Neurochemical Research, Vol. 14, No. 11, 1989, pp. 1081–1088

Maternal Alcohol Consumption and Undernutrition in the Rat: Effects on Gangliosides and Their Catabolizing Enzymes in the CNS of the Newborn

V. V. T. S. Prasad[1,2]

(Accepted July 18, 1989)

Consumption of fifteen percent alcohol, during gestation did not cause any decrease in the total calorie or fluid intake of the rats maintained on normal dietary regimen. However, the alcohol consumption by gestating mothers resulted in a decreased contents of both DNA and protein in the CNS of the *in utero* alcohol exposed pups at birth. DNA content was also found to be less in the undernourished pups compared to the normal pups. On the other hand an increase in the total gangliosides and a decrease in the ganglioside catabolizing enzymes was observed in the brain and spinal cord of alcoholic pups at birth. However undernutrition resulted in a decrease in the content of total gangliosides both in brain and spinal cord. Maternal alcohol consumption and undernutrition had also resulted in an altered proportions of the individual ganglioside fractions.

KEY WORDS: CNS; alcohol; undernutrition; DNA; gangliosides; glycosidases.

INTRODUCTION

Gangliosides are a family of sialo glycosphingolipids, which are normal constituents of mammalian cell membranes (49). Gangliosides are particularly abundant in neuronal membranes (8, 29, 34, 49, 59) but are also present at much lower levels in myelin (40, 49, 51, 52). The functions of gangliosides are not yet clearly understood. However, by virtue of their asymmetric localization on the outer surface of the lipid bilayer of membranes (24) and because of the striking changes in brain gangliosides concentration and composition during development (25, 55) gangliosides are believed to play an important role in several cellular events. These functions include differentiation of neuronal cells (18, 33, 38), growth (20), regeneration, transformation (19), cell-cell interaction (62), receptor sites for peptide hormones (30), release and reabsorption of neurotransmitters (5, 18). Gangliosides have also been known to affect the kinetics of certain membrane bound enzymes (33) suggesting their importance in neural membrane function.

Although, "Growth-Spurt" which consists of axonal and dendritic growth, synaptogenesis (7), glial multiplication and myelination (11, 60) is a postnatal event in rat brain (16), development of nervous system begins around the end of first week of gestation (27). Neuronal multiplication takes place in the last week of gestation (17) and except for certain microneurons (3, 4) adult number of neurons is reached by the third postnatal day with the cessation of neuroblast multiplication (12).

The presence of gangliosides in the nervous system has been reported in the fetal and newborn rats (25, 56, 57) though their maximum deposition corresponds to the peak period of dendritic arborization and synaptogenesis (56). This indicates that the metabolic pathways of gangliosides in nervous tissue are operative even during gestation.

Teratogenic effects of alcohol have been well documented (32) and especially the developing CNS has been shown to be susceptible to the prenatal alcohol

[1] Department of Biochemistry, Faculty of Science, M. S. University of Baroda, Baroda 390 002, India.
[2] Address reprint request to: V. V. T. S. Prasad, Department of Pharmacology, University of Florida, Gainesville, FL 32610, USA.

0364-3190/89/1100-1081$06.00/0

exposure (37). Furthermore, maternal undernutrition has also been shown to decrease the cell number (63) and particularly multipolar neurons of the rat CNS (46). Since chronic alcoholism often has been associated with undernutrition, it is of interest to study the specific contributions of alcohol, undernutrition and the interaction of these two in producing abnormalities.

Studies were therefore, planned to determine the effects of maternal alcohol consumption and undernutrition on the offspring at different developmental stages.

EXPERIMENTAL PROCEDURE

Experimental Design and Animals. Virgin female rats of Charles-Foster strain weighing 180-200 g were divided into four groups. Control group (Normal Protein:NP) was fed 20% casein diet and drinking water *ad lib*. Second group of rats (Normal Protein Alcohol:NPA). were fed 20% casein diet and 15% alcohol in drinking water. Third group (Low Protein:LP) of rats were fed 6.5% casein diet and drinking water *ad lib*. and low protein-alcohol(LPA) rats were fed 6.5% casein diet and 15% alcohol in drinking water *ad lib*. The four groups of rats were maintained on respective regimen for four weeks prior to breeding and during gestation. After recording the body weights, pups at birth, were sacrificed by decapitation. Intact whole brains and spinal cords were quickly dissected out and tissue weights were recorded. For ganglioside analysis tissues were transferred to tubes containing cold chloroform:methanol mixture (1:1v/v).

Biochemical Analysis

(A) *Estimation of Maternal Plasma Alcohol Levels*. Tail blood samples were collected in the morning at the end of pregnancy. Alcohol was assayed by the alcohol dehydrogenase method of Bernt and Gutman, 1974(6).

