

Alcohol-Induced Alterations in the Palate Epithelium of the Rat Fetus

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Maternal alcoholism (ethanol and sugar cane brandy) during gestation induces delayed cellular growth and differentiation in fetal rat palate epithelium, with increased nuclear, cytoplasmic and cellular volumes, increased epithelial and keratin thickness and decreased cellular numerical density.

Key Words: palate, rat, fetus, alcoholism, stereology.

Introduction

Prenatal exposure to alcohol was seen to result in pre- and postnatal growth deficiencies, increased fetal death and increased incidence of congenital abnormalities (Tze and Lee, 1975). This study was undertaken to provide details of the structural changes occurring in the palate epithelium of rats injected with ethanol and sugar cane brandy.

Material and Methods

Nulliparous female Wistar rats weighing 220-240 g were acclimated to laboratory conditions before mating. Vaginal smears were used for the determination of estrus activity in the female over a one-week period. All animals were bred overnight to males of the same strain. Appearance of sperm in the vaginal smear on the following morning established day 1 of gestation.

A 25% (v/v) ethanol or sugar cane brandy solution was injected intraperitoneally at a dose of 0.03 ml/g body weight at 9, 10, and 11 days of pregnancy. The control female was injected with saline at the same dose.

On day 20 of gestation, the pregnant rats were sacrificed by ether inhalation. The fetuses were freed from the uterus and decidua, and immediately removed with fine forceps and a pair of iridectomy scissors. Isolated fetuses were fixed for 24 h at room temperature

in a solution of 85 ml 80% alcohol, 10 ml formol, and 5 ml glacial acetic acid. After fixation, the heads were dissected and posteriorly embedded in paraffin. Histological sections, 6 μ m thick, were prepared and stained with hematoxylin and eosin.

Karyometry

A light camera (Zeiss, Jena), projecting images of the nuclei with a magnification of 2,600x, was used to obtain the nuclear volume of basal and spinous cells of the palate epithelium. The orthogonal diameters were measured in drawings of 50 nuclei per animal for calculation of nuclear volume by the formula proposed by Valeri et al. (1967):

$$V = 0.0297906 D_1.D_2.(D_1.D_2)^{1/2}$$

Stereology

Stereological analysis was performed with a curvilinear test system of Merz (1968). The grid had a test line system composed of semicircles in a square lattice. The following parameters were calculated after point counting (720 per animal): cytoplasmic and cellular volumes, nuclear/cytoplasm ratio, keratinized surface density, epithelial thickness, keratin thickness, free surface/basal surface ratio, and numerical density (Weibel, 1969).

Statistical analysis

Comparison of the results for the experimental groups and the controls was made by the nonparametric Mann-Whitney test.

Results

The mean fetal body weight was 5.18 g for the control group, 2.16 g for the ethanol-treated group and 2.49 g for the sugar cane brandy-treated group. The mean body weights of ethanol and sugar cane brandy groups were significantly smaller than that of the control group, whereas the difference between the treated groups was not significant.

Morphology

Histologically, the hard and soft palates of animals treated with ethanol or sugar cane brandy were thicker and less differentiated than in control animals (Figures 1-3). The epithelium was composed by larger cells, with scanty but larger light-stained nuclei. The keratohyaline granules were inconspicuous. The palate glands showed a reduced development (Figures 1B, 2B, 3B). Cleft palate was not observed in any animal of the experimental groups.

