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EFFECT OF ORAL ADMINISTRATION OF ALCOHOL ON THE DEVELOPING RAT EMBRYOS

By

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ABSTRACT

Pregnant rats were forced to ingest 0.015 ml of 25% ethanol/gm body weight daily starting from day 7 (D7-group) or day 9 (D9-group) until day 15 of gestation. The number of surviving embryos significantly decreased and more embryos were resorbed in alcohol-treated groups. The mean weight of ethanol-and saline-treated surviving embryos did not differ significantly. Alcohol administration in the D7-group produced exencephaly and hydrocephaly while in D9-group only hydrocephalic abnormality occurred. Alcohol treatment in both groups produced destruction mainly in the neuroepithelium of the cerebral cortex. Numerous degenerating cells and necrotic fragments were observed in the cerebral cortex of D7-group. Moreover, the cytoplasm of the neuroepithelial cells of cerebral cortex of D7-and D9-groups was highly vacuolated.

INTRODUCTION

The morphological teratogenicity of alcohol has been well documented in humans and other experimental animals. Maternal alcohol consumption in humans results in a group of abnormalities known as the fetal alcohol syndrome "FAS" (Jones and Smith, 1973). The characteristic features of this syndrome are pre-and postnatal growth retardation, facial dysmorphology, central nervous abnormalities and skeletal and cardiovascular anomalies (Jones and Smith, 1973, 1975; Clarren and Smith, 1978). Little (1977) and Hanson *et al.* (1978) have shown that consumption of 350 mg/kg of absolute alcohol per day is sufficient to produce offspring with FAS.

The effects of ethanol have been studied extensively during recent years in embryos of rats and mice (Brown *et al.*, 1979; Randall and Taylor, 1979; Sulik *et al*, 1981; Bannigan and Burke, 1982; Sulik and Johnston, 1983; Webster *et al*, 1983; Nakatsuji and Johnson, 1984; Sanchis *et al.*, 1987), chick (Sandor, 1968),

amphibians (Naktsuji, 1983) and in monkey offspring (Scott and Fradkin, 1984; Clarren *et al.*, 1987).

The timing, duration, dose and route of alcohol administration and the species or strain used may influence the resulting effects (Borges and Lewis, 1981). Among the previous literature, the dose and the route of alcohol administration in mice vary from one intraperitoneal (IP) injection (Bannigan & Burke, 1982), two IP injections, 4 or 6 hours apart (Sulik & Johnston, 1983; Webster *et al.*, 1983; Nakatsuji & Johnson, 1984), one or two oral doses, 4 hours apart (Webster *et al.*, 1983) and in diet from gestation day 5 through gestation day 10 (Randall & Tylor, 1979). However, the effect of a daily administration of ethanol on the developing rat embryos has not been investigated.

Drinkers do not intake alcohol intermittently but usually in continuous manner. Hence, the aim of the present investigation is to study the effect of daily alcohol administration via the oral route on the developing rat embryos.

MATERIAL AND METHODS

Virgin female rats weighing 250-300 gm were placed with males (one male for three females) for two hours in the early morning. The presence of sperms in the vaginal smear at the end of this period indicated that mating had occurred and was referred to as 0 day 0 hour of gestation.

Twenty five% ethanol in normal saline solution was given in a single daily dose of 2.9 mg/gm body weight (0.015 ml/gm of body weight), by intragastric intubation. Alcohol administration was initiated on day 7 (D7-group) or day 9 (D9-group) of pregnant rats and continued through day 15 of gestation. Pregnant control rats were orally intubated daily with 0.015 ml/gm body weight of normal saline solution. Alcohol-treated and control pregnant rats were sacrificed on days 12 and 15 of gestation by cervical dislocation, and the uteri were removed and placed in a normal saline solution. Embryos were freed from the uterine tissue and the number of surviving embryos was counted. Survivors were weighed, examined for external malformations and then fixed in Zenker's or Bouin's fluids. Fixed embryos were then dehydrated, cleared, embedded in paraffin, serially sectioned at 6 microns and stained with hematoxylin and eosin. The significance of the differences between alcohol-treated and control embryos were tested using the Student's t-test.

