CRANIOFACIAL ALTERATIONS IN ADULT RATS AFTER ACUTE PRENATAL ALCOHOL EXPOSURE

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Background: Exposure during pregnancy to alcohol (ethanol) produces a number of adverse effects. One of them) is fetal alcohol syndrome. The hallmark in fetal alcohol syndrome (FAS) is craniofacial dimorphisms and the changes in craniofacial measurement are dependent on the alcoholic dose and its time of exposure. Since prenatal ethanol exposure can alter craniofacial development in rodents and reliably produce long-term behavioral effect in them, the present study was designed to extend the same changes in the Sprague Dawley species. ***Methods:*** The albino rat was studied to determine whether gestational exposure to ale tol (Ethanol) produces permanent craniofacial effect On gestational day (GD7-I0) 25% ethanol was injected intraperitonealy to pregnant rats. Various dimensions for skull and face of adult male rats were taken. ***Results:*** Both vertical and coronal dimensions were altered in the exposed animals. ***Conclusions:*** This study demonstrates that exposure to ethanol on a critical gestational period produces permanent craniofacial defects.

INTRODUCTION

Exposure during pregnancy to alcohol (ethanol) produces a number of adverse effects. One of them is fetal alcohol syndrome.

The hallmark of fetal alcohol syndrome (FAS) is craniofacial dimorphisms and the changes in craniofacial measurement are dependent on the alcoholic dose and its time of exposure.

Although all alcohol exposed children do not display craniofacial anomalies, but alcohol affected children are often correctly differentiated from normal children by an experienced examiner ". Prenatal alcohol exposure in non-human primate produces off springs, which tend to be micro, or macro cephalic. A convincing mode of human malformation has been documented in primate species.[4]

Since prenatal ethanol exposure can alter craniofacial development in rodents and reliably produce long term behavioral effect in them, the present study was designed to extend the same changes in the Sprague Dawley species.

MATERIAL AND METHODS

Adult male and female albino rats of Sprague Dawley strain were kept in the laboratory for a week to acclimatize them to the laboratory condition. Then male and female rats in a ratio of I to 3 respectively were allowed to mate overnight, 8 males and 24 females. Presence of sperm in vaginal swab on next morning was taken as surest sign of copulation and day zero of pregnancy. The weight range of animals was 173.3 to 178.3 gm.

Females with sperm positive swat were divided into two groups, A and B for control and alcohol exposed respectively at random, containing 12 females in each group A-females were given normal saline in a dose of 3ml 100 gm body weight intraperitonealy. And group B animals were given 25% alcohol intraperitonealy in a dose of 3ml/100 gm body weight on day 7-10 of gestation.

Each group was kept in a separate cage after marking both the cages and animals. Just prior to the expected parturition, breeding cages were checked twice a day for newborn pups. Shortly after birth, pups were weighed and examined for any obvious malformations. Off springs were raised with their biological mother.

At 100 days of age, control and experimental male offspring were sacrificed by decapitation. Eight animals from each litter were studied and statistically analyzed. Heads were treated with 10% potassium hydroxide (KOH) for soft tissue removal. The following direct skull measurements were taken.

1. ***Mandibular height at the coronoid process***. A vertical measurement from coronoid process to the base of the mandible (Fig. 1.D).

2. ***Premaxillary height.*** Height of the premaxillary bone at the frontal premaxillary parietal bones, (Fig. l.C)

3. *Superior cranial width.* The greatest distance between the lateral surfaces of the parietal bone. (Fig. l.A).

4. ***Rostral width.*** Between lateral asepsis of the premaxillary bones at the point of junction with the maxilla, (Fig 1. A).

5. ***Palatal width.*** The distance between the postalabur occlusal fossae on the right and left second maxillary teeth, (Fig. 1 B).

6. ***Superior cranial length***. From the anterior margin of the nasal bone to the posterior lambdoidal suture in the midline. (Fig. I.A).

Table-l: Skull measurements of group A, control, albino rat off springs.

S.NO	Vertical Measurement (mm)		Coronal Measurement (mm)			Para Sagittal Measurement			(mm)
	Mandibular Length	Premaxillary Length	Superior Cranial width	Rostral Width	Palatal Width	Cranial Width	Palatal Length	Maxillary Length	Mandibular Length
1	10	8	12	7	6	33	27	32	17
2	10	8	13	7	6	37	37	33	18
3	11	8	13	7	6	38	38	31	16
4	11	9	12	7	6	37	37	32	16
5	9	7	14	6	7	32	26	30	17
6	12	8	12	6	6	39	39	35	15
7	10	8	12	8	6	35	35	31	16
8	11	7	12	8	7	37	37	31	15
Total	**84**	**63**	**100**	**56**	**50**	**288**	**275**	**255**	**I29**

Table-2: Skull measurements of Group B. alcohol exposed, albino rat offsprings.