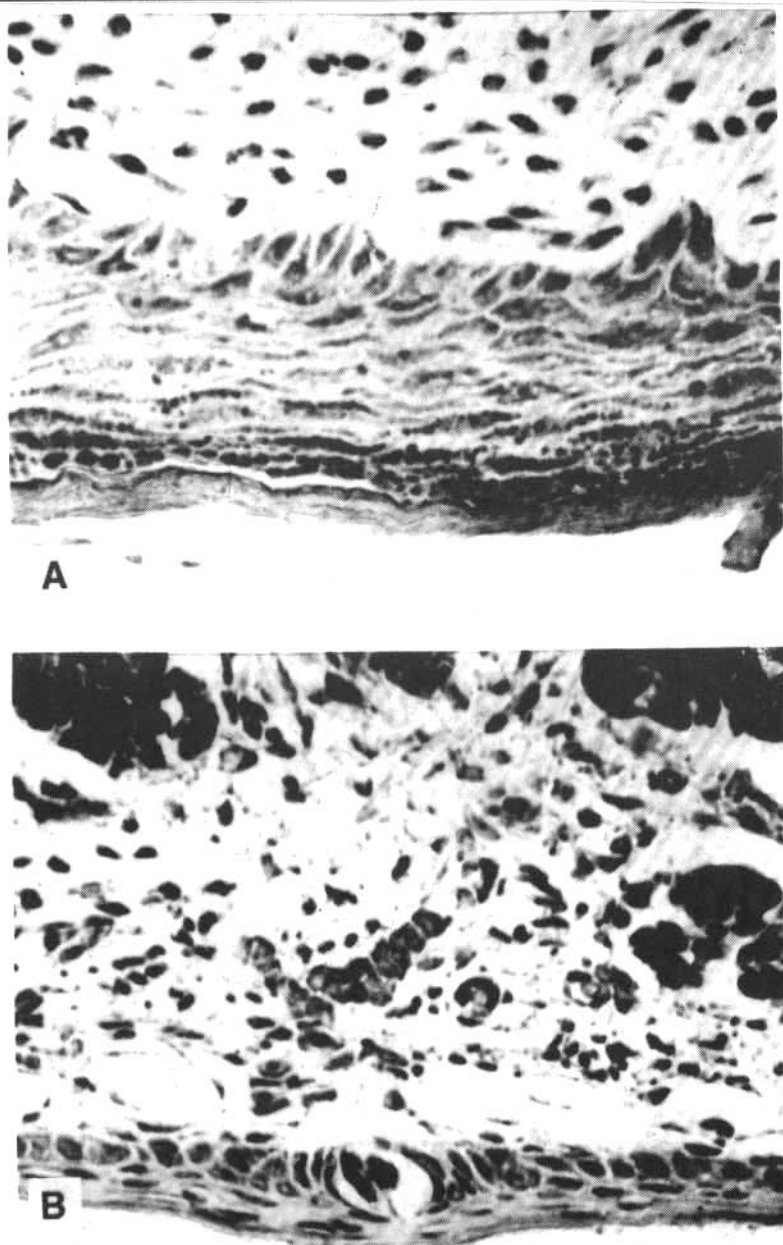


Figure 1 - Histological picture of hard (A) and soft (B) palate epithelium of the control fetus. Hematoxylin and eosin (40 X).

