

References and Notes

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A Cytoplasmically Transmitted, Diet-Dependent Difference in Response to the Teratogenic Effects of 6-Aminonicotinamide

Abstract. *The frequency of congenital cleft palate produced by maternal treatment with 6-aminonicotinamide during pregnancy is lower in the C57BL/6J than in the A/J inbred mouse strain. In the C57BL/6J strain the frequency is lower when the mothers are maintained on Purina Lab Chow than when they are on Breeder Chow. A/J females do not show this effect of diet. There is a matroclinous reciprocal cross difference in frequency of induced cleft palate which persists in the back-cross when the F_1 mothers are maintained on Lab Chow, but not on Breeder Chow.*

The nicotinamide analogue, 6-aminonicotinamide, has been shown to produce clefts of the secondary palate (as well as other kinds of malformations) in the offspring of pregnant mice injected with it (1). The analogue also caused a transient paralysis of the mother several hours after treatment, and an increase in the frequency of resorptions. Concurrent administration of nicotinamide prevented both the teratogenic and maternal effects. When the

nicotinamide was given 2 hours after the 6-aminonicotinamide, cleft palates were produced, but there were no signs of maternal distress and no increase in resorption rate. This approach had the additional advantage that the embryo was exposed to the teratogen for a precisely defined period (2).

The highest frequency of cleft palate was obtained following treatment 13½ days after conception (3). At that time, the inbred A/J strain gave higher fre-

quencies of cleft palate after treatment than the C57BL/6J strain, and there was a matroclinous reciprocal cross difference in the frequency of induced cleft palate in the F_1 offspring of crosses between the two strains. That is, the offspring of A/J females mated to C57BL/6J males and treated on day 13½ of gestation had more cleft palates than those of C57BL/6J females mated to A/J males. F_1 females from A/J mothers and C57 fathers (AC) and from C57 mothers and A/J fathers (CA) were back-crossed to A/J males and treated with 6-aminonicotinamide. The frequency of cleft palates was higher in the offspring of AC females than in those of CA females. This suggested that part of the difference between the strains in response of the palate to this teratogen stemmed from factors that are transmitted through the cytoplasm (3). During subsequent study of this question the difference between the two types of back-cross surprisingly vanished. In retrospect, the disappearance of the effect seemed related to a change in diet from Purina Lab Chow to Purina Breeder Chow. Lab Chow is said to have about twice as much niacin as Breeder Chow (4).

The results of further experiments are presented in Table 1. The back-crosses described above were repeated on the two diets. Details of the methods used are presented in the publications cited. The cleft palate frequency induced by maternal treatment with 6-aminonicotinamide was the same in the offspring of the AC (42.9 percent) and CA (45.9 percent) females maintained on Breeder Chow and of AC females on Lab Chow (44.5 percent), but was significantly decreased in the offspring of CA females on Lab Chow (25.8 percent). There is therefore a difference which results in a lower frequency of cleft palate induced by 6-aminonicotinamide in the offspring of CA females than in those of AC females. This difference is manifested when the treated mothers are maintained on Lab Chow but not on Breeder Chow. It is not clear whether the difference resides in the response of the mother to the teratogen, or in that of the embryo. In either case, since the nuclear genes of the two types of F_1 females are presumably identical, the difference must be transmitted through a cytoplasmic factor.

Such a factor ought to be present in the inbred strains as well, and this ap-

Table 1. Frequencies of cleft palate following injection into female mice on day 13½ of gestation of 6-aminonicotinamide (6AN) dissolved in distilled water in a concentration of 225 mg/100 ml followed 2 hours later by nicotinamide (NIC) in a concentration of 85 mg/100 ml. Abbreviations: B.C., Breeder Chow; L.C., Lab Chow.

Cross		Diet	6AN (mg/kg)	NIC (mg/kg)	Number of females	Number of offspring	Cleft palate	
♀	♂						No.	%
AC	A	B.C.	19	7.3	16	133	57	42.9
CA	A	B.C.	19	7.3	18	157	72	45.9
AC	A	L.C.	19	7.3	23	200	89	44.5
CA	A	L.C.	19	7.3	30	295	76	25.8
A	A	B.C.	14.25	5.48	14	102	57	55.9
A	A	L.C.	14.25	5.48	14	82	53	64.6
C	C	B.C.	19	7.3	18	117	79	67.5
C	C	L.C.	19	7.3	15	108	12	11.1

pears to be the case. The offspring of C57BL/6J females, mated to C57BL/6J males, had a lower frequency of cleft palate induced by 6-aminonicotinamide when the treated females were maintained on Lab Chow (11.1 percent) than when on Breeder Chow (67.5 percent). In the A/J strain, on the other hand, the frequency of induced cleft palate is not significantly different in the offspring of females maintained on Breeder Chow (55.9 percent) and Lab Chow (64.6 percent).