RESULTS

The mean number of survivors during the course of the experiment is reported in Tables 1 and 2. The number of surviving embryos was smaller in alcohol-treated mothers on gestational day 12 in both D7-($p<0.02$) and D9-($p<0.05$) groups and during day 15 in both D7-($p<0.01$) and D9($p<0.02$) groups than in the

Table 1

The effect of acute alcohol treatment at day 7 and continued through day 15 of gestation on the developing rat embryos.

Observations	Embryos at day 12 of gestation		Embryos at day 15 of gestation	
	control	alcohol-treated	control	alcohol-treated
Mean number of survivors ± S.E.	9.8 ± 0.636	7.4 ± 0.562 ($p<0.02$)	10.6 ± 0.634	6.8 ± 0.814 ($p<0.01$)
Mean number of resorbed ± S.E.	0.0 ± 0.000	2.8 ± 0.374 ($p<0.001$)	0.2 ± 0.245	4.0 ± 0.707 ($p<0.001$)
Mean body weight (gm) ± S.E.	0.025 ± 0.002	0.022 ± 0.001 ($p>0.2$)	0.173 ± 0.021	0.209 ± 0.006 ($p>0.2$)
Mean thickness of cerebral cortex (microns)±S.E.	120.0 ± 7.949	102.75 ± 8.220 ($P>0.2$)	150.0 ± 10.946	138.0 ± 5.336 ($p>0.4$)

Table 2

The effect of acute alcohol treatment at day 9 and continued through day 15 of gestation on the developing rat embryos.

Observations	Embryos at day 12 of gestation		Embryos at day 15 of gestation	
	control	alcohol-treated	control	alcohol-treated
Mean number of survivors±S.E.	10.4±0.678	8.2±0.583 (P 0.05)	10.80.583	7.4±±0.927 (P 0.02)
Mean number of resorbed±S.E.	0.2±0.2	1.6±0.4 (p 0.02)	0.00±0.00	2.2±0.583 (p 0.01)
Mean body weight (gm) ± S.E.	0.025±0.002	0.022±0.001 (p 0.2)	0.170±0.014	0.190±0.005 (p 0.2)
Mean thickness of cerebral cortex (microns)±S.E.	121.0±6.347	104.8±7.696 ($P>0.2$)	151.0±11.539	146.0±10.689 ($p>0.9$)

corresponding control groups. Also a significant resorption incidence was observed on days 12 and 15 of gestation in both D7- and D9-groups (Tables 1 and 2).

On the other hand, slight changes, but insignificant, in the embryo body weight (excluding resorptions) were observed in both D7- and D9- groups (Tables 1 and 2).

Incidence of Malformations

On day 12 of gestation, the developing D7- and D9-embryos (74 and 82 embryos respectively) showed no morphological malformations, except that the forebrains of some embryos were somewhat narrower and smaller than those of the saline-treated controls (Figs, 1,2 and 3). After 15 days of gestation, eight embryos (11.76%) showed exencephally (Fig. 5) and 3 embryos (4.41%) showed hydrocephaly in the D7-group. In the D9-group, 11 embryos (14.86%) showed hydrocephaly (Fig. 6) but no exencephalic abnormalities were detected. On the other hand, the saline-treated controls showed no incidence of morphological abnormalities.

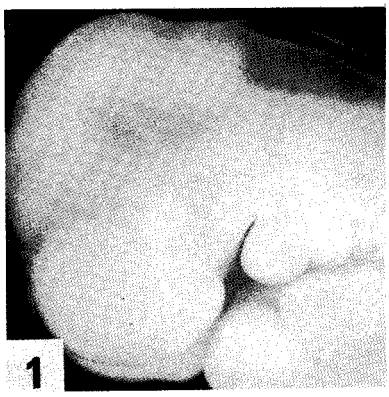
HISTOLOGY

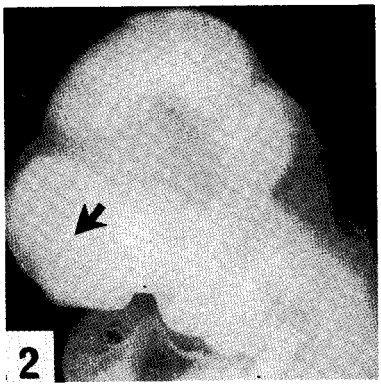
1-12-day embryos

In saline-treated embryos, beginning either after 7 or 9 days of gestation, the cerebral cortex of the brain consisted of stratified epithelial cells each with an oval nucleus containing one or more nucleoli. The nuclei of the inner ependymal layer showed highly mitotic figures (Fig. 7). The apical borders of the neuroepithelium presented the normal ruffled appearance due to the numerous pseudopodial processes (Fig. 7).