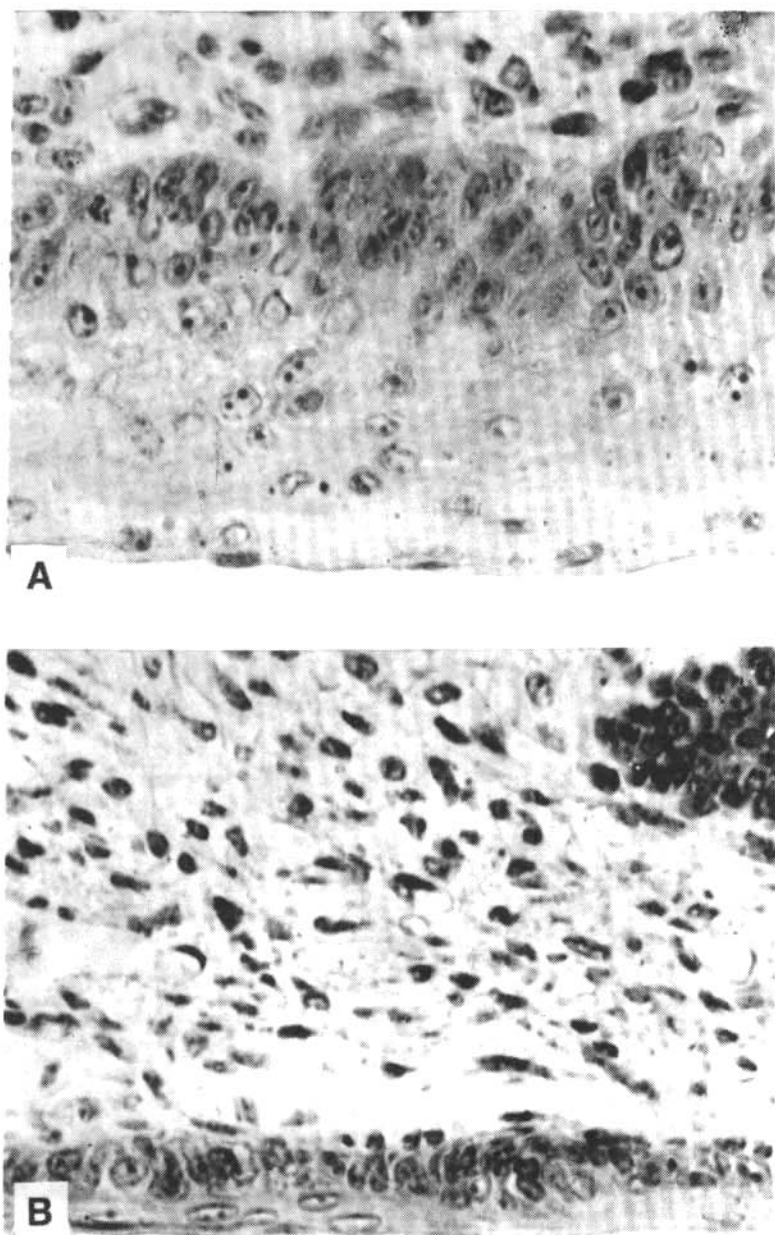


Figure 2 - Histological picture of hard (A) and soft (B) palate epithelium of the ethanol-treated fetus. Hematoxylin and eosin (40 X).

