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METHANOL NEUROPATHY: A HISTOLOGICAL STUDY ON LONG-EVANS RATS. M. Hoque, W.C. Monte, and C.S. Johnston. Food and Nutrition laboratory, Arizona State University. Tempe; AZ 85287

Methyl alcohol (methanol), a highly toxic substance, is in food products containing the artificial sweetener, aspartame. Since aspartame is a very popular artificial sweetener, a large segment of our population is currently consuming chronic, low doses of methanol. This study examined the neurotoxic effect of methanol on the central nervous system of rat pups. Methanol was administered daily by the gavage technique to 20 female Long-Evans rats (1.28 g/kg body weight) beginning two weeks prior to mating and throughout gestation and lactation. A control group of ten female rats received same volume of distilled water. Prenatal methanol exposure induced brain defects (hydrocephalus), eye defects, spina bifida occulta, and stillborn pups. All experimental pups showed massive axonal degeneration in multiple regions of brain. Histological examination of the brain using reduced silver staining techniques, revealed massive fiber degeneration of the cerebellar cortex, deep cerebellar nuclei, and cranial nerve nuclei. Additional regions of axonal degeneration were found in the hippocampus, corticospinal tract and optic chiasm. These results show that by using sufficiently sensitive neurohistological technique, the neurotoxicity of methanol is revealed in the mammalian central nervous system. Although the daily dosage used in this study is high (approx. equivalent to 3 liters of an aspartame-sweetened beverage), pregnant or lactating women, should limit consumption of aspartame-sweetened food products.

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Results

Experiment I: Physical Growth, Malformation and External Deformity

There were no significant difference in body weight gain among the two groups of pregnant dams. However, prenatal exposure to methanol significantly reduced body weight of the offspring at birth, relative to control group (Table 1).

The incidence of malformation was significantly increased in the methanol treated group (Table 2). Fourteen of the 20 litters had one or more fetuses with malformation of external morphology ($p < 0.001$; 55 of the 263 total fetuses). The external malformations included brain defects, eye defects and spina bifida occulta. The incidence of brain defects was significant ($P < 0.005$); specifically, seven of the 20 litters had thirteen fetuses with hydrocephalus, and one little had two fetuses with aencephaly and one fetus with exencephaly. The incidence of eye defects was significant ($p < 0.01$); specifically, four of the 20 litters had eleven fetuses with anophthalimia, and one litter had two fetuses with microopthalmia. The incidence of spina bifida occulta was significant ($p < 0.005$); thirteen of the 20 litters had one of more fetuses with this spinal deformity. The incidence of still born was also significant ($p < 0.005$); fourteen of the 20 litters had one or more still born fetuses (25 out of 263 total fetuses).

The incidence of pups mortality rate was higher in the first week of nursing period, twelve of the 20 litters had one or more dead fetuses within first week of their birth. All of the dead pups had severe breathing problem for two/three days and lost weight before they passes away. This strongly suggests that methanol or it's metabolic products either formaldehyde or formic acid can cause severe problem in pups during nursing period.

Overall, prenatal methanol exposure significantly affected mean fetal birth weight and also affected the incidence of brain defects, eye defects, spina bifida and stillborn was significant.

Histological Examination

In this study, methanol was administered orally to rats during gestational and lactational period was neurotoxic at 1.28 g/kg dose level. Histopathological examination indicates that methanol causes damage to the central nervous system of rats and their offspring. The effect of prenatal methanol exposure during lactation period were observed under light microscope.

All pups showed massive degeneration in widely distributed regions of the central nervous system (See Table 3). Microscopic examination revealed that degenerating fibers in the different layers of cerebellar cortex, several cranial nerve nuclei, and in the brain stem. All experimental pups showed axonal damage independent of the sex of pups. Microscopic examination indicates anterior magdola and periventicular gray have degenerating fiber. Inferior colliculus and autorhinal cortex of the pups brain have beaded axon.

The normal nerve fibers of the control rat brain were photographed using light microscope (See Figure 6). All experimental pups showed massive degeneration in the

different layer of cerebellar cortex (See Figure 7). Degeneration was also found in subliculum. Enthorhinal cortex also had some degeneration; and the hippocampus were filled with degenerating fibers throughout the region (See Figure 8).