	Vertical Measurement (mm)		Coronal Measurement (mm)			Para Sagittal		Measurement (mm)
S.NO	Mandibular Length	Premaxillary Length	Superior Cranial width	Rostral Width	Cranial With	Palatal Length	Maxillary Length	Mandibular Length
1	10	6	12	7	5	36	30	35
2	9	7	12	7	5	30	28	35
3	8	6	11	7	6	38	25	37
4	10	6	12	8	5	30	25	35
5	10	8	10	6	5	34	30	39
6	9	9	13	6	4	33	20	34
7	9	6	10	5	5	23	30	36
8	11	7	12	6	5	20	18	20
Total	76	55	92	52	40	244	206	281

Table-3: Mean, Standard Deviation & T-Values of group A and Group B albino rat off springs skull measurement

Measurements (mm)	Group	A control	Group Bal-exposed		t-values (df 14)	P Value
	Mean	SD	Mean	SD		
Vertical Measurement i) Mandibular Height	10.5	0.86	9.5	0.86	2.22	P< 05
ii) Premaxillary Height	7.87	1,11	6.87	1.11	2.22	P < .05 .
Coronal Measurements i) Superior Cranial Width	12.5	0.86	11.5	1.11	2.22	p < 05
ii) Rostral Width	7.0	0.7	6.5	0.86	2.77	P<01
iii) Palatal Width	6.2	0.5	5.0	0.5	5.00	p< 01
Para Sagittal Measurements i) Cranial Length	36.0	2.5	30.5	5.9	2.39	p< 05
ii)Palatal Length	34.3	5.6	25.75	4 4	3.42	p < 01
iii)Maxillary Length	31.8	1.5	35.1	2.4	3.11	P <01
iv)Mandibular Length	16.1	1.0	18.3	1.1	3 85	P<01

7. Palatal length. From the most anterior point on the premaxillary suture to the most posterior point on the palatine suture, (Fig. l.B).

8. Maxillary length. From internal acoustic meatus to point A, (Fig. l.C).

9. Mandibular length. The distance between the incisive foramen and the notch- between the coronoid and the angular processes, (Fig. 1. D).

RESULTS

Exposure to alcohol is deleterious during pregnancy. Intraperitoneal injection of 25% alcohol during pregnancy resulted in reduced intrauterine weight gain in exposed offspring was (5.10 ± 0.117gm) when compared with weight in control animals (7.00 ± 0.2015 gm) showing highly significant ($P < 0.001$) reduction.

At day 100 of life no significant reduction was observed in weight. All the other observations are shown in fig. 1 and in tables 1-3.

DISCUSSION

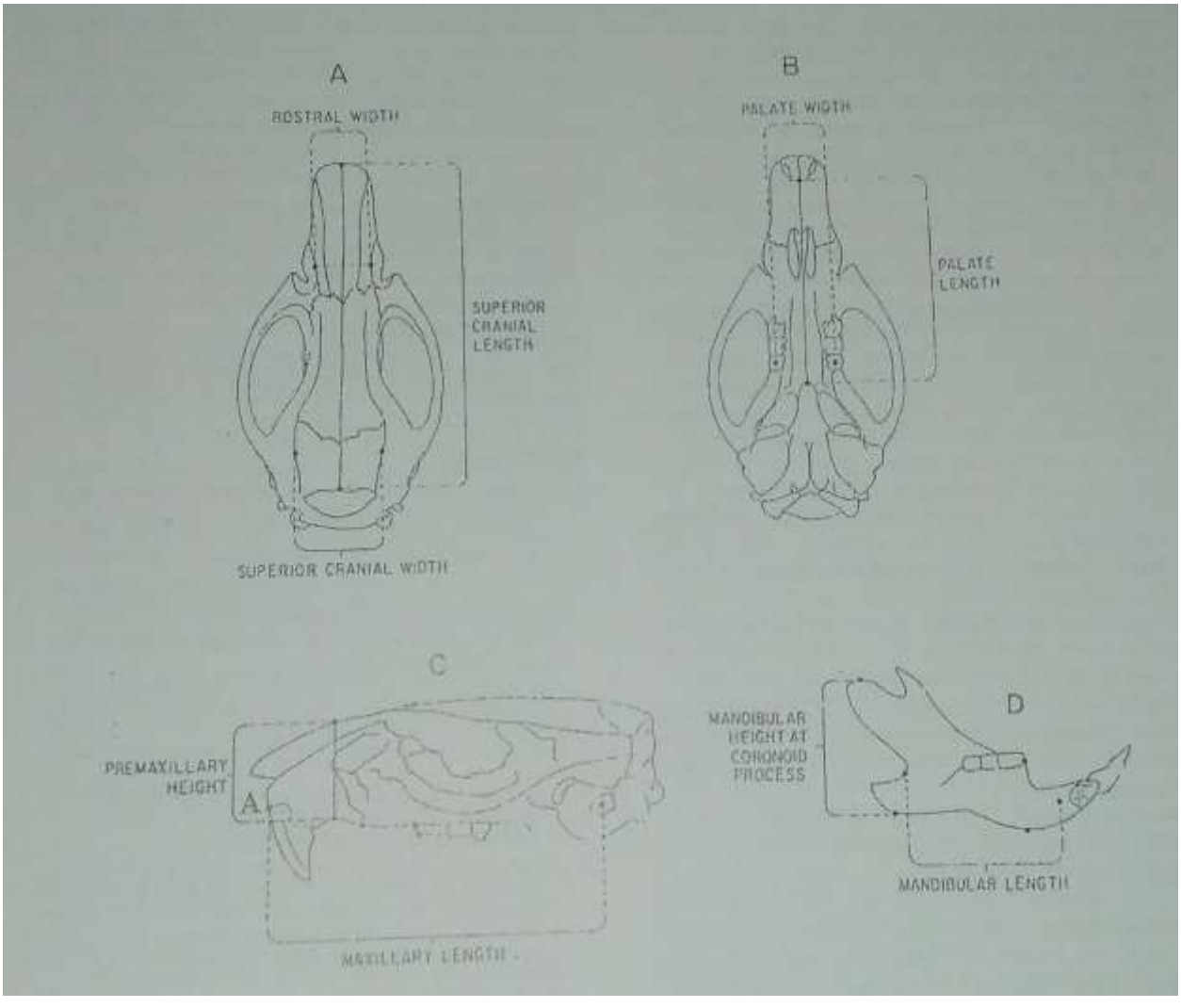
Various skull measurements were taken directly from the dried and KOH treated adult albino rat skulls, both of control and experimental animals.

