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20 March 1972; revised 12 May 1972 ■

Fertility Impairment in Mice on a Low Fluoride Intake

Abstract. *Female mice maintained on a low fluoride diet over two generations showed a progressive decline in litter production. Mice receiving the same diet supplemented with fluoride reproduced normally and at consistent intervals. Addition of fluoride to the intake of females with demonstrated impaired fertility restored their reproductive capacity.*

Satisfactory evidence of a deficiency state with respect to fluorine has not been demonstrated despite several investigations with this purpose (*1*). We report here a progressive decline in fertility and a delayed onset of sexual maturity in female mice maintained on a low fluoride intake.

Weanling female albino mice were divided randomly into two groups and were given either deionized water (58 animals) or deionized water containing 50 parts per million of fluoride as sodium fluoride (55 animals). Both groups received a low fluoride diet (*2*) containing 0.1 to 0.3 ppm of fluoride. Commercial rodent food contains 30 to 80 ppm of fluoride. The mice were first mated at 8 weeks of age and were maintained in groups of four earmarked females and one male per cage. The males were housed constantly with the females for 25 weeks. To confine the dietary effects to the females, the male mice were fed laboratory chow prior to breeding and were transferred every 6 weeks between the female animals that were on a low fluoride diet and those on a high fluoride diet. Litter production was observed over the 25-week period to a maximum of four litters. Each litter was reduced to six pups and all pups were removed at 5 days of age to promote a rapid breeding rate. The age at which each mouse gave birth to its first and subsequent litters and the number of pups per litter were recorded. Body weights of 100 newborn pups and 50 5-day-old pups from each fluoride group were obtained.

Litter production was also assessed in second generation females taken mainly (*3*) from the fourth litters of the first-generation mice. These mice were maintained on the same fluoride intakes as their mothers and an experimental plan identical to that for the first generation was followed. The second-generation group which was on a low fluoride diet contained 38 females and the group receiving a high fluoride diet contained 44 animals.

Mice in both generations of both fluoride groups weighed approximately 13 g at weaning and 25 g at 8 weeks, when they were first mated. The mean age at which mice gave birth to their first litter was not significantly different from 13 weeks for both generations of the high fluoride group and for the first generation of the low fluoride group. However, a highly significant ($P < .005$) delay in the birth of the first litter occurred in the second-generation animals on the low fluoride intake—16.0 ± 0.87 (standard error of the mean) weeks versus 13.0 ± 0.52 (S.E.M.) weeks for the high fluoride second-generation animals.

Fertility was essentially complete in both generations of mice in the high fluoride group (Table 1); 96 percent of the first-generation and 90 percent of the second-generation mice given the high fluoride intake produced four litters within the experimental period. Neither of these values is significantly less than 100 percent. However, mice in the low fluoride group showed a progressive impairment in reproductive capacity. Thus, while all animals of the first generation produced one litter, progressively fewer mice gave birth to additional litters and less than 50 percent produced four litters. The decrease in reproduction was more severe in the second-generation mice on the low fluoride intake and a progressive decline in litter production was again apparent. Almost 20 percent of animals in this group failed to produce even one litter and more than 50 percent failed to produce four litters. The differences in litter production between high and low fluoride groups were significant for the third and fourth litters of the first generation and for all four litters of

Table 1. Influence of maternal fluoride intake on litter production over two generations. The percentage of mice producing a given litter in the 25-week experimental period and, in parentheses, the number of mice at risk, is given for each litter. Significance level of differences between high and low fluoride groups was determined by comparison of binomial distributions (*8*). Abbreviation: n.s., not significant.

Litter	Percentage of mice producing litter		Significance levels
	High fluoride diet	Low fluoride diet	
	First generation		
First	98.1 (55)	100.0 (58)	n.s.
Second	98.1 (53)	91.4 (58)	n.s.
Third	96.0 (51)	72.7 (55)	$P < .001$
Fourth	96.0 (51)	48.1 (54)	$P < .001$
	Second generation		
First	97.7 (44)	81.5 (38)	$P < .05$
Second	95.2 (42)	66.7 (36)	$P < .001$
Third	95.1 (41)	54.2 (35)	$P < .001$
Fourth	90.0 (40)	45.7 (35)	$P < .001$

Table 2. Influence of fluoride on recovery of fertility. The table lists the number of mice at risk in parentheses and the percentage of mice producing a given litter in 20 weeks following transfer to a high fluoride intake or retention on the low fluoride intake. The 5-week transfer group represents those mice transferred to the recovery study after 5 weeks of breeding on a low fluoride intake without producing a litter. The 15-week transfer group was placed in the recovery study after producing only one or two litters in 15 weeks of breeding while on the inadequate diet.