(B) *Ganglioside Analysis*. Total gangliosides were isolated and purified by the method of Seyfried *et al*. 1978 (45) and ganglioside N-acetyl-neuraminicacid (NANA) was assayed by the method of Warren, 1959 (59) as modified by Skoza and Mohes, 1976 (48). For the estimation, aliquots containing 3-40μg ganglioside- NANA in 0.9ml were digested at 80°C for 2 hours with 0.1 ml of 1N sulphuric acid. The samples were kept at room temperature for 10-15 minutes and 0.25ml of 25 uM sodium periodate was added and vortexed. After 30 minutes 0.25 ml of 2% sodium arsenite was added and vortexed. And after the disappearance of yellow colour 0.5ml of 6% thiobarbituric acid was added and the samples were kept in boiling water for 7.5 minutes. After cooling the samples to room temperature 2.5ml of Dimethyl sulfoxide was added and the colour was read at 532 and 549 nm against a blank which consisted of distilled water in place of sample or standard. Interference was corrected using the following equation (59)

$$\text{Optical Density at } 549\text{nm} \times 0.09 - \text{Optical Density at } 532\text{nm} \times 0.03 = \text{Optical density due to NANA.}$$

For the separation of different fractions of gangliosides, 20 × 20cm glass plates coated with 0.25mm layer of silica gel-G were used. The plates were developed by one ascending run with chloroform:methanol:water(58:38:9) that contained 0.02%, $CaCl_2.H_20$ The bands, marked after exposure to iodine vapoures were scarped from the plates and ganglioside-NANA was estimated. Since the concentration of monosialogangliosides like GM_2 and GM_3 are extremely low the bands corresponding to monosialogangliosides were pooled together for the estimation and the fraction was designated as GM. The bands were confirmed as gangliosides by using resorcional-HCl reagent, on a duplicate set (53). The identity of individual ganglioside fractions was confirmed by simultaneously running commercially obtained reference bovine brain ganglioside mixture from Sigma chemical company, U.S.A. A typical thin layer chromatogram of the separation of gangliosides is shown in Figure 1.

(C) *Preparation of Tissue Homogenate for Enzyme Assays*. A10% (w/v) homogenate was prepared by homogenizing tissue with ice cold glass distilled water for 3 minutes at 2,000-3,000 rpm upon ice using a Potter-Elvehjem homogenizer. The homogenate was used for enzyme assays.

Enzyme assays

β-D-Galactosidase (EC 3.2.1.23) and β-D-Glucosidase (EC 3.2.1.21) were assayed according to the method of Gatt and Rapport, 1966 (22). β-D-Galactosidase assay system contained 3 micromoles of *p*-Nitrophneol-β-D-galactopyronoside and 100 micromoles of either glycine-HCl, pH3.1 buffer or acetate, pH4.5 buffer. Final volume of 1ml was made up with glass distilled water. β-D-Glucosidase assay system contained 5.6 micromoles of p-Nitrophenyl-β-D-glucopyranoside and 100 micromoles of acetate buffer pH5.0 in a total volume of 1ml made up with distilled water. Total β-D-Hexosaminidase (EC 3.2.1.30) activity was assayed according to the method of Frohwein and Gatt, 1967 (21) by incubating with 50 micromoles of glycine-HCl, pH3.5 and 4 micromoles of p-Nitrophenyl-N-Acetyl-β-D-glucosaminide in a total volume of 0.5 ml. Enzyme unit for the above mentioned enzymes is defined as nmol of *p*-nitrophenol liberated per minute under the assay conditions.

The sialidase (E.C. 3.2.1.18) activity using endogenous substrate alone was assayed according to the method of Irwin *et al*. 1973 (26), by incubating with 50 micromoles of acetate buffer pH 4.5, for 2 hours at 37°C. The reaction was terminated by adding 0.25ml of 25mM chilled sodium periodate solution and 0.75 ml of chilled glass distilled water. Tubes were centrifuged and free NANA in the supernatant was measured as mentioned earlier. Enzyme unit for sialidase is defined as nmol of NANA liberated per minute under assay conditions.

All the enzyme assays were carried out at optimal conditions. Substrate and enzyme blanks were included in the assays.

Protein and DNA were estimated by the method of Lowry *et al*., (35), and Schneider, 1957 (44), respectively.

Statistical comparison between group means was performed by Student's *t* test (10).

Materials: All reagents used were of analytical grade. p-Nitrophenyl-β-D-galactopyranoside, p-Nitrophenyl-β-D-glucopyranoside, *p*-Nitrophenyl-N-Acetyl-β-D-glucosaminide, Alcohol dehydrogenase, sephadex G-50, DEAE-sephadex A-25 and Bovine brain ganglioside mixture were from Sigma Chemical company, U.S.A. Silica Gel-G was obtained from E. Merck, Germany.