In alcohol treated D7-group, an insignificant decrease in the cerebral cortical thickness occurred (Table 1). The neuroepithelium consisted of stratified epithelial cells containing irregularly shaped and darkly stained nuclei. Numerous vacuoles were also visible in the cytoplasm of other neuroepithelial cells. Moreover, numerous cells showed different stages of degeneration with many darkly stained particles suggestive of nuclear and cytoplasmic debris of these cells. In many places, the apical border of the neuroepithelium was more or less smooth with no pseudopodial processes (Fig. 8).

In the D9-group an insignificant decrease of the cerebral cortex thickness was shown in day 12 of gestation (Table 2). The neuroepithelium exhibited a normal appearance except that the cells were less dense and contained numerous vacuoles. The mitotic figures in the ependymal layer as well as the pseudopodial processes of the apical border were less numerous as compared with those of the corresponding controls (Fig. 9).





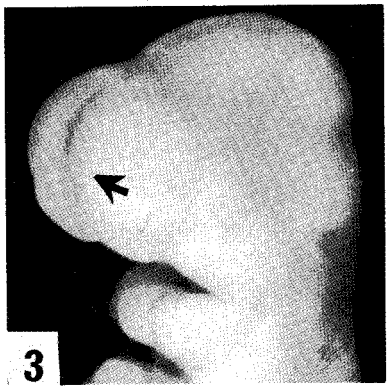
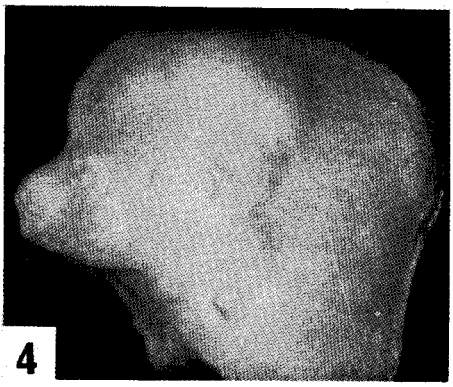
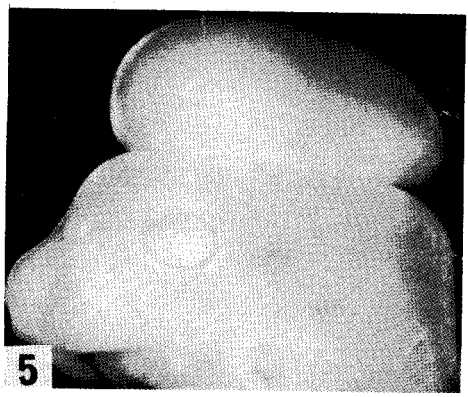
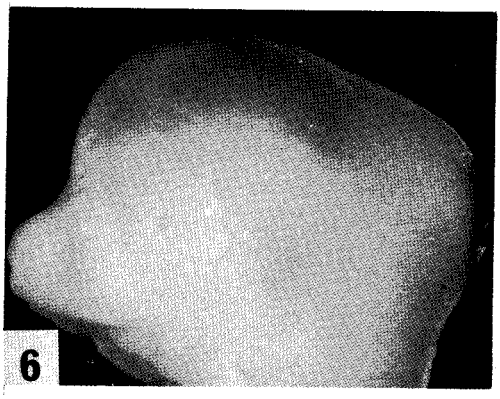


Fig. 1-3: Rat embryos on day 12 of gestation showing the heads of control (Fig. 1), D7-embryo with small prosencephalon (arrow) (Fig. 2) and D9-embryo with reduced prosencephalon (arrow) (Fig. 3)







Figs. 4-6: Rat embryos on day 15 of gestation showing the heads of control (Fig. 4), D7-embryo showing exencephaly (Fig. 5) and D9-embryo with hydrocephaly (Fig. 6).

2-15-day embryos

On day 15, the ependymal layer of the cerebral cortex of control embryos proliferated actively and the neuroepithelium appeared much thicker and contained more cell layres. The nuclei of the inner layer, the ependymal layer, show high mitotic figures (Fig. 10).