These findings support the hypothesis that there is a factor, transmitted through the cytoplasm, which makes C57BL/6J mice on a Lab Chow diet more resistant than A/J mice to the cleft palate-producing effect of 6-aminonicotinamide. Since mitochondria are transmitted through the egg cytoplasm, and since 6-aminonicotinamide forms an inactive nicotinamide-adenine dinucleotide analogue that interferes with oxidative phosphorylation in mitochondria (5), it is reasonable to postulate that the cytoplasmic factor is associated with a difference in the mitochondria of the two strains (6).

This example of the extrachromosomal transmission of a metabolic characteristic adds another to the few cases of maternal inheritance reported in mammals (7). It also adds a new complexity to the question of gene-environment interactions in general and drug-induced malformations in particular.

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Metacontrast and Evoked Potentials: A Reappraisal

Abstract. Stimulation of the parafoveal retina may give rise to visual evoked responses generated in large part by stray light impinging upon the fovea. This effect appears to account for the absence of changes in the visual evoked response to parafoveal stimulation during metacontrast suppression. When the central retina is directly stimulated, the spatiotemporal interactions associated with brightness suppression during metacontrast may be readily demonstrated in a late component of the visual evoked response.

Schiller and Chorover (1) failed to detect changes in the human visual evoked response (VER) associated with the reduction of brightness induced by metacontrast. They concluded that the VER, although varying with alterations in stimulus intensity, does not necessarily reflect changes in subjective brightness. However, it is possible that the negative finding was due to the specific conditions of the previous study and that it does not imply a general insensitivity of the VER to brightness changes in metacontrast. Although the effectiveness of photic stimuli for eliciting VER's falls off rapidly with distance from the fovea (2), even stimuli projected onto the blind spot may produce evoked responses. These are apparently due to stray light, for they are eliminated by an illuminated background field (3). A likely reason for the failure of the VER's recorded in the previous study to reflect metacontrast suppression was the use of parafoveal stimuli presented on a dark background.

To evaluate the possibility that stray light striking the fovea might have contaminated the evoked responses recorded to parafoveal stimulation, we repeated the metacontrast experiments under the following four conditions. The disk and ring stimuli were projected onto the fovea and at a point 5° from fixation, in each case against both a dark background as in the previous study and against an illuminated surround about one-fifth as bright as the stimuli themselves (4). In addition to the 5° stimulus configuration employed by Schiller and Chorover, we used a disk and ring slightly less than half that size (2.35° in total subtense), providing a stimulus closer to foveal dimensions, yet producing a sizable VER at low stimulus intensities. Due to the limitations of our apparatus, we used a luminance (5.8 millilamberts) considerably less than that used in the previous study. This served to strengthen our results, since the absolute amount of stray light was reduced.

The VER's to presentation of a 5°

disk are shown in Fig. 1. The adapting field, which attenuates the effects of intraocular stray light (5), virtually eliminated the VER to the parafoveal stimulus, whereas it neither reduced the amplitude nor increased the latency of the foveal VER's. With the smaller stimuli, VER's to parafoveal stimulation were either small or absent, even without the illuminated surround. These results strongly suggest that VER's produced by the larger parafoveal stimuli were contaminated by stray light impinging upon the relatively potent fovea.

We found that subjective metacontrast suppression was readily observed under all four conditions, generally with delays between presentations of the disk and ring (disk-ring delay) of 30 to 90 msec. The VER's, however, showed patterns of behavior different from the perceptual effects. No change in the VER's to the parafoveal stimuli as a function of disk-ring delay was seen, confirming the observations of Schiller and Chorover. In contrast, there was a distinct alteration in the VER's to foveal stimulation during metacontrast suppression (Fig. 2). This consisted of a reduction in size of a VER compo-

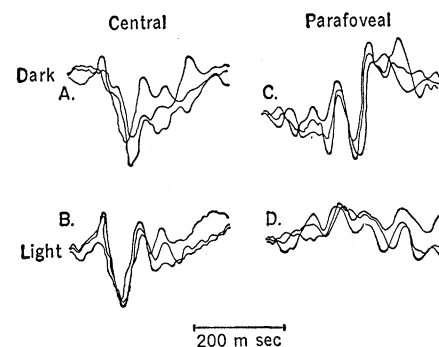


Fig. 1. Visual evoked responses for subject L.S. Stimulus diameter 5°, luminance 5.8 millilamberts. (A) Central projection, dark background; (B) central projection, adapting surround; (C) projection onto parafoveal region, 5° nasal to foveal center, dark background; (D) parafoveal projection, adapting surround. Time marker 200 msec. Positivity at superior occipital electrode represented by upward deflection.