RESULTS

In the preliminary studies fifteen percent alcohol was found to be isocaloric to the control rats and it did not affect the water intake.

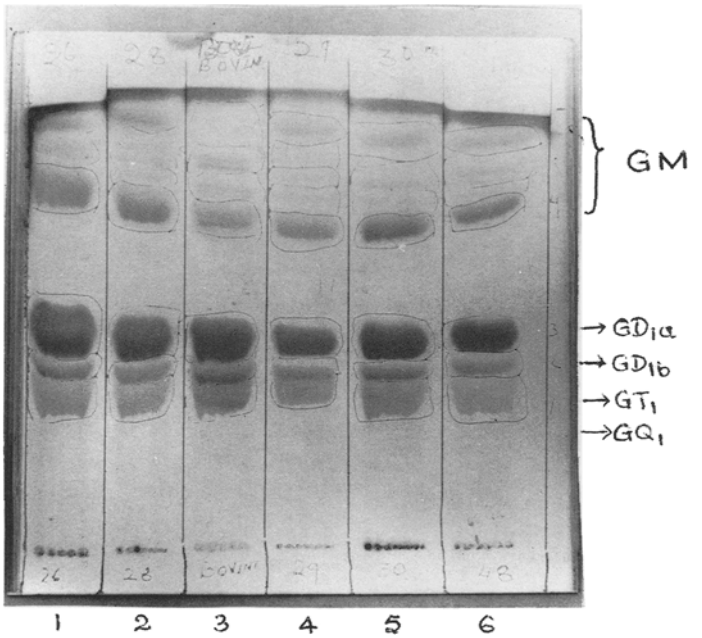

Fig. 1. Thin layer chromatogram of brain gangliosides. TLC conditions were exactly as described in the Experimental Procedure section. Numbers 1,2,4,5,6 are rat brain gangliosides and Number 3 is bovine brain gangliosides.

(a) The results of maternal alcohol consumption and undernutrition on DNA and protein of the progeny are shown in Table I. The brain and spinal cord weights of NPA, LP and LPA pups were significantly less compared to the NP pups. Weight deficits were also observed in LPA pups compared to the LP pups.

DNA and protein concentrations (mg/g tissue) and contents (mg/total tissue) were significantly decreased in both brain and spinal cord of the NPA pups compared to the NP pups resulting in to a significant increase in protein/DNA ratio. Similarly, a decrease in DNA concentration and content was also observed in both brain and spinal cord of the LPA pups compared to the LP pups. However, there was no significant change in protein concentration in spinal cord of the LPA pups compared to the LP pups (Table I).

DNA content of both brain and spinal cord of LP pups showed a marked decrease when compared to the NP pups but the concentration remained unchanged. A decrease was also observed in both concentration and content of protein in brain and spinal cord of LP pups compared to the NP pups. Protein/DNA ratio showed a significant decrease in the brain of LP pups compared to the NP pups.

(B) The effects of maternal alcohol consumption and undernutrition on total ganglioside concentration and pattern are presented in Table-II. Both brain and spinal cord of NPA and LPA pups showed a significant increase in the amounts of total gangliosides compared to the NP and LP pups, respectively. However, LP pups showed a significant decrease in the amount of total gangliosides compared to the NP pups. These results were reflected in both concentration (microgram NANA/g tissue) and content (microgram NANA/tissue) as shown in the Table II.

Five major gangliosides, namely, GQ_1, GT_1, GD_{1b}, GD_{1a} and GM were estimated. In NP pups brain gangliosides were present in the order $GT_1 > GD_{1a} > GM > GD_{1b} > GQ_1$ and in the undernourished pups the order was $GT_1 > GD_{1a} > GM > GQ_1 > GD_{1b}$. An increased proportion of GT_1 and GQ_1 and decreased proportion of GD_{1a} and GM was observed in the brains of NPA pups compared to the NP pups. On the other hand LP pups showed a higher proportions of GQ_1 and GM and lower proportions of GT_1, GD_{1b} and GD_{1a} in brain compared the NP pups. An increase in GD_{1a} proportion and a decrease in GQ_1 proportion in the brains of LPA pups compared to the LP pups was observed.

In the spinal cord of control pups the ganglioside pattern was $GT_1 > GD_{1a} > GM > GD_{1b} > GQ_1$ and in the undernourished pups the pattern remained same but showed a significant increase in GD_{1a} and a decrease in GM proportions over the controls. An increase in the proportions of GQ_1 and GT_1 and a decrease in GM pro-

Table I. Changes in DNA and Protein levels in CNS Tissues of Rat Pups Due to Maternal Alcohol Consumption and Protein Restriction

