the effect on brain in F_2 animals, we favor the following one: Due to protein restriction of F_0 mothers before delivery, the F_1 offspring are born handicapped, not only with regard to the brain (*1*) but also in other respects. Zeman (*7*) and Hall and Zeman (*8*) have reported that the offspring of rats simi-

larly protein-restricted during pregnancy suffer from retardation of kidney development and altered kidney function. They may also lack the vigor to suckle (*9*). Lee and Chow (*10*) have reported that the restricted progeny showed reduced feed efficiency and low nitrogen balance; they excreted more amino acids than the controls. Thus, such progeny (F_1) may indeed suffer from *cryptic malnutrition*, even when postnatally given full access to normal food (groups B and C). Groups A, D, and E may represent a more overt malnutrition in F_1, since their nursing mothers were on a protein-restricted diet. In both cases, the progeny (F_2) of F_1 had a cerebral cell deficiency (Table 1), in accordance with our original findings (*1*).

Many investigators have implied that protein deprivation before and after birth results in mental impairment and brain cell deficiency in children [for reviews see (*2, 11*)]. If the results with rats have a bearing on the situation in humans, one must consider that, even after nutritional rehabilitation, a cerebral deficiency may last for at least one generation longer.

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DNA Polymerase Required for Rapid Repair of X-ray–Induced DNA Strand Breaks in vivo

Abstract. *A much higher yield of DNA single-strand breaks was obtained in the DNA polymerase–deficient mutant* Escherichia coli *K-12* pol A1 *after a given dose of x-rays than had been found before in* Escherichia coli. *The increased yield of single-strand breaks was due to the absence of a rapid repair system, which had not been described in* Escherichia coli *K-12. This absence probably accounts for the x-ray sensitivity of the* pol A1 *mutant. The rapid repair system can be reversibly inhibited in* pol+ *cells.*

There is currently a dichotomy in the published data concerning the radiation energy required to produce one single-strand break in DNA. Although many authors favor ~ 60 ev per break [see summary in (*1*)], those who have worked with the chromosome of *Escherichia coli* have all (*2–7*) obtained data quantitatively similar to those of McGrath and Williams (*2*), which yield values of 300 to 600 ev per break. Alexander *et al.* (*8*) have shown that in *Micrococcus radiodurans* approximately 90 percent of the DNA single-chain breaks produced by aerobic x-irradiation are rapidly repaired in buffer (*p*H 8.6) at 30°C but not at 0°C; the remaining breaks are repaired when the cells are subsequently incubated in growth medium at 30°C.

These observations with *M. radiodurans* have led to the suggestion (*1*) that in *E. coli* many of the DNA single-strand breaks are rapidly repaired before the samples can be analyzed by sedimentation. However, a greater number of breaks were not detected even when *E. coli* were held at 0°C under normal experimental conditions (phosphate buffer, *p*H 7) from the start of irradiation until their lysis on the alkaline sucrose gradient (*2, 5*), an observation that we have confirmed in this laboratory.

Radiation studies on the DNA polymerase–deficient mutant, *pol A1* (*9*), indicated that it was very sensitive to killing by aerobic x-irradiation, and there was an unexpectedly high yield of DNA single-strand breaks (for a given dose of x-rays) compared with the parent strain (*10*). This suggested that *pol A1* might be defective in a "rapid repair" system which had not been demonstrated in *E. coli*. In confirmation of this we observed that in *E. coli pol*+ cells the apparent energy requirement for the production of a single-strand break in DNA is dependent upon the irradiation conditions and the time taken thereafter to lyse the cells. When irradiated aerobically at 0°C in 0.05*M* phosphate buffer at *p*H 8.0, and lysed within about 1 minute of the end of irradiation, *pol*+ cells showed a requirement of 80 to 140 ev per break —about five times lower than previously observed at room temperature and *p*H 6.9 (*3, 7*)—and this energy requirement was comparable with that observed for *pol A1* cells. The yield of breaks in *pol A1* cells was not affected by these different irradiation conditions, nor by the speed with which subsequent lysis was carried out. These results suggest that the *pol A* gene controls a repair system which *rapidly* rejoins x-ray–induced DNA single-chain breaks, even in buffer.

The DNA polymerase–deficient mutant *E. coli* K-12 P3478 (*pol A1*) (*9*)

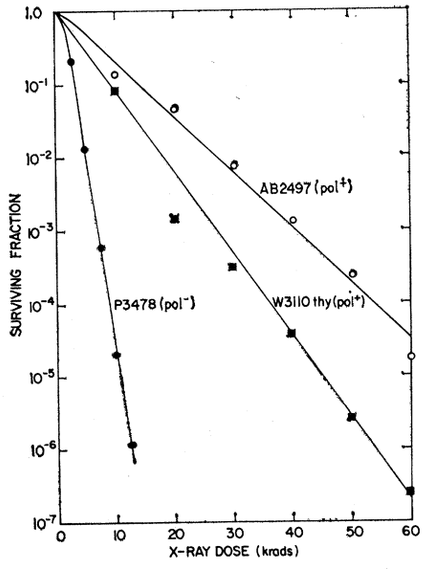

Fig. 1. Survival curves of *E. coli* K-12 *pol*+ (W3110 *thy* and AB2497) and *pol*− (P3478) cells exposed to 50-kv (peak) x-rays. Cells in exponential growth in glucose-salts-BCA medium were collected at ~ 10^8 cell/ml, washed, resuspended, and irradiated at room temperature (~ 25°C) in aerated 0.05*M* phosphate buffer, *p*H 6.9. They were then diluted in water and plated on glucose-salts-BCA-agar plates.