In the D7-group, a few necrotic particles were still present and the mitotic figures were very scant. The cerebral cortex decreased slightly in thickness (Table I), but the neuroepithelial cells contained oval nuclei which may be considered a sign of repairing. In addition, numerous small vacuoles were still visible in the cytoplasm of the cerebral cortex. The intercellular spaces of the neuroepithelium were more enlarged than those of the control embryos (Fig. 11).

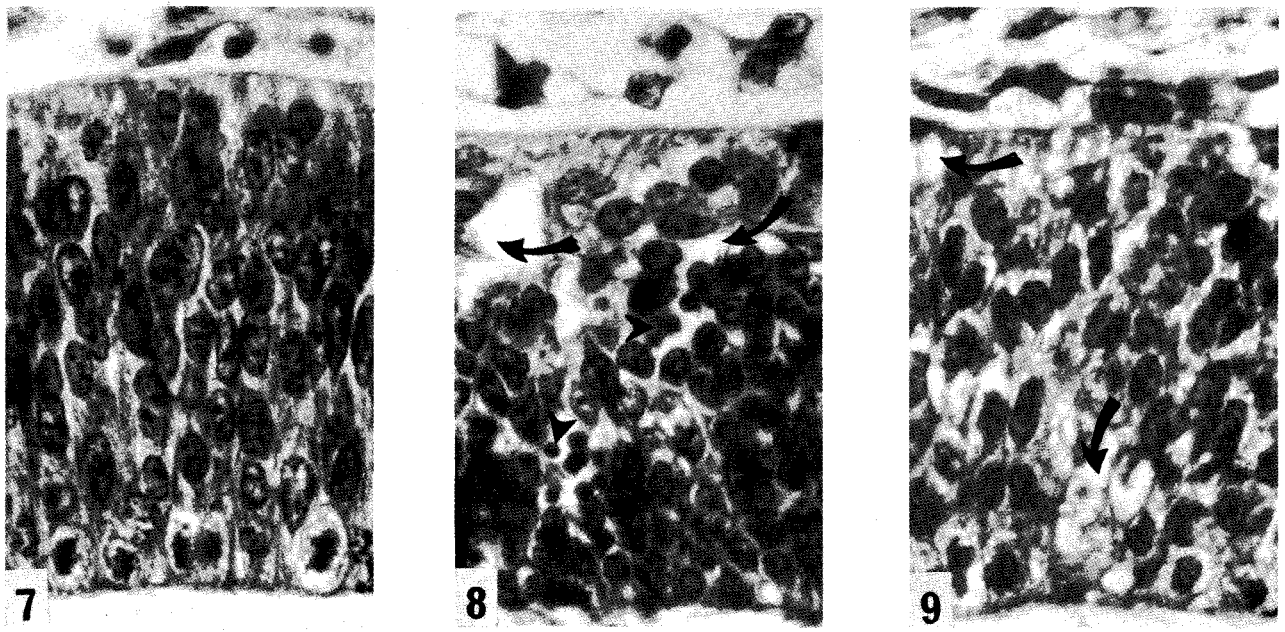
In the D9-group, the cerebral cortex slightly decreased in thickness (Table II) as compared with that of corresponding control. The neuroepithelial cells contained small rounded nuclei, and many vacuoles were observed in the cytoplasm of these cells especially near the inner limiting membrane. The mitotic figures in the ependymal cell layer were fewer (Fig. 12) than in corresponding control.