Group	Tissue	Tissue weight (g)	DNA		Protein		Protein / DNA
			mg/g tissue	mg/tissue	mg/ g tissue	mg/ tissue	
NP	W . B	0.248	3.47	0.86	63.3	15.7	18.3
	(n=6)	±0.005	±0.063	±0.013	±0.69	±0.35	±0.41
	S . C	0.039	3.03	0.117	51.6	1.99	17.0
	(n=3)	±0.0007	±0.057	±0.04	±0.53	±0.035	±0.25
NPA	W . B	0.217[d]	2.98[d]	0.649[d]	61.4[d]	13.4[d]	20.6[d]
	(n=6)	±0.002	±0.1	±0.024	±1.29	±0.38	±0.27
	S . C	0.028[d]	2.26[d]	0.064[d]	48.7[c]	1.37[d]	21.4[d]
	(n=3)	±0.0004	±0.09	±0.0026	±0.64	±0.025	±0.52
LP	W . B	0.204[d]	3.51	0.73[d]	62.1	12.7[d]	17.2[d]
	(n=6)	±0.004	±0.066	±0.021	±0.6	±0.027	±0.13
	S . C	0.0232[d]	2.92	0.0677[d]	50.8	1.17[d]	17.6
	(n=6)	±0.009	±0.055	±0.0015	±0.73	±0.027	±0.3
LPA	W . B	0.191[b]	3.1[d]	0.595[d]	59.2[e]	11.3[d]	18.9[d]
	(n=6)	±0.003	±0.05	±0.055	±0.86	±0.28	±0.55
	S . C	0.018[h]	1.95[h]	0.035[h]	47.1	0.847[h]	24.2[h]
	(n=3)	±0.0002	±0.0058	±0.0	±1.58	±0.03	±0.76

NP - Pups born of mothers fed 20% casein diet; NPA - Pups born of mothers fed 20% casein diet and 15% alcohol in drinking water. LP - Pups born of mothers fed 6.5% casein diet; LPA - Pups born of mothers fed 6.5% casein diet and 15% alcohol in drinking water.
n = number of observations. Values are mean ± SD.
Levels of significance was tested by student's 't' test. Values significantly different from NP group; [a] $P < 0.05$; [b] $P < 0.02$; [c] $P < 0.01$; [d] $P < 0.001$. Values significantly different from LP group; [e] $P < 0.05$; [f] $P < 0.02$; [g] $P < 0.01$; [h] $P < 0.001$.

portions in the NPA pups compared to the NP pups was observed. In the LPA pups, GD_{1b} proportion besides GM also showed a significant decrease when compared to the LP pups.

The activities of β-D-Galactosidase (pH 3.1 and 4.5), β-D-Glucosidase, β-D-Hexosaminidase and Sialidase (units/g tissue) in brain and spinal cord of NP, NPA, LP and LPA pups are shown in Table-III. Both brain and spinal cord of NPA and LPA pups showed a highly significant decrease ($P<0.001$) in the β-D-Galactosidase (pH 3.1 and 4.5) β-D-Glucosidase, β-D-Hexosaminidase and Sialidase activities compared to the NP and LP pups, respectively. The enzyme activities were also found to be significantly high in the brains of the LP pups when compared with the NP pups. Spinal cord of LP pups also showed similar increase in the activities of these enzymes excepting β-D-Galactosidase (pH 4.5) activity.

DISCUSSION

Though several researchers have studied teratogenic effects of alcohol administered to pregnant rats, in many of these studies alcohol dose was relatively high (43, 47, 55) with alcohol contributing as high as 28-36% of total calorie intake. Administration of alcohol in high doses was shown to be associated with a decrease in the intakes of total calorie (13), food (13, 61) and fluid (2). Therefore 15% alcohol in drinking water which gave comparable blood ethanol concentration with out causing a decrease in total calorie, food or fluid intakes of the alcoholic rats, was chosen for this study.

The observed deficits in cell number and an increase in cell size as judged by the DNA content and protein to DNA ratio, respectively in the brains and spinal cords of alcohol exposed pups are in agreement with the earlier reports by others (9, 61, 63). Woodson and Ritchey, 1979 (61) showed a significant decrease in DNA and somewhat larger cells in the fetal brain exposed to alcohol, *in utero*. Bursey 1972 (9) reported a reduction in brain DNA concentration of the offspring (at 8 weeks of age) (when the rats were fed alcohol during perinatal period. The reductions observed in the DNA and protein contents and cell size due to undernutrition in the present study are also in line with the observations made by others (46, 61).