Litter	Percentage of mice producing litter	
	High fluoride diet	Low fluoride diet
	5-week transfer	
First	100.0 (15)	78.6 (14)
Second	100.0 (15)	78.6 (14)
Third	93.0 (14)	61.5 (13)
Fourth	85.8 (14)	38.5 (13)
	15-week transfer	
First	100.0 (12)	91.0 (11)
Second	100.0 (12)	81.8 (11)
Third	100.0 (11)	63.6 (11)
Fourth	91.0 (11)	54.5 (11)

the second generation. No differences were found between the two groups in weight of the pups at birth (1.5 g) or at 5 days (3.6 g), or in the number of pups per litter (eight to ten).

The fluoride concentrations in the ash of the humeri of mice of the low fluoride group at the conclusion of the experimental period averaged 0.010 percent in the first generation and 0.009 percent in the second generation. The corresponding values for the high fluoride group were 0.78 and 0.77 percent.

The ability of added fluoride to restore normal fertility to mice previously rendered subfertile on a low fluoride intake was also investigated. Weanling female mice were maintained on the low fluoride diet plus deionized water and mated at 8 weeks of age, as before. After 5 or 15 weeks of exposure to males, female mice, of demonstrated impaired fertility (Table 2), were divided into two groups. Half of the animals at each breeding time period were transferred to an intake of 50 ppm of fluoride in the water, while the remaining animals were retained on the low fluoride intake. After 7 days separation from males (*4*), the mice were remated and litter production was assessed over 20 weeks.

Mice retained on the low fluoride intake continued to show impaired reproduction. Only 40 to 50 percent produced four litters during the 20-week recovery period (Table 2). By contrast, mice transferred to the high fluoride intake showed an improvement in litter production. In five instances all animals produced two or three litters and approximately 85 to 90 percent of these animals produced four litters in the 20-week period after transfer to the high fluoride intake. These last values are not significantly less than 100 percent, demonstrating that fertility was restored in this group.

The infertility resulting from a restricted intake of fluoride and the delay in production of the first litter in the second generation of low fluoride mice point clearly to a deficiency state with respect to fluoride. While complete infertility of all mice on the low fluoride intake was not demonstrated, it is possible that all mice in this group would have become infertile if the intake of fluoride could have been more severely restricted.

The failure of previous studies (*1*) to demonstrate a role of fluoride in reproduction may be attributed to the small numbers of animals involved and the short duration of the studies, since in this work the infertility developed slowly in each generation. The delayed production of the first litter in the second generation of the low fluoride group may have been a result of an irregular estrous cycle or the onset of sexual maturity may have been retarded. A similar finding in manganese deficiency has been ascribed to a delayed onset of sexual maturity (*5*).

The basis of the infertility in mice receiving a low fluoride intake is not known. Infertility is a relatively common manifestation of deficiency in trace elements, including deficiencies of copper, zinc, manganese, iodine, and selenium (*6*). Thus it may represent, at least in part, a nonspecific response to the stress of a nutritional deficiency.

This study demonstrates that fluorine satisfies the major criteria for an essential trace element: (i) A deficiency state, characterized by a delayed production of the first litter and a progressive infertility, has been produced in mice on a diet low in fluoride. (ii) The deficiency is prevented and cured by addition of fluoride alone to the diet. (iii) The deficiency correlates well with low tissue (bone) levels of fluoride. Thus, there is good evidence that fluorine is an essential element, at least in the diet of the mouse.

Note added in proof: After this report was sent to the printer we received a copy of a publication by Schwarz and Milne (*7*) in which it was found that addition of 2.5 to 7.5 ppm of fluoride to highly purified amino acid diets (basal fluoride contents, 0.04 to 0.46 ppm) significantly enhanced the growth rates of young mice given these diets and improved the pigmentation of their incisor teeth. The amounts of added fluoride are within the range of the fluoride contents of human diets.

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18 May 1972

Coordinated Development of β-Glucuronidase and β-Galactosidase in Mouse Organs

Abstract. *Changing concentrations of β-glucuronidase and β-galactosidase are coordinated during the development of mouse liver, heart, and brain. Although coordinate, the developmental patterns for the two enzymes are under independent control by genetic elements apparently linked to the respective structural genes.*

Mutations affecting developmental changes in enzyme concentration are known for mouse β-glucuronidase (*1*) and corn esterase (*2*). We now report that in mice the developmental pattern for β-glucuronidase is shared by β-galactosidase, and that an analogous developmental mutant of β-galactosidase exists.

β-Glucuronidase and β-galactosidase are easily detectable in liver, brain, and heart of mice of the DBA/2J and C57BL/KsJ strains as early as the 14th day of gestation. In each organ there is a distinctive pattern of changes in enzyme activities until stable adult levels are reached at 40 to 50 days of age (Fig. 1). Parallel development of the two enzymes in liver and heart is apparent. There is some difference in early development of the two enzymes in brain, but after 10 days of age the patterns are quite similar. β-Glucuronidase and β-galactosidase thus appear to share a common developmental program in mice of these strains.

The β-glucuronidase present in liver, brain, and heart of adult mice is coded