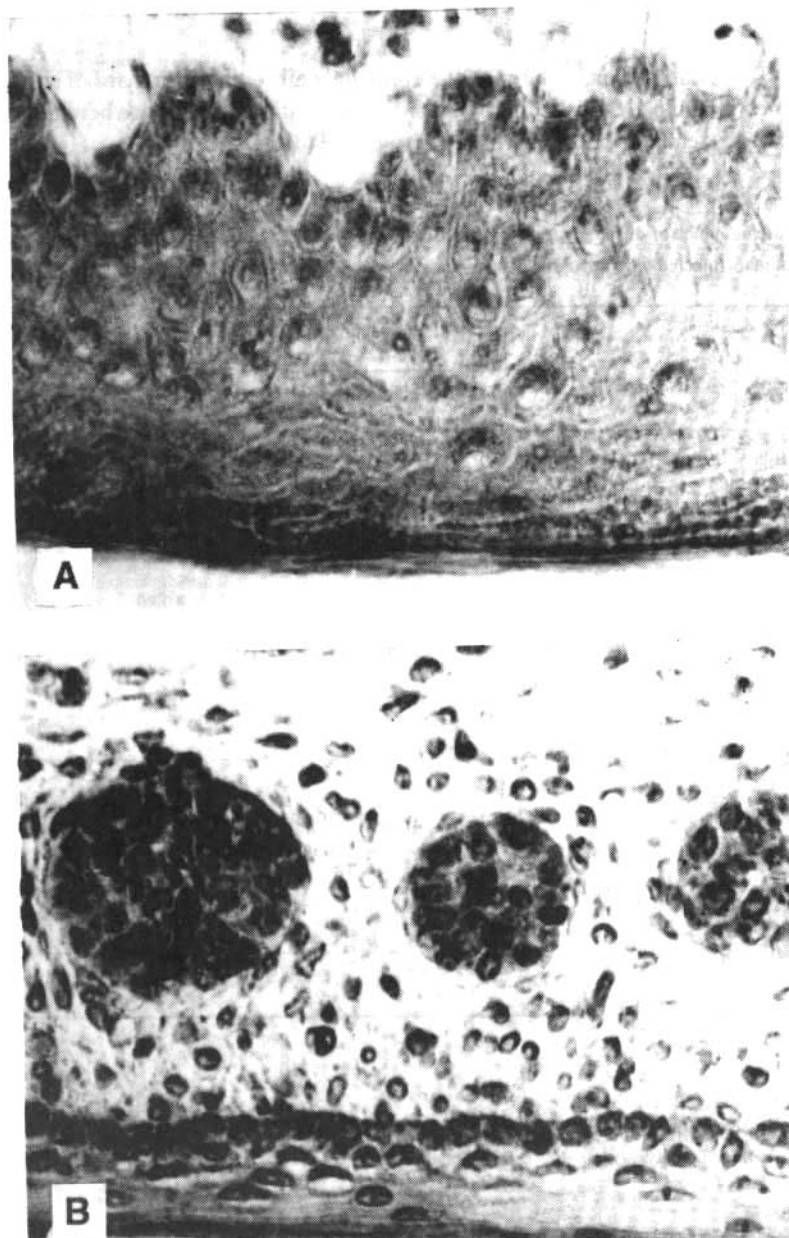


Figure 3 - Histological picture of hard (A) and soft (B) palate epithelium of the sugar cane brandy-treated fetus. Hematoxylin and eosin (40 X).

Nuclear volume

The mean nuclear volume of the epithelial cells either from hard or soft palate can be observed in Table 1. Statistical analysis showed significant difference between the control group and the treated groups only in the hard palate (Table 2).

Table 1 - Mean values of different stereological parameters studied in hard and soft palate epithelium of control rats and animals injected with ethanol and sugar cane brandy (SCB).

Parameter	Hard palate			Soft palate		
	Control	Ethanol	SCB	Control	Ethanol	SCB
Nuclear volume (μm^3)	73.56	134.09	110.58	45.22	80.40	71.11
Cell volume (μm^3)	507.53	844.12	749.30	275.38	414.04	449.84
Cytoplasm volume (μm^3)	433.97	710.03	638.72	230.16	333.64	378.73
Nuclear/cytoplasm ratio	0.1699	0.1900	0.1725	0.2040	0.2450	0.1874
Keratinous surface density (mm^2/mm^3)	12.38	0.91	0.94	19.80	1.82	0.00
Epithelial thickness (μm)	55.56	94.34	114.60	27.92	42.32	56.01
Keratin thickness (μm)	5.44	0.33	0.24	2.83	0.12	0.00
Free surface/basal surface ratio	0.9127	0.0653	0.0833	0.5893	0.0678	0.0000
Numerical density (N/mm^3)	2.03×10^6	1.26×10^6	1.43×10^6	3.96×10^6	2.49×10^6	2.27×10^6

Table 2 - Comparison of the results for the experimental groups: control x ethanol (C x E), control x sugar cane brandy (C x S), and ethanol x sugar cane brandy (E x S). Nonparametric Mann-Whitney test.

Parameter	Hard palate			Soft palate		
	C x E	C x S	E x S	C x E	C x S	E x S
Nuclear volume	2*	4*	8	7	8	8
Cell volume	1*	3*	10	4*	3*	8
Cytoplasm volume	0*	4*	10	5	2*	8
Nuclear/cytoplasm ratio	8	12	7	7	8	3*
Keratinous surface density	0*	0*	10	1*	0*	8
Epithelial thickness	0*	0*	5	0*	0*	0*
Keratin thickness	0*	0*	9	0*	0*	10
Free surface/basal surface ratio	0*	0*	10	0*	0*	8
Numerical density	1*	3*	10	4*	3*	8

* $P < 0.05$

Nuclear/cytoplasm ratio

The mean values of the nuclear/cytoplasm ratio in the epithelial cells from the hard palate (Table 1) showed no differences between groups (Table 2). The mean values of the nuclear/cytoplasmic ratio for the epithelial cells of the soft palate (Table 1), on the other hand, were significantly different only between the treated groups (Table 2).

Cytoplasm and cellular volume

The cytoplasm volume and the mean cell volume of the hard palate epithelium (Table 1) were significantly smaller in the control group than in either of the treated groups (Table 2). The cytoplasm volume of the soft palate epithelial cell (Table 1) showed significant difference only between control and sugar cane brandy groups (Table 2), whereas the mean cell volume (Table 1) was significantly smaller in the control group than in the treated groups (Table 2).

Density of keratinous surface

Table 1 shows the mean values of the surface density for the hard and soft palate keratinizing epithelium. Statistical analysis showed the existence of a significant difference between the control group and the treated groups, in hard and soft palate (Table 2).

Thickness of the epithelium

The hard palate epithelium (Table 1) was significantly thicker in treated groups than in control (Table 2). The thickness of the soft palate epithelium (Table 1) was significantly different in the three experimental groups, when compared to one another (Table 2).