Total gangliosides increased significantly both in the brain and spinal cord of the offspring due to maternal alcohol consumption (Table-II). It is of interest to note that the total ganglioside content (microgram NANA/ tissue) is significantly high in NPA and LPA pups compared to NP and LP pups, respectively, in both brain and spinal cord, in spite of a significant decrease in the

Table II. Effect of Maternal Alcohol Consumption and Undernutrition on Rat Brain and Spinal Cord Gangliosides at Birth

			Total gangliosides		ganglioside species (% of total ganglioside NANA)				
Group	Tissue	wet weight (g)	μg NANA/g tissue	μg NANA/ tissue	GQ_1	GT_1	GD_{1b}	GD_{1a}	GM(x)
	W B	0.248	264	65.1	5.9	36.2	13.1	29.2	15.6
	(n=8)	±0.005	±8.0	±2.6	±0.095	±0.88	±0.31	±1.48	±1.23
NP	S C	0.040	166	6.6	7.8	36.1	12.6	25.3	18.2
	(n=6)	±0.0064	±13.9	±0.52	±0.3	±0.606	±0.6	±0.83	±0.59
	W B	0.224[c]	369[d]	83[d]	8.8[c]	43.4[d]	12.2	21.8[d]	13.8[c]
NPA	(n=8)	±0.0046	±27.6	±2.3	±1.8	±1.73	±1.73	±1.5	±0.9
	S C	0.028[d]	286[d]	8.0[c]	12	25.8[d]	17[d]	32.5[c]	12.6[d]
	(n=8)	±0.0022	±19.9	±0.92	±1.23	±1.21	±1.5	±1.32	±1.6
	W B	0.204[c]	237[c]	48.2[d]	14.9[d]	32.6	10.4	24.6[d]	17.5[a]
	(n=8)	±0.008	±16	±2.4	±1.8	±1.52	±1.1	±0.87	±1.7
LP	S C	±0.025[d]	135[b]	3.4[d]	8.04	35.9	12.2	29.1[d]	14.6[d]
	(n=8)	±0.0083	±3.5	±0.23	±0.65	±0.58	±1.0	±1.87	±1.1
	W B	0.190	278[g]	52.7[f]	4.3[g]	41.9[h]	10.5	30.8[h]	12.6[h]
	(n=8)	±0.01	±25.5	±3.8	±1.2	±2.2	±1.05	±0.88	±1.29
LPA	S C	0.0187[h]	244[g]	4.6[h]	14.6[h]	25.0[h]	8.6[h]	41.6[h]	10.2
	(n=8)	±0.0015	±24.6	±0.275	±1.23	±0.84	±0.34	±1.59	±1.2

NP - Pups born of mothers fed 20% casein diet; NPA - Pups born of mothers fed 20% casein diet and 15% alcohol in drinking water; LP - Pups born of mothers fed 6.5% casein diet; LPA - Pups born of mothers fed 6.5% casein diet and 15% alcohol in drinking water.

W.B - whole brain; S.C Spinal cord

Values are mean ± S.D; n = Number of observations. Each brain sample was obtained by pooling brains from 2-3 pups. Each spinal cord sample was obtained by pooling spinal cords from 12 - 16 pups.

NANA was estimated in 5 major ganglioside species. GM (x) represents total monosialo gangliosides. Levels of significance was tested by student's '*t*' test. Values significantly different from NP group [a] $P < 0.05$; [b] $P < 0.02$; [c] $P < 0.01$; [d] $P < 0.001$. Values significantly different from LP group : + $P < 0.05$ [f] $P < 0.02$; [g] $P < 0.01$; [h] $P < 0.001$.

tissue weights. The increase in total gangliosides is in line with the reported increase in hepatic lipid content in fetal and neonatal rats born of mothers fed ethanol during gestation (43) and also with the observed increase in the concentration of cerebral lipids including gangliosides due to alcohol treatment (42). As the gangliosides are majorily located in plasma membranes (31) the increase in total gangliosides, in *in utero* alcohol exposed pups may be accounted by the increase in the cell size observed in the present study. In contrast, maternal undernutrition caused a decrease in CNS total gangliosides which may be attributed to the observed decrease in cell number and cell size in the undernourished pups (Table-I). Deficits in total brain gangliosides have been reported in malnourished children (15). However, it has been shown that feeding mothers with a low protein diet during gestation caused no changes in total brain ganglioside levels of the offspring (28). These differences may be due to the variations in the amount of protein in the diets and the duration of exposure.

In the present study, the ganglioside composition was also found to be affected due to maternal alcohol consumption and undernutrition, and the effects of the two were found to be different. An increase in GT_1, and GQ_1 and a decrease in GD_{1a} and GM was observed in the brains of NPA pups compared to the NP pups, whereas in the brains of undernourished pups the GT_1 and GD_{1a} were found to be decreased and GQ_1 and GM increased compared to the control pups. The alterations due to alcohol were found to be more profound in GT_1 and GQ_1 which increase during the latter part of prenatal development (25). In this connection, Vrbaski *et al.* 1984 (58) also reported an increase in GT_1 and a decrease in the GD_{1a} proportions in adult rat brain subjected to chronic alcohol consumption and the changes were attributed to the presence of ethanol itself. In the malnourished children, GD_{1a} fraction of ganglioside was found to be decreased in the forebrain compared to the normal children (14, 36). The observed decrease in the proportion of GM in the brain and spinal cord of alcoholic pups is in line with the reported decrease in GM_1 proportion in the myelin of in utero alcohol exposed pups (23).