DISCUSSION

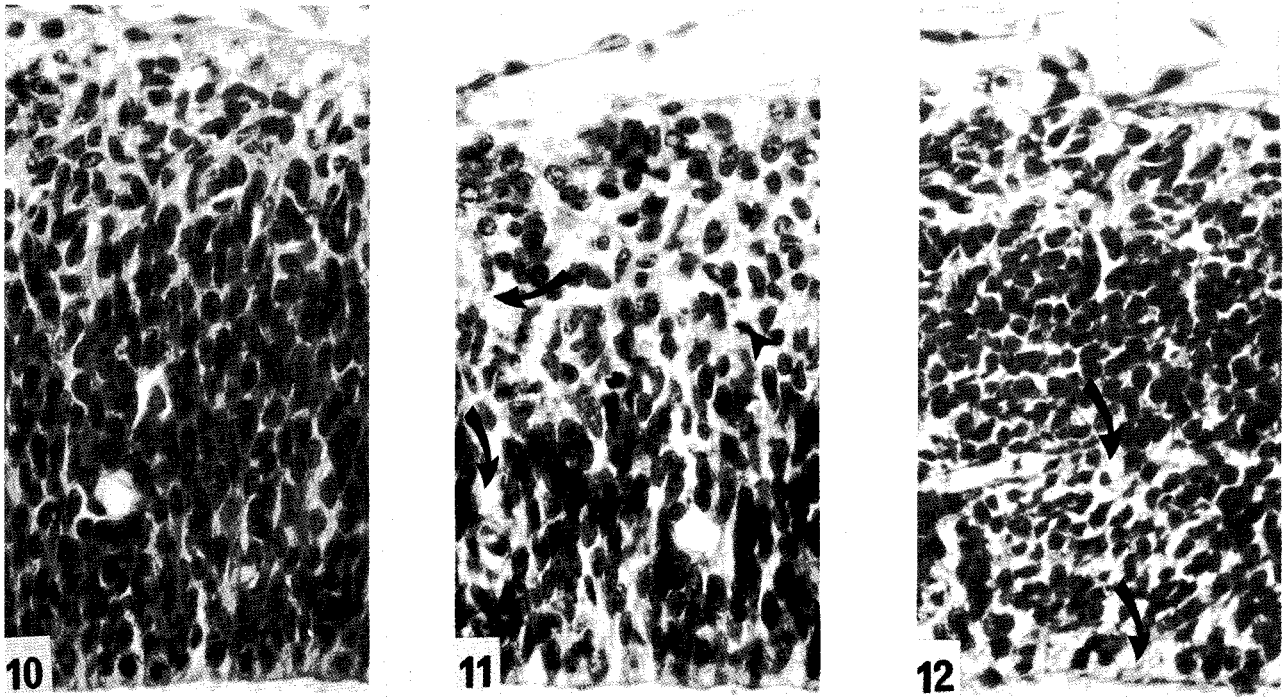
In the present study, a daily dose of alcohol closely resembling that used by the average alcoholic drinker was given orally to the pregnant rats beginning with 7 or 9 days after gestation. Alcohol administration caused a higher embryo mortality than in controls. Similar results were obtained in mice embryos (Randall and Taylor, 1979; Bannigan and Burke, 1982; Webster *et al.*, 1983).

A large number of rat embryos was resorbed in alcohol-treated embryos than in controls. Similar results were obtained in mice embryos (Randall and Taylor, 1979; Bannigan and Burke, 1982; Webster *et al.*, 1983). Moreover, the frequency and severity of resorption incidence were greater in the D7-group than in the D9-embryos. This may be due to a higher sensitivity of early embryonic stages to alcohol and/or to the long term exposure of D7-group to the alcohol. Also, Clarren *et al.* (1987) reported that administration of ethanol orally once per week to gravid *Macaca nemestrina* increased the rate of spontaneous abortion at and above 1-8gm/kg body weight. Hence, daily drinking alcohol by pregnant women, especially at early stage and before being aware of pregnancy, may induce abortion.

The result of the present investigation showed no significant changes in the embryo body weight after alcohol administration. This result confirms the findings reported by Kronick(1976), Randall and Taylor (1979) and Bannigan and Burke (1982). On the other hand, Scott and Fradkin (1984) found that birthweight of cynomolgus monkeys *Macaca fascicularis* was significantly lowered when the mothers received 5



Figs. 7-9: Transverse sections through the fore-brains of developing rat embryos on gestation day 12 of control (Fig. 7), D7-embryos (Fig. 8) and D9-embryo (Fig. 9) showing the cytoplasmic vacuoles (arrows) and necrotic particles (arrowheads). X 700



Figs. 10-12: Transverse sections through the fore-brains of developing rat embryos on gestation day 15 of control (Fig. 10), D7-embryos (Fig. 11) and D9-embryo (Fig. 12) showing the cytoplasmic vacuoles (arrows) and necrotic particles (arrowheads). X 700

gm/kg/day of ethanol by intragastric intubation from day 20 to 150 of gestation.