Keratin thickness

Table 1 shows the mean values of the keratin thickness in the epithelia from hard and soft palate. Statistical analysis showed that the keratin layer is significantly thinner in the treated groups than in the control group, in the two palate regions studied (Table 2).

Free surface/basal surface ratio

Table 1 shows that the values for this ratio were significantly smaller in the treated groups than in the controls, either in hard or soft palate (Table 2).

Numerical density

The number of cells by cubic millimeter of epithelium of hard and soft palate (Table 1) were significantly smaller in the treated animals than in the controls (Table 2).

Discussion

In the present report, epithelial alterations were observed in hard and soft palates of fetuses whose mothers were injected with ethanol or sugar cane brandy during pregnancy. These alterations were statistically demonstrated on data obtained by karyometry (larger nuclear volume in the epithelia), and stereology (larger cell and cytoplasmic volumes, increased thickness, thinner keratin layer and smaller numerical density). All these findings demonstrate a delayed differentiation of the analyzed tissue in the treated animals.

These alterations were probably due to a diminution of the cell content of both DNA and RNA, as well as of proteins, in the growing fetus. The diminution of DNA, RNA and proteins suggests an impairment of cell division and protein synthesis.

It is known that alcohol can exert its prejudicial effects on fetal development by several mechanisms. Thus, it is well established that alcohol and its principal metabolite, acetaldehyde, rapidly cross the placental barrier (Ho et al., 1972), and that the two substances can be teratogenic, since it was demonstrated *in vitro* that they inhibit the synthesis of nucleic acid and proteins (Brown et al., 1979).

Ethanol, alone, is relatively non-toxic. Its direct pharmacologic effects result from narcosis of the cell membranes, giving rise to a general depression of the cell function. All cell types are affected by the same mechanism, but the effect is more evident in those cells with excitable membranes. When a large volume of ethanol is ingested and the metabolic pathways of the liver become saturated, the plasma level of acetaldehyde increases. Acetaldehyde is a very reactive compound, which affects many tissues joining its own effects and inherent toxicity to the effects and toxicity generally attributed to ethanol (Lieber, 1976).

Another important fact related to the effect of maternal alcoholism on the embryo and fetus is that the increase of maternal ethanol consumption has different consequences on the maternal and fetal organisms. Thus, as alcohol is very soluble in water and lipids, it easily crosses any biologic membrane, and rapidly equilibrates the total water volume in the mother-placenta-fetus trinomial. Nevertheless, according to the kinetics of the amniotic fluid circulation, and both the absence in the embryo and the small activity in the late fetal period of enzymes responsible for the biotransformation of drugs (Pikkarainen and Raiha, 1967), the amniotic fluid serves as an alcohol and acetaldehyde reservoir (Ho et al., 1972). In this manner, the action of these substances on the embryo or fetus is prolonged for a long time after their maternal absorption.

During normal fetal development, there is a continuous increase of the protein, lipid, and electrolyte content, as well as a gradual diminution of water in different tissues (Spray and Widdowson, 1950). In fetus under the action of ethanol, there is an increased content of water and sodium, and diminution of the content of free lipids (Abel and

Greizerstein, 1979). This fact can explain the increased volume of the epithelial cells observed in the present report. On the other hand, the placental $\text{Na}^+ - \text{K}^+$ -ATPase is diminished in alcoholic animals, producing water retention in the fetus.

Besides the pharmacologic effects directly related to alcohol or its metabolites, this substance can also act indirectly to alter prenatal development. Since alcohol abuse often leads to a concomitant undernourishment (Shaw and Lieber, 1979), the importance of inadequate nutrition in the etiology of the fetal alcoholic syndrome was exhaustively studied, in an attempt to delimit the responsibilities of each one of the causal agents.

Moreover, it was demonstrated that when there is a diminution of the maternal-fetal transfer of zinc (Ghishan et al., 1982), the teratogenic effects of ethanol can be aggravated (Keppen et al., 1985).

Conclusions

The results of the present report suggest that prenatal direct exposure to alcohol acts on embryogenesis, resulting in developmental alterations characterized by the diminution of body weight as well as by the delayed differentiation of diverse organs and tissues, including the epithelia of oral structures.

Acknowledgment

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