The interactive effects of alcohol and undernutrition were again different on the ganglioside composition when comapred to the effects of either alcohol or undernutrition, alone. For e.g. in brain proportions of GQ_1 was

Table III. Effect of Maternal Alcohol Consumption and Undernutrition on Enzymes of Ganglioside Catabolism in Rat Brain and Spinal Cord at Birth

Group	Tissue	β-D-Galactosidase Activity at pH 3.1	β-D-Galactosidase Activity at pH 4.5	β-D-Glucosidase	β-D-Hexosaminidase	Sialidase
		(Enzyme units / g tissue weight)				
NP	W . B ($n=15$)	134 ±4.7	85 ±4.0	185 ±13.4	1519 ±168	2.25 ±0.028
	S . C ($n=10$)	261 ±8.0	139 ±5.8	196 ±18.2	1161 ±101	1.22 ±0.23
NPA	W . B ($n=15$)	110[d] ±5.6	69[d] ±7.3	151[d] ±13.6	1067[d] ±96.9	1.16[d] ±0.206
	S . C ($n=15$)	204[d] ±3.6	122[d] ±1.7	165[d] ±7.5	1320[d] ±191	0.079[d] ±0.28
LP	W . B ($n=8$)	128[c] ±4.1	88[c] ±0.98	225[d] ±4	2725[d] ±185	3.3[d] ±0.03
	S . C ($n=6$)	221[d] ±2.9	143 ±15.6	216[d] ±2.4	3280[d] ±55	246[d] ±0.16
LPA	W . B ($n=6$)	110[h] ±5.4	69[h] ±4.0	210[h] ±3.9	2390[g] ±176	2.65[h] ±0.09
	S . C ($n=6$)	204[g] ±2.9	124[g] ±1.0	180[h] ±6.0	3157[g] ±66	1.9[h] ±0.07

NP - Pups born of mothers fed 20% casein diet; NPA - pups born of mothers fed 20% casein diet and 15% alcohol in drinking water; LP - Pups born of mothers fed 6.5% casein diet; LPA - Pups born of mothers fed 6.5% casein diet and 15% alcohol in drinking water.

n = Number of observations; Values are mean ± SD. Each spinal cord sample was obtained by pooling spinal cords from 3-6 pups.

Enzyme unit is defined as n moles of product liberated per minute under assay conditions. Levels of significance was tested by student's 't' test.

Values significantly different from NP group [a] $P < 0.05$; [b] $P < 0.02$; [c] $P < 0.01$; [d] $P < 0.001$.

Values significantly differnt from LP group, [e] $P < 0.05$; [c] $P < 0.02$; [g] $P < 0.01$; [h] $P < 0.001$.

increased in the NPA pups and decreased in the LPA pups compared to the NP and LP pups, respectively, whereas the maternal undernutrition caused a marked increase in the proportions of GQ_1 in the brains of LP pups compared to the NP pups (Table-II). Some of the changes observed in the ganglioside composition appear to be tissue specific, too. For example, the spinal cord of the NPA pups showed an increase in GD_{1a} proportion and a decrease in GT_1 proportion compared to the NP pups. In brain, however, GD_{1a} was decreased and an increase in the GT_1 proportion was observed in the NPA pups compared to the NP pups.

Ganglioside catabolism proceeds in a step wise manner and the enzymes known to be involved in the catabolism are Sialidase, β-D-Galactosidase, β-D-Hexosaminidase and β-D-Glucosidase. Sialidase activity was assayed against endogenous substratate as the use of endogenous substrate may reflect the enzyme-substrate interactions as it actually occurs in the cells (26). In the present study activity of β-D-Galactosidase was assayed both at pH 3.1 and 4.5 as our preliminary studies have suggested a possible excistence of β-D-galactosidase isozymes in rat brain (41). In contrast to the changes in the total gangliosides, these enzyme activities in the CNS showed an increase following *in utero* exposure to alcohol and a decrease due to maternal undernutrition.

Apparently, undernutrition may not be the primary cause of the abnormalities observed in the pups exposed to alcohol, for the following reasons. First, the pregnant rats (NPA group) were receiving the recommended amounts of protein, total calories and water (39). Secondly, the differences observed in the NPA and LP pups were quite different. Thus the abnormalities observed in the NPA pups may not be due to undernutrition but may be a direct consequence of alcohol treatment.