Alcohol intake also produced some morphological abnormalities in the head region including reduction of forebrain, hydrocephaly (in D7-and D9-embryos) and exencephaly (in D7-group). The variation in the type of malformations with the day of administration suggests that certain organogenic stages of the embryos may be more sensitive to alcohol than other stages. On the other hand, no evidence of facial defects was observed in any of the ethanol exposed embryos. This result confirms that of Scott and Fradkin (1984) in cynomolgus monkeys. Also, pregnant mice fed a chocolate-flavored liquid diet provided with ethanol from gestation day 5 through gestation day 10 produced limb and eye defects, hydrocephaly and exencephaly but no facial abnormalities were noted (Randall and Taylor, 1979). However, the typical midfacial abnormalities could be produced in mice by a brief period of maternal alcohol intoxication during gastrulation (Sulik *et al.*, 1981; Sulik & Lauder, 1983). Moreover, two IP injections, 4 or 6 hours apart, of 25% ethanol on gestation day 7 produced eye abnormalities, deficient derivatives of medial nasal prominences, exencephaly (Sulik & Johnston, 1983), mandibular and maxillary hypoplasia, narrow face and/or absence of mandible (Webster *et al.*, 1983). The latter authors also reported that two IP injections, 4 or 6 hours apart, of alcohol on gestational day 9 produced mainly defects of limbs besides a few cases of exencephaly, omphalocoeles and facial defects. The route of alcohol administration rather than the amount of alcohol administered or the stage of embryonic development at the time of exposure may be critical factor in producing facial defects in the developing rat embryos. This hypothesis is under investigation.

Administration of alcohol to pregnant rats produced extensive cellular damage of the cerebral cortex with no alteration of the neuroepithelial thickness. However, Bannigan and Burke (1982) reported retardation of the neuroepithelium of alcohol-treated embryos. The presence of cytoplasmic vacuoles in D7-and D9-groups suggests that ethyl alcohol may be toxic to the cells, affecting the cytoplasm or the cell membrane of the neuroepithelium (Bannigan and Burke, 1982). Moreover, ethanol treatment caused susceptible effect for cell divisions of ependymal layer. These results suggest that a long exposure period of early rat embryos can produce destructive effects of the cerebral cortex.

On gestation day 15, some neuroepithelial cells showed signs of repairing. The capacity of repairing was demonstrated by the smaller number and size of vacuoles and cellular debris. However, the intercellular spaces were still large as compared with those in saline-treated embryos. Bannigan and Burke (1982) reported that, fifty hours after alcohol injection, the neuroepithelium was completely cleared of debris which resulted from cell necrosis. They also suggested that the intercellular spaces may not have been caused directly by ethanol but might be due to sodium

retention. Further physiological and cytological studies are required to explain the mechanisms of alcohol cytotoxicity to the neuroepithelium.

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تأثير الكحول على نمو أجنة الفئران

حمـــزه احمـد الشـــبكة

تم في هذا البحث دراسة تأثير جرعة يومية مقدارها ٠,٠١٥ مل من ٢٥٪ كحول اثيلي على أجنة الفئران . وقد أعطيت هذه الجرعة لمجموعتين من الأمهات أحدهما بعد ٧ أيام والأخرى بعد ٩ أيام من الحمل .

وبدراسة تأثير الكحول على الأجنة عمر ١٢ ، ١٥ يوماً وجد أن عدد الأجنة في الأمهات المعالجة بالكحول يقل بوضوح عن عددها في غير المعالجة كما تزداد عملية الامتصاص للأجنة في الأمهات المعالجة بالكحول بينما لا يوجد تغير واضح في وزن أجنة الفئران المعالجة بالكحول أو غير المعالجة . وبدراسة الشكل الخارجي للأجنة وجد أن الكحول يحدث بعض التشوهات مثل صغر المخ الأمامي في الأجنة عمر ١٢ يوماً أما الأجنة عمر ١٥ يوماً فإن الكحول يحدث إنتفاخ في رأس بعض أجنة المجموعتين وبروز في رأس بعض أجنة الفئران المعالجة في اليوم السابع من الحمل . كما أثبتت الدراسة الهستولوجية أن الكحول يحدث تحطيم لخلايا قشرة المخ في أجنة الفئران المعالجة بالكحول في اليوم السابع من الحمل كما توجد بين خلايا قشرة المخ في أجنة الفئران المعالجة بالكحول فراغات كثيرة وكبيرة بالمقارنة بمثيلتها في أجنة غير معالجة .