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ADVERSE EFFECTS OF ALCOHOL CONSUMPTION, ON FOETAL AND POSTNATAL MICE. *

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Summary

Long -term multigeneration effects of the 20% (group A) and 10% (group B) alcohol in water on Razi: NIH (S) mice were measured in 3 generations (1,2). Growth rate, food and liquid consumption, reproduction, litters resorption malformation, hematology and urine analysis were taken on 2 treatment and a control group. Chi-square test and t-test were used to analyze the data.

Average weight gain in AF_1, AF_2, AF_3 and food and liquid consumption in all treatment groups were signifieantly different. Number of offspring per litter neonatal weight and viability index were significantly decreased in all of the A, BF_2 and BF_3 groups. The resorption and malformation incidence had a close relation with alcohol doses and duration of consumption. Intestinal and uterus haemorrhage and mammary tumour have been seen in second and third generations. The mean rate of liver weight to total body weight was signifieantly different. Hematology showed slight differences. Urine analysis showed no

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significant differences. In all group A subjects maturation index was very low. Some of the mature offspring of A subjects were dwarfs. This can be explained genetically. Ongoing research is being conducted to confirm the above findings.

Introduction

In 1973 (1) the fetal alcohol syndrome (FAS) was described by Jones et al, in children born by mothers with a history of chronical alcohol consumption. FAS is characterized by develomental and psychomotor delay, pre-and postnatal growth retardation, craniofacial, cardiac and joind defects. Heart defects occur in about 30 per cent of children and facial characteristics are as follow (2):

1. Short palpebral fissures
2. Low nasal bridge
3. Epicanthic folds
4. Short nose
5. Indistinct philtrum.
6. Narrow upper lip
7. Small chin
8. Flat midface
9. Ptosis
10. Strabismus

Many investigations of effect of ethanol on prenatal development in animals were made in the past. The result of injecting ethanol into air sac at early stage of chicken embryo was growth retardation with generalized malformation of the central nervous system(3). In the mouse, investigators have produced eye defects, cardiac and neural abnormalities and varying degrees of isolated hyperplasia of the lung(4). Different doses of alcohol resulted to digit anomalies, cardiovascular, urogenital and head malfotmation (5). In rat, microcephaly, developmental retardation and malformation have been noted (6). Uptill now all the reports confined

themselves to short term studies. In our study long-term multigeneration effects of 20% and 10% alcohol in water on Razi: NIH (S) mice were measured in 3 generations. Growth rate, food and liquid consumption, reprocuction, litters resorbtion, malformation, hematology and urine analysis were taken on 2 treatment and a control groups. Chi-sqare test and t-test were used to analyze the data.

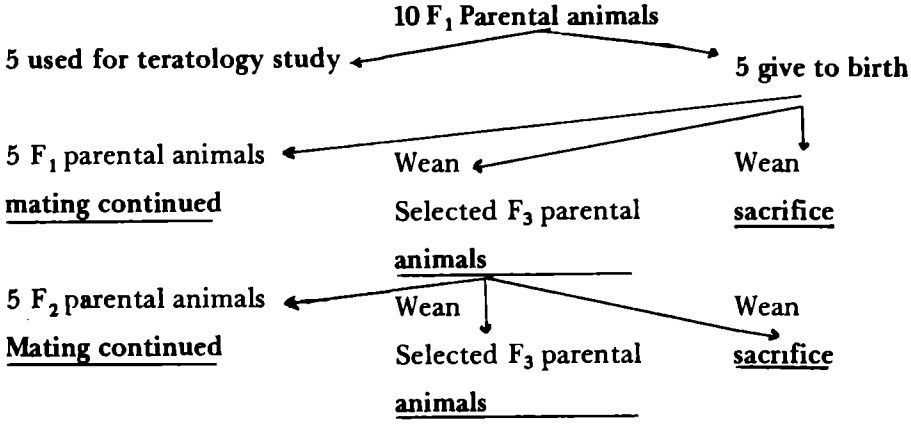
Material and Methods

30 male and 30 female Razi: NIH(S) mice weighing 30–35 grams were divided into one control, C, and two experimental groups A and B. The latter groups received 20% and 10% alcohol in water respectively. After adaptation period each female and male from each group were housed per cage. The mating day, as judged by the detection of the vaginal plug, was called day 1 of pregnancy. After plugging all the males were seperated and housed per cage for further mating. The amount of consumed food, water, alcohol solution and animal weight were determined every other day during test. On 19th day of pregnancy, 50 per cent of pregnant mice of each group have been taken for teratology, blood and urine analysis. Blood samples for hematocrit, hemoglobin, red and white blood cell count were taken from orbital sinus. Urine was analysed by reagent strips. The animals were sacificed by cervical dislocation and the content ofThe vterus examined. Resorption, dead fetuses, external malformation, fetuses' weight and numbers were recorded. Relative organ weight of liver, kidny and spleen were measured. The other 50 per cent of pregnant females in each group and in each generation were allowed to proceed to natural births. In the natural birth litters, the pups were counted and weighed. Litters containing more than 10 pups were reduced to that number. The cages were inspected every day and observations put down. Viability and maternal index were determined. This method was continued in 3 generations. Chi-square test and t-test

were used to analyse the data. The protocol for 3 generation study is diagramed in figure (1).