In conclusion, the results suggest that the increase in total gangliosides due to prenatal alcohol exposure may be at least, in part, due to the decreased activities of the enzymes related to ganglioside catabolism. This probably would reflect an overall decrease in the catabolic activity of ganglioside metabolism in the CNS of the pups born of mothers consuming alcohol a month prior to conception and during gestation. Similarly the decrease in the levels of total gangliosides in the under-

nourished pups may be correlated, at least in part, to the increased catabolic activity due to undernutrition. However, in the absence of data on enzymes involved in ganglioside synthesis, it is not possible to arrive at any conclusive explanation to the alterations observed in the ganglioside composition in the experimental rats.

The changes observed in the rat brain, at birth due to maternal alcohol consumption and undernutrition, may reflect mostly those occurring in neuronal cell population as the neuronal multiplication is mostly a prenatal event (3, 4, 17), prior to glial formation which is a postnatal event (16). However, the functional significance of these abnormalities are yet to be elucidated.

The above mentioned findings indicate that the maternal alcohol consumption during gestation even in relatively moderate doses could be deleterious to the offspring with alcohol causing deficits in the CNS cell number and effecting abnormalities in the total gangliosides level and their composition, in the CNS of the alcoholic pups.

ACKNOWLEDGMENT

This research was supported by the University Grants Commission's special assistance programme to the Department of Biochemistry, M. S. University of Baroda, India.

REFERENCES

1. Abel, E. L. 1978. Effects of ethanol on pregnant rats and their offspring. Psycho pharmacology. 57:5–11.
2. Abel, E. L., and Dintcheff, B. A. 1978. Effects of prenatal alcohol exposure on growth and development in rats. Pharmacol. Exp. Ther. 207:906–921.
3. Altman, J. 1968. Autoradiographic and histological studies of postnatal neurogenesis II. J. Comp. Neurol. 128:431–474.
4. Altman, J., and Das, G. D. 1966. Autoradiographic and histological studies of postnatal neurogenesis I. J. Comp. Neurol. 126:337–389.
5. Behr, J. P., and Lehn, J. M. 1973. The binding of divalent cations by purified gangliosides. FEBS Letters. 31:297–300.
6. Bernt, E., and Gutman, I. 1974. Ethanol determination with ADH and NADP. Pages 1499–1502, in Bergmeyer, H. U. (ed.). Methods of enzymatic analysis, Academic Press Inc., N.Y.
7. Bloom, F. E. 1972. The formation of synaptic junction of developing rat brain. Pages 101–120, *in* Pappas, G.D. and Purpura, D. P. (eds). Structure and Function of Synapses. Raven Press, N.Y.
8. Burton, R. M., and Gibbons, J. M. 1964. Lipid composition of rat brain synaptic vesicle fraction. Biochim. Biophys. Acta. 84:220–223.
9. Bursey, R. G. 1972. Effect of maternal ethanol consumption during gestation and lactation on the development and learning performance of the offspring. Ph.D. dissertation. Clemson university.
10. Colton, T. 1974. Inference on means. Pages 99–150, *in* Statistics in Medicine, Little Brown and Company, Boston.
11. Davison, A. N. 1977. Biochemical, morphological and functional changes in the developing brain. "Biochemical correlates of brain structure and function". Pages 1–13, *in* Davison, A.N. (ed). Academic Press, New York.
12. Davison, A. N. and Dobbing, J. 1968. The developing brain. Pages 253–286, *in* Davison, A. N. and Dobbing, J. (eds.), Applied Neurochemistry. Blackwell, Oxford.
13. Detering, N., Reed, W. D., Ozand, P., and Karhasan, A. 1979. The effects of maternal ethanol consumption in the rat on the development of their offspring. J. Nutr. 109:999–1009.
14. Dickerson, J. W. T. 1980. Protein deficiency and the brain. In: Food and Health: Science and Technology. Pages 487–500 *in* Birth, G. G. and Parker, K. J., (eds.). Applied Science Publishers, London.
15. Dickerson, J. W. D., Meart, A. and Yusuf, H. K. M. 1982. Effect of malnutrition on brain growth and development, Pages 73–108, *in* Dickerson, J. W. T. and Mc Gurk, H. (eds.). Brain and Behavioural Development. Surry Univ. Press, Guildford.
16. Dobbing, J. 1968. Vulnerable periods in developing brain. Pages 287–316 *in* Davison, A. N. Dobbing, J. (eds) Applied Neurochemistry. Blackwell, Oxford.
17. Dobbing, J., and Sands, J. 1979. Comparative aspects of the brain growth spurt. Early human develop. 3:79–83.
18. Ferrari, G., Fabris, M., and Gorio, A. 1983. Gangliosides enhance neurite outgrowth in pc 12 cells. Develop. Brain Res. 8:215–221.
19. Fishman, P. H., Brady, R. O.. and Aaronson, S. A. 1976. A comparison of membrane glycoconjugates from mouse cells transformed by nurine and primate RNA sarcoma viruses. Biochemistry. 156:201–208.
20. Fishman, P. H., Moss, J. and Manganiello, V. C. 1977. Synthesis and uptake of gangliosides by choleragen-responsive human fibroblasts. Biochemistry. 16:1871–1875.
21. Frohwein, Y. Z., and Gatt, S. 1967. Isolation of β-N-acetyl hexosaminadase, β-N-acetyl glucosaminidase and β-N-acetyl galactosaminidase from calf brain. Biochemistry. 6:2775–2782.
22. Gatt, S., and Rapport, M. M. 1966. Isolation of β-galactosidase and β-glucosidase from brain. Biochim. Biophys. Acta. 113:567–576.
23. Gnaedinger, J. M., Noronha, A. B. and Druse, M. J. 1984. Myelin gangliosides in developing rats. The influence of maternal ethanol consumption. J. Neurochem. 32:1281–1285.
24. Hanson, H. A., Holmgren, J., and Svennerholm, L. 1977. Ultra structural localization of cell membrane GM_1 ganglioside by cholera toxin. Proc. Natn. Acad. Sci. U.S.A. 74:3782.
25. Hilbig, R., Leuke, H. and Rahman, H. 1983-1984. Brain gangliosides during the life span (Embryogenesis to senescence) of the rat. Dev. Neurosci. 6:260–270.
26. Irwin, L. N., Mancini, J., and Hills, D. (1973). Sialidase activity against endogenous substrate in rat brain. Brain Res. 53:488–491.
27. Jacobson, M. 1978. The germinal cell and histogenesis of the nervous system. Pages 27–55, *in* Developmental Neurobiology, 2nd Edn. Plenum Press, New York and London.
28. Karlson, I., and Svennerholm, L. 1978. Biochemical development of rat forebrain in severe protein and essential fatty acid deficiencies. J. Neurochem. 31:657–662.
29. Lapetina, E. G., Soto, E. F., and De Robertis, E. 1967. Gangliosides and acetyl choline esterase on isolated membranes of the rat brain cortex. Biochim. Biophys. Acta. 135:33–43.
30. Lee, G., Alaj, S. M., Brady, R. O., and Kohn, L. D. 1976. The structure and function of glycoprotein hormone receptors. Ganglioside interactions with human chorionic gonodotrophin. BBRC. 73:370–377.
31. Ledeen, R. W. 1978. Ganglioside structures and distribution: Are they located at nerve endings? J. Supramol. Struct. 8:1–17.
32. Lemoine, P., Haronsseau, H., Bortery, J. P., and Menuet, J. C. 1968. Les enfants alcolianes: anomalies observees a proposade 127 cas. Quest. Med. 25:476–482.
33. Leon, A., Faccil., Benvegnu, D., and Toffano, G. 1982. Morphological and biochemical effects of gangliosides in neuroblastoma cells. Devlopl. Neurosci. 5:108–113.
34. Lowden, J. A., and Wolfe, L. S. 1964. Studies on brain gan-