Methanol was toxic to the dams at the 1.28/kg dose level. All experimental animals showed massive degeneration in numerous regions of the brain (See Table 3). Microscopic analysis indicated massive degeneration in the white matter on the ventral-most aspect of the brain. In gray matter there also appears to be a number of broken axons and this region has degeneration throughout the entire ventral surface. Light microscopic examination showed complete absence of detectable neuronal cell body.

All experimental animals showed massive degeneration throughout the medullary layer of the cerebellum. Microscopic analysis indicates beaded axons all over the cerebellur medulla. In the cerebellur cortex, axons found were beaded and degenerated. Degeneration also found in the cerebellum and brain stem especially in the ventral and lateral most tracts. Degenerating fiber were found in motor nucleus of the trigeminal nerve (and particularly the nucleus of spinal tract of the trigeminal (5th) nerve), the trapezoid body, the trigeminal nerve, corticospinal tract and rubrospinal tract. Exceptionally heavy degeneration was observed in the flocculus and in the ventral-most aspect of the periventricular gray (dorsal raphe nucleus). There was also extensive damage to the dorsal and ventral cochlear nuclei. The optic chiasm showed areas of degeneration. All experimental animals exhibited beaded axons and massive degeneration throughout the hippocampus regions. The thalamus, hypothalamus, cingulate cortex, substantia nigra and the reticular formation showed no signs of degeneration in any animals (See Table 4).

Experiment II

In this study, methanol was administered orally to adult rats for six weeks was neurotoxic to rats. Microscopic examination indicates extensive destruction of myelin throughout the cerebral hemispheres of the rat brain. Histological examination showed massive degeneration in the white matter on the ventral-most aspect of the brain. In the gray matter there also appeared lost of broken axons and this region has degeneration throughout the entire ventral surface.

Beaded axons were found in cerebellar medulla, cerebellar cortex and brain stem, especially medulla, cerebellar cortex and brain stem, especially in the ventral and lateral most tracts. Microscopical examination showed degeneration in motor nucleus of the trigeminal nerve. The spinal tract, trigeminal, trapezoid were filled with degenerating fiber.

Optic chiasm have patchy fibers of degeneration. Hippocampus were filled with massive degeneration. The locus and extent of the brain damage was independent of the sex of the experimental rats. The thalamus, hypothalamus substantia nigra reticular formation and cingulate cortex showed no signs of degeneration in any experimental rats (See Table 4).

Whereas, rats received mixture of solution (10% ethanol and 10% methanol) does not exhibit any axonal damage to the central nervous system. Oral administration of equal volume of ethanol/methanol mixed solution showed no signs of degeneration in any rat brain.

Discussion

In this neurotoxicological evaluation of methanol administered orally to rats during pregnancy and nursing period, methanol was neurotoxic at 1.28 g/kg dose levels, chronic low doses methanol are capable of inducing axonal damage in the brain of rats and their offspring.

Methanol induced a decrease in fetal birth weight. Prenatal exposure of methanol also causes malformation of external morphology in pups. These malformation consists of brain defects (hydrocephalus, aenaphaly and exencephaly). The incidence of brain defects were significant. The incidence of still born pups were significant. Methanol also causes eye defects such as microophalmia and anophthalmia. Spina bifida occulta also observed I pups due to prenatal exposure to methanol (1.28 gm/kg of body weight, at 20% (w/v)) aqueous solution. The dose used in this study caused malformation and growth retardation due to prenatal exposure. Although the rat is the generally preferred model for terotogenicity studies (Beaudoin, 1980), it is not preferred for studies of methanol toxicity (Roe, 1982). This is because differences in susceptibility to methanol exists in animals and humans (Roe, 1982; Tyson and Schoenberg, 1914). Unlike the rat, humans lack two specific enzyme uricase (EC.1.7.3.3) and formyltetrahydrofolate synthetase (EC 6.3.4.3) involved in the detoxification of methanol's toxic metabolites, formaldehyde and formic acid (Roe, 1982). It has been shown that the toxicity of methanol in humans is due to its metabolic products, either formaldehyde or formic acid, rather than methanol per se (Tephly and McMartin, 1982). The rat neither accumulates formate I the blood nor develop metabolic acidosis, after large doses of methanol (6 gm/kg), but humans does (Roe, 1982; Tephly and McMartin, 1982).

In this study, methanol was administered orally to rats during prenatal and postnatal period, methanol was neurotoxic at 1.28 g/kg of body weight. Histopathological examination revealed that methanol can induce damage to the central nervous system in both rats and their offspring.