Result and Discussion

Hemetology analysis (Tab. 1) shows no significant differences except in number of neutrophils of A group. Urine analysis for urobilinogen, blood, bilirubin, ketones, glucose, protein and pH showed no differences. The maternal performance (tab. 2) was influenced by alcohol consumption in three generations. Alcohol had apparent effect on viability of the offsprings, as there were significant differences in the average number of liveborn animals per pregnant female in A group. Neonatals' weights were significantly different in A group and results bear a close resemblance to viability index. During sukling a lot of suklings were dead in the tested groups, especially in A groups. There were a few mothers who held all suklings until weaning age.



This condition depended on two factors which are concluded from our daily observations. 1. The mothers were nervous and majority of them failed to gather offsprings in one place for suckling, so the offsprings were retarded and their weight was significantly different from C groups. These retardation phenomena can be comparable with human beings. Table 3 shows the relative organ weights of spleen, kidny and liver. Liver and kidney's relative weight in group A have meaningful difference from C group. In investigation of the interior organs stomach bleeding have been seen. In most cases liver was big and pale. The weight gain (Tab. 4) of three generations of each group is compared with control group. The weight gain of AF_2 and AF_3 are significantly different from control group. Food intake and liquid consumption (Tab. 5a) were not essentioally influenced by different doses of alcohol during adaptation period. Food intake and liquid consumption during lactation and pregnancy (Tab. 5b and 6) show significant differences in all groups. Now the question arises that insufficient food intake has any relation to such made adverses. Through the use of pair fed control, the animals studied have clearly shown that it is the alcohol and not the diet that produces malformation and growth deficiency (7). The incidence of external abnormalities of fetuses in control and treatment groups are shown in table 7. The resorption and malformation had a close relation with alcohol doses and duration of alcohol consumptions. Litter size and feotal weight (Tab. 9, 10) in A groups are more affected than B groups. It was found that 19 day mouse fetuses had a pattern of malformation similar to those reported in children with foetal alcohol syndrom. These similarities included prenatal growth deficiency evidenced by low foetal weight (Tab. 10) face deformation and prenatal loss and growth retardation. In excencephalia case. brain was grown completly out of skull. With considering high lipid solubility of alcohol, it is conceivable that the effect was resulted during neurola. Validity of this interpretation awaits further study. Table 8 shows incidence of mammary tumors in each

group. The incidence of mammary tumor in treatment group was much higher than control group. The tumor samples were sent to pathology department and were distingished as endocar cinoma. Some of the cases were quite progressed and diameter of tumors were 2–3 centimeters and some were in the first stage of growth and their diameters were not exceeding a few millimeters. The relation of mamary tumor and alconol consuption in this study was an interesting and disputable subject.

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Tab 1.

Hematology

	Control	A(20%)			B(10%)		
		F_1	F_2	F_3	F_1	F_2	F_3
Hematocrite	49.6 ± 1.51	48.6 ± 1.14	48.8 ± 0.83	49.2 ± 1/48	48.8 ± 0.83	49.2 ± 0.83	49 ± 1.51
Hemoglobin	14.6 ± 0.31	14.36 ± 0.31	14.56 ± 0.31	14.56 ± 0.55	14.14 ± 0.72	14.7 ± 0.16	14.24 ± 0.76
W.B.C[1]	8.88 ± 4.62	6.86 ± 3.54	7.62 ± 2.99	8.01 ± 3.41	9.46 ± 3.61	8.21 ± 2.37	9.32 ± 2.12
R.B.C[2]	9.18 ± 2.20	8.90 ± 1.33	9.45 ± 1.33	9.45 ± 3.01	9.07 ± 4.18	7.98 ± 4.04	8.79 ± 3.85
Lymphocyte	80.8 ± 2.68	73 ± 8.42	79.2 ± 5.14	80.2 ± 5.31	81.6 ± 6.14	79.3 ± 3.50	77.21 ± 6.10
Neutrophil	17.8 ± 3.56	24 ± 7.48[a]	19.3 ± 4.18	18.5 ± 3.45	15.4 ± 4.33	17.2 ± 4.12	19.1 ± 4.01
Monocyt	1 ± 0.70	1.6 ± 1.51	1.5 ± 0.86	1.3 ± 1.42	1.4 ± 1.14	2.85 ± 1.33	3.01 ± 1.14