Statistical analysis showed significant decrease in craniofacial skeleton in alcohol exposed animals (tables 1,2 and 3).

Earlier research has demonstrated that the rat skull attains most of its length during postnatal period [6]. Since ethanol was not administered during the postnatal period this deficiency in various lengths of skull must be due to some factor other than the actual process of laying down bone.

FIGURE -1: CRANIOFACIAL EFFECTS OF ETHANOL IN PART

A: Dorsal View
B: Ventral View
C: Lateral View
D: Lateral View of Mandible



Our results are consistent with the hypothesis that the ethanol exposure on a specific day of gestation (day 7-JO in this case), alters the pattern of development,

producing a holoprosencephalic conformation. In addition, chronic ethanol exposure may then exacerbate the problem by producing overall growth retardation. This hypothesis is based on two concepts. First, the work of Sulik *et at..*[1] demonstrating that high doses of ethanol, by an unknown mechanism, can produce an abnormal craniofacial pattern which resembles holoprosencephaly. Secondly, ethanol is known to produce growth retardation both clinically and in the rodent model. However, other factors should also be considered particularly since our own study utilized much lower dose of ethanol i.e. 25% instead of high doses of ethanol in the Sulik et al[7] studies.

Early work by Moss [6] demonstrated that normal brain growth is necessary for normal skull growth. Removal of forebrain tissue from the newborn rat resulted in narrowing of the angle of the cribriform plate with the inferior aspect of the frontal bone. Although, for technical reasons, we were unable to take this measurement in the present study, there is ample evidence that prenatal exposure to ethanol results in rodents with deficient forebrain regions and smaller brain weights [7-S]. Therefore, ethanol's effects on skull growth may be secondary to the effect of inhibiting proliferation of the neural crest cells of the forebrain or perhaps throughout the entire brain, in addition, under-nutrition during a restricted period of development has, in itself, shown to produce asymmetrical growth patterns in the brain. Since craniofacial skeletal growth is dependent upon brain group, S particularly during the initial organgenic period (GD 7-10) in combination with the effects of ethanol on metabolism at the cellular level, may be sufficient to account for the results observed in the present study.

Whatever the mechanism whereby ethanol produces the craniofacial dimorphisms, our findings support and extend the findings of Sulik et if and Edwards and Edwards[10], that ethanol exposure during gestation in the mouse and rats respectively produces a pattern of craniofacial anomalies which resembles that of human FAS. These anomalies include a narrow forehead, flat mid face, short nose, narrow palpebral fissures, and a diminished vermilion border. Although cephalographsflateral radiographs) were not analyzed geometrically in the present study, changes consistent with the flat mid face and short nose observed in the human FAS are evident when the ethanol exposed crania are viewed laterally, the narrow forehead and palpebral fissures of the human FAS arc consistent with the reductions seen in superior cranial and rostral widths in the present study. Therefore, the craniofacial changes observed in our study match those commonly seen in human FAS.

In conclusion, prenatal ethanol exposure produces a pattern of abnormal cranial and mandibular skeletal growth in the rat. While mechanisms were not addressed in the study, the date are consistent with the hypothesis that prenatal ethanol exposure induces a hormonal and/or nutritional imbalance and produces an abnormal pattern of protein synthesis either in the osteoblasts themselves or in the neural crest cells[11], thus producing an abnormal pattern of cranial growth. Whatever the cause, these data confirm the prenatal ethanol exposure at levels, which produce behavioral changes in the rat also produces specific craniofacial malformations that persist to adulthood and closely resemble the craniofacial features of the human FAS.

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