Exposure of the developing brain to methanol via the maternal diet, delays the onset of myelination in the cerebellur cortex, and also causes axonal damage in cerebellum. In this study suggests that the hippocampus of the pup's brain is especially sensitive to the neurotoxic effect of chronic methanol exposure. Until now, there is no literature addressing the long term methanol exposure in any species, due to high resistance to methanol found in nonprimates. Knowledge of methanol neruotoxicity is needed because increased level of methanol are being consumed daily from foods and beverages sweetened with aspartame. Pregnant women concerned about limiting their weight gain using large amounts of this sweetener and subsequently ingesting increased amounts of methanol.

Since prenatal exposure of methanol causes damage to hippocampus in rats, there is a strong possibility that it causes damage in human fetus brain. The hippocampus plays critically important roles in learning and memory in all mammals, including humans. The hippocampus plays a critical role in placing new memories in storage. The hippocampus has numerous connections with most portions of the vertebral cortex as well as with the basic structures of the

limbic system - the anygdala, the hypothalamus, the septum, and the mammillary bodies. Almost any type of sensory experiences causes instaneous activation of different parts of the hippocampus and hippocampus in turn distributes many outgoing signals to the hypothalamus and other parts of the limbic signals to the hypothalamus and other parts of the limbic system, especially through the formix, one of its major output pathways. It has been observed that in the person bilateral removal of the hippocampus, in medical problem (especially for treatment of epilepsy), these person can perform most previously learned activities satisfactorily (Guyton, 1986). However, they cannot learn anything new. Destruction of the hippocampi also cause some deficit in previously learned memories (retrograde amnesia) a little more so far recent memories than far memories of the distant past (Thompson, 1985).

Exposure of the developing brain to alcohol via the maternal diet delays the onset of myelination in cerebral cortex (Jacobson et al., 1979) and alters axonal distribution in rat (West et al., 1981) Alcohol is the potential teratogenic substance, and as such it is the prime cause of mental retardation in the Western World (clarrena and Smith, 1978). Consumption of alcoholic beverages by women during pregnancy has been widely described as a significant threat to normal fetal development. The children with fetal alcohol syndrome, the central nervous dysfunction may be a result of abnormal brain development. It is not yet clear how much and how often ethanol must be consumed by pregnant women to cause impaired brain development in their children.

In 1981, Barnes and Walder have demonstrated that prenatal ethanol exposure can permanently alter the number of dorsal hippocampal pyrimidal cells in the absence of observable physical alterations in physical growth in rat. Results of nutritionally control experiment suggested that the developing nervous system is particularly sensitive to the toxic effects of ethanol (Barnes and Walker, 1981).

Furthermore, Clarren et al. (1978) suggested that abnormalities of brain development can occur in the absence of detectable external abnormalities is supported by a report of postmortem neuropathological case study of human neonates that were exposed to ethanol during gestation. In addition, the study suggested that in utero exposure to ethanol can result in structural brain abnormalities as well as in an abnormally small brain in human (Clarren et al., 1978). The developing nervous system is particularly sensitive to the effects of prenatal ethanol exposure is also supported by the observation of behavioral deficits (Riley et al., 1979 and 1986) and delayed cerebellar development in exposed offspring in the absence of other observable teratology (Knornguth et al., 1979).

Significant number of rat pups dies within first week of lactation period. This strongly suggests that metabolic products of methanol can pass through milk and can cause physiological imbalance and cause severe breathing problems in pups and subsequently can lead to death. In the human central nervous system, the initial multiplication of neurons occurs very early in gestation and the “brain growth spurt” begins about 15-18 weeks before birth, ending about the third to fourth post-natal year of life (Dobbing and Sands, 1973). On the other hand, the neuronal cell multiplication phase in rat occurs throughout the last half of gestation. The “brain growth spurt” is entirely post-natal life (Dobbing, 1974). Since if

exposure to methanol occurs during the most vulnerable period of CNS development and the period of rapid growth, the effects of methanol have far-reaching health consequences.

The human brain undergoes less post-natal development than the rat brain; indeed, the entire gestation period of the rat is only equivalent to the first and second trimesters in human (Dobbing & Sands, 1979).

Studies with prenatal exposure to ethanol showed that ethanol adversely affects the hippocampus in fetal and adult rats (Barnes & Walker, 1981; Riley & Walker, 1978; Riley et al., 1986) and human (McLardy, 1975). West ethanol during a period equivalent to the first and second trimesters in humans results in abnormal neuronal circuitry in the central nervous system.