1. White Blood Cell X 1000
2. Red Blood Cell X 1000000
a. r = 0.36 P 0.06

Tab. 2

Maternal performance

	Control	A(20%)			B(10%)		
		F_1	F_2	F_3	F_1	F_2	F_3
Viability[a] index	9.4 ± 0.69	5 ± 2.50[1]	5.4 ± 2.45[2]	6.25 ± 1.48[3]	7.2 ± 1.78	7.6 ± 0.70	8.8 ± 0.76
Neonatal weight	1.78 ± 0.19	1.17 ± 0.22[4]	1.11 ± 0.25[5]	1.19 ± 0.27[6]	1.29 ± 0.1	1.34 ± 0.27	1.73 ± 0.15
Lactation[b] index	9.4 ± 0.69	3 ± 2.20[7]	3.66 ± 2.44[8]	3.37 ± 2.26[9]	6.88 ± 2.26	6.9 ± 1.79	8.44 ± 1.50
Weanling weight	20.44 ± 1.18	10.28 ± 0.98[10]	10.25 ± 1.2[11]	11 ± 1.16[12]	12.15 ± 1.5[13]	12.36 ± 1.04[14]	12.95 ± 1.183[15]

a. Average number of survivors to day 4 per pregnant female.
b. Average number of survivors at weanling age.
1. Significantly different from control value P 0.04
2. Significantly different from control value P 0.05
3. Significantly different from control value P 0.06
4. Significantly different from control value P 0.06
5. Significantly different from control value P 0.06
6. Significantly different from control value P 0.05
7. Significantly different from control value P 0.06
8. Significantly different from control value P 0.05
9. Significantly different from control value P 0.05
10. Significantly different from control value P 0.04
11. Significantly different from control value P 0.02
12. Significantly different from control value P 0.0
13. Significantly different from control value P 0.02
14. Significantly different from control value P 0.03
15. Significantly different from control value P 0.03

Tab 3.

Relative organ weight.

	Control	A(20%)	B(10%)
Spleen	0.0051 ± 0.0008	0.0068 ± 0.001	0.0058 ± 0.001
Kidney	0.0160 ± 0.0010	0.0485 ± 0.001^{a}	0.0158 ± 0.001
Liver	0.0870 ± 0.0080	0.1154 ± 0.020^{b}	0.0949 ± 0.008

a. Significantly different from control value P 0.05
b. Significantly different from control value P 0.05

Tab 4.

Weight gain

	F_1	F_2	F_3
Control	0.9 ± 0.17	0.46 ± 0.03	0.44 ± 0.28
A (20%)	0.58 ± 0.16^{a}	0.31 ± 0.19^{b}	0.21 ± 0.30^{c}
B (10%)	0.85 ± 0.19	0.57 ± 0.20	0.59 ± 0.39

a. Significantly different from control value P 0.06
b. Significantly different from control value P 0.05
c. Significantly different from control value P 0.05

Tab. 5a

Food and liquid consumption before Pregnancy

	Food	Liquid
Control	6.25 ± 0.87	6.4 ± 1.09
A (20%)	4.61 ± 0.87	4.8 ± 1.38
B (10%)	5.68 ± 1.22	7 ± 1.10

Tab 5b.

Food and liquid consumption during lactating

	Food	Liquid
Control	15.24 ± 2.45	19.6 ± 3.62
A (20%)	6.59 ± 1.70[a]	6.1 ± 1.01[b]
B (10%)	11.79 ± 2.27[c]	13.5 ± 2.48[d]

a. Significantly different from control value P 0.04
b. Significantly different from control value P 0.03
c. Significantly different from control value P 0.05
d. Significantly different from control value P 0.05

Tab. 6.

Food and liquid consumption during pregnancy

	Food	Liquid
Control	9.65 ± 3.07	12.1 ± 4.98
A (20%)	4.66 ± 0.97[a]	5.27 ± 1.01[b]
B (10%)	9.2 ± 2.69	11.6 ± 3.77

a. Significantly different from control value P 0.05
b. Significantly different from control value P 0.05

Tab. 7

Fetuses abnormalities

	Control			A(20%)			B(10%)		
	F_1	F_2	F_3	F_1	F_2	F_3	F_1	F_2	F_3
Total fetuses	62	65	60	50	52	54	60	59	61
Hyperplasia				1					
Deformed tail					5	7			7
Deformed nose			1	2	4	4		3	1
Hematoma		1		1	23	18		8	10
Excencephalia					2				1
Deformed					3				1
Total deformed fetuses		1	1	8	49	42		11	20

Tab. 8

Tumor incidence

Number of animals	10
Control	2/10
A (20%)	8/10
B (10%)	6/10

Tab. 9

Litter size

	Control	A(20%)	B(10%)
F_1	14.8 ± 1.78	9.6 ± 1.94^a	10.8 ± 1.64^b
F_2	13.9 ± 1.82	10.6 ± 2.96^a	11 ± 1.87
F_3	14.7 ± 2.01	10.2 ± 2.58^a	12.2 ± 3.56

a. Significantly different from control value P 0.05
b. Significantly different from control value P 0.06

Tab. 10

Fetus weight

	Control	A(20%)	B(10%)
F_1	1.2693 ± 0.11	0.8817 ± 0.11^a	0.9203 ± 0.08
F_2	1.3201 ± 0.25	0.8452 ± 0.05^a	1.1519 ± 0.44
F_3	1.2945 ± 0.17	0.9183 ± 0.10	0.9640 ± 0.10

a. Significantly different from control value P 0.05