gliosides. Evidence for the localization of gangliosides specifically in neurons. Canad. J. Biochem. 42:1587–1603.
35. Lowry, O.H., Rosenbrough, N. J., Farr, A. L., and Randal, R. J. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265–275.
36. Merat, A. 1971. Effects of protein-calorie malnutrition on brain gangliosides. Ph.D. thesis, University of Surrey, England.
37. Miller, M. W. 1986. Effects of alcohol on the generation and migration of cerebral cortical neurons. Science. 233:1308–1310.
38. Moskal, J. R., Gardner, D. A., and Basu, S. 1974. Changes in glycolipid glycosyltransferases and glutamate decarboxylase and their relationship to differentiation in neuroblastoma cells. Biochim. Biophys. Res. Commun. 61:751–758.
39. National Research council (NRC) 1978. Nutrient requirements of the laboratory rat. Pages, 7–37 *in* Nutrient requirements of laboratory animals. 10. 3rd Edn., National Academy of Science, U.S.A.
40. Norton, W. T., and Autilio, L. A. 1966. The lipid composition of purified bovine brain myelin. J. Neuro Chem. 13:213–222.
41. Prasad, V. V. T. S., and Ramakrishnan, C. V. 1982. Studies on β-galactosidases of rat brain. Abst. 51st Ann. Meet. Soc., Biol. Chem. India.
42. Rawat, A. K. 1974. Lipid metabolism in brains from mice chronically fed ethanol. Res. Commun. Chem. Pathol. Pharmacol. 8:461–469.
43. Rawat, A. K. 1978. Effects of maternal ethanol consumption on hepatic lipid biosynthesis in foetal and neonatal rats. Biochem. J. 174:213–219.
44. Schneider, W. C. 1957. Determination of nucleic acids in tissues by pentose analysis. Pages 680–684. *in* Colowick, S. P. and Kaplan, N. O. (eds). Methods in ezymology. Vol. III., Press Inc., New York.
45. Seyfried, T. N., Glaser, G. H., and