Permanent motor dysfunction due to methanol intoxication was reported in the literature (Guggenheim, 1971). In report of case history of a young girl who developed rigidity, akinesia, tremor, and pyramidal tract signs following ingestion of methanol in a suicidal attempt. Permanent neurologic sequelae other than optic atropy was result of methanol ingestion (Guggenheim, 1971).

In this study, fiber degeneration was present in the corticospinal tract, rubrospinal tract and red nucleus due to chronic oral methanol exposure in rat. The corticorubrospinal pathway serves as an accessory route for the transmission of relatively discrete signals from the motor cortex to the spinal cord. When the pyramidal fibers are destroyed without destroying this other pathway, discrete movements can still occur, except that the movements of the fingers and hands are considerably impaired. Therefore, the pathway through the red nucleus to the spinal cord is associated far more with the pyramidal system than with the vestibuloreticulospinal system that controls mainly the axial and girdle muscle of the body (Guyton, 1986; Thompson, 1985).

Corticospinal tract also known as pyramidal tract, is responsible for the fire control of movements, such as movements when there is damage to an isolated area of the precentral gyrus or anywhere along the pyramidal tract, there will be a complete loss of all movements of the affected body part, including reflexes. Gradually, over the next one or two weeks the reflexes return, as well as some other movements, but fine movement will be lost permanently (Kalat, 1984; Thompson, 1985).

Massive degeneration are found in cerebellar cortex, deep cerebellar nuclei and cranial nerve of due oral methanol exposure in rat. The cerebellum is involved in synergic control of skeletal muscles and plays an important role in the coordination of voluntary muscular movement. Cerebellar cortex is a large structure in the posterior of the brain, is highly important for motor control (Thompson, 1985). Cerebellum plays a key role in all voluntary ballistic movements in humans, damage to the cerebellum causes difficult with a variety of rapid movements, including and doing most athletic skills (Angevine & Cotman, 1981; Kalat, 1984; Thompson, 1985). Cerebellum is also particularly important for the control of eye movement. When there is damage to certain parts of the cerebellar cortex, the person will have difficult with the initial, rapid movement. When there is damage to the cerebellar nuclei, depending on the location of the damage, there may be difficulty with the brief hold segment. Cerebellar damage normally caused by alcohol (ethanol) intoxication. They often fail the

finger-to-nose test; the finger either overshoots or undershoots the target. The reason for the resemblance is that while alcohol interferes with the functioning of all parts of the brain, the cerebellum is generally among the areas most severely affected during the early stages of intoxication (Kalat, 1984).

Microscopic analysis indicates that methanol induces damage to the several cranial nerve nuclei. By mean of the cranial nerves, the medulla mediates a number of life-preserving reflexes, including breathing, heart rate, vomiting, salivation, coughing, sneezing, and gagging (Angevine & Cotman, 1981; Thompson, 1985).

Ethanol is one of the most commonly used neurotoxic drugs in the world. Currently, its abuse constitutes a serious health problem. In the United States it is stimulated that nine million Americans suffer from chronic alcoholism. It is now well established that chronic alcoholic patients suffer central nervous system disorders (Nakada & Knight, 1984). Up to 27 percent of the brains of chronic alcoholic patients show evidence of cerebellar degeneration at autopsy (Torvik et al., 1982). The process is far more frequent in men than women and has been referred to as "alcoholic cerebellar degeneration." The cause of alcoholic cerebellar degeneration is unknown.

Autopsy revealed cystic resorption of the putamen and the frontocentral subcortical white matter in addition to widespread neuronal damage throughout the cerebrum, cerebellum and spinal cord in human brain stem due to methanol poisoning (McLean, 1980).

Demyelination in cerebral white matter of patients was due to methanol intoxication (Sharpe et al., 1982). Optic damage found in methanol intoxicated patient was due to cerebral white matter damage was reported by Sharpe et al.

Microscopic examination indiciates degeneration in cranial nerve neclei and optic chiasm. In methanol toxicity, metabolic acidosis followed by ocular toxicity are well known features in human (Benton & Calhoun, 1952; Bennett, 1953; Erlanson, 1965; Roe 1943). In methanol toxicity, damage to the optic chiasm and cranial nerves (particularly second nerve) may be responsible for ocular toxicity and blindness. Pre-natal exposure of methanol may possibly be responsible for eye defects and blindness in rat's pups.

In experiment II, rats received 10% ethanol and 10% methanol by oral administration did not have any damage to the central nervous system. In one study investigated the effect of ethyl alcohol on the oxidation of methanol was observed by Zatman (1946). He found that the oxidation of methanol with alcohol dehydrogenase was considerably inhibited by ethyl alcohol. No oxidation took place when ethanol and methanol were present in equimolar quantities. A definite inhibition of methanol oxidation is demonstrable even when the molar relationship of ethanol to methanol is only 1:16 (Roe, 1950). Possibly same mechanism works here to protect brain from damaging effect of methanol by inhibiting alcohol dehydrogenase by ethanol.

In methanol poisoning, clinical investigation showed that symptoms and sings late in the course of methanol poisoning indicated a state of tissue hypoxia (Roe, 1982). Methanol toxicity also cause severe metabolic acidosis, edema, anoxia and ocular toxicity.

In methanol poisoning the most vulnerable organs are eye and central nervous system. The gray matter contains most neurons and has a higher metabolic needs for oxygen so that the preferential effect on brain tissue and in eye happen in most cases of methanol poisoning.

A generalized acidosis occurs in methanol poisoning with generalized hypoxia affecting the body as a whole, and this will be reflected in the circulating blood and may affect all tissues, including the cerebral white matter. Possibly such generalized acidosis induce edema of the cerebral white matter, with a modest degree of hypoxia may induce degeneration of white matter.

The brain swelling caused by edema may be so severe that in some such instances the great increase in intracranial pressure results in a marked decrease in blood circulation in the brain (Feigin et al., 1973).

From the Thesis of Lynette Black

Teratology of Methanol on Long Evens Rats

Statistics

For the maternal data, multivariate analysis (with baseline weight as a covariant) was used for comparisons of maximum weight gain, average daily weight gain, and average placental weight across groups.

Multivariate analysis is an appropriate statistical technique to use with a wide variety of experimental designs [64]. Specifically, this technique should be used when there are more than two experimental variables to consider; in analyses involving more than two variables, there will be second order interactions to consider in addition to the main effects of each variable. Including a variable in the analysis as a covariant is often necessary to hold this "nuisance" variable constant.

Data for fetal body weights, number of implants, and number of corpora lutea were evaluated by analysis of variance. Group means for fetal body weight were than compared with the Students' t-test [64]. Number of resorptions, runts, dead fetuses, total malformed fetuses, and external abnormalities were analyzed using a 2 x 2 contingency table [65].

Chi-square analysis (which may involve a 2 x 2 contingency table in the analysis) is an appropriate statistical technique to use when the data is discontinuous, is at the nominal level, and/or has a skewed distribution [65]. With this analysis, it can be determined if independent samples from two different populations have the same frequency distribution.

In all analyses, the litter was used as the experimental unit, and probability of $p \leq 0.05$ was accepted as significant. Variables, statistics, and p values can be referred to in Appendix C.

Results

Methanol was not toxic to the dams even at the highest dosage. No outward symptoms of toxicity such as unsteady gait, pulmonary congestion, paralysis, or hair loss were noted in the dames following treatment. Average placental weight and body weight in terms of maximum weight gain between Day 1 and Day 20 of gestation and average daily weight gain were not significantly affected by methanol treatment.

Exposure of the dams to methanol had no significant effect on the numbers of corpora lutea, implantations, or dead fetuses. Also methanol exposure did not significantly affect the ration of male to female fetuses. However, at the higher dosage, 3.0 g/kg, methanol significantly depressed mean fetal birth weight below that of the control group ($p \leq 0.05$; 2.31 ± 0.48 g Vs 3.38 ± 0.21g) (Table I). At the lower dosage, 0.3g/kg, the mean fetal birth weight

was not significantly depressed. The number of litters having runts increased significantly with methanol administration in a dose-related manner (Table 1). At the higher dosage level, 24 of the 26 litters had one or more runts ($p<0.001$; 262 out of 336 total fetuses). At the lower dosage level, six of the 2.1 litters had one or more runts (p 0.05; 18 out of 272 total fetuses).

Methanol administration also significantly increased total resorptions (post-implantation loss) in a dose-related manner (Table 1). At higher dosage, 22 of the 26 litters had one or more early/late resorptions, ($p<0.001$; 39 total resorptions out of 374 implantations). At the lower dosage, four of the 21 litters had one or more early/late resorptions (p 0.05; four total resorptions out of 276 implantations). At the higher dosage, the number of early resorptions was significant, ($p<0.001$): at the lower dosage, the number of early resorptions was not significant. The number of late resorptions was not significant at either dosage.

The incidence of malformations was significantly increased in the treatment group exposed to the higher dosage (Table 1). Twenty of the 26 litters had one or more fetuses with malformations of external morphology ($p<0.001$; 51 of the 336 total fetuses); the external malformations included brain defects, eye defects, and spina bifida occulta. The incidence of brain defects was not significant; specifically, one of the 26 litters had two fetuses with hydrocephalus, and one litter had one fetus with aencephaly and one fetus with exencephaly. The incidence of eye defects was significant ($p<0.05$); specifically, five of the 26 litters had eight fetuses with anophthalmia, and three litters had five fetuses with microopthalmia. The incidence of spina bifida occulta was also significant; 19 of the 26 litters had one or more fetuses with this spinal deformity ($p<0.001$; 40 out of 336 total fetuses).

The incidence of malformations in the lower treatment group receiving 0.3 g/kg was not significant (Table 1). Only one fetus from one of the 21 litters had a malformation of external morphology (spina bifida occulta).

No incidence of specific skeletal anomalies - missing, extra, wavy, or fused ribs and missing bones in the upper and lower limbs- was found in either the treatment groups or the controls. Because a more thorough and complete analysis of the fetal skeletons was not possible or practical in this study, a statement regarding the statistical significance of skeletal malformations in the fetuses of dams treated with 0.3 g/kg and 3.0 g/kg methanol cannot be made.

Overall, methanol exposure significantly affected mean fetal birth weight and the incidence of resorptions and runts. Methanol exposure affected the incidence of external malformations only at the higher dosage of 3.0 g/kg. Brain defects, eye defects, and spina bifida occulta comprised the external malformations at this dosage; the incidence of eye defects and spina bifida was significant.

Table 1

INFLUENCE OF METHANOL ON PREGNANT LONG-EVANS RATS[a]

Group	**O**	**1**	**3**
No. pregnant/ No. bred	22/24	21/22	26/27
x Max. wt. gain + SD (g)[b]	130.56 ± 13.14	120.34 ± 19.17	110.64 ± 45.47
x Daily wt. gain +SD (g)	6.95 ± 0.70	6.33 ± 19.17	6.88 ± 0.81
x Placental st. + SD (g)	0.52 ± 0.18	0.52 ± 0.14	0.44 ± 0.21
x Corpora lutea/dam + SD	17 ± 2	18 ± 4	17 ± 3
x Implants/ dam + SD	13.2 ± 2	13 ± 4	14 ± 5
x Fetal weights + SD (g)	3.38 ± 0.21	3.27 ± 0.27	2.31 ± 0.48
Sex ratio (M: Fe)	54:46	54:46	54:46
Litters (fetuses) observed	22 (296)	21 (272)	26 (336)
Litters (%) w/ early resorptions/No.	0	3(14)*/3	21 (80)**/39
Litters (%) w/ late resorptions/ No.	0	1 (5)/1	3 (12)/3
Litters (%) w/ total resorptions?No.	0	4 (19)*/4	22(88)**/39
Litters (%) w/ runts[c] /No. fetuses (%)	0	6 (29)*/18 (6)	24 (92)**/262 (78)
Litters (%) w/ dead fetuses/No. fetuses (%)	0	0	1 (4)/1 (0.3)
No. litters (%) affected/No. fetuses (%) affected			
Total external malformations	0	1(5)/1 (0.4)	20 (77)**/51 (15)
Brain	0	0	2 (8)/4 (1)
Hydrocephalus	0	0	1 (4)/2 (0.6)
Aencephaly	0	0	1 (4)/1 (0.3)
Excencephaly	0	0	1 (4)/1 (0.3)
Eye			6 (23)*/13 (4)
Mitroophthalmia	0	0	5 (19)*/8 (2)
Anophthalmia	0	0	3 (12)*/5 (1)
Spina bifida occulta	0	1 (5)/1 (0.4)	19 (73)**/40 (12)

[a] Rats were treated wotj ,atjamp; frp, Day 6 - Day 15 of gestation:
0 = controls, 1 = 0.3 g/kg methanol, 3 = 3.0 g/kg methanol

[b] Weight gained between Day 1 and Day 20 of gestation

[c] Runts - features whose weights are less than the mean of the base control minus 3-fold standard deviations.

* significantly different from control group at $p < 0.05$
** significantly different from control group at $p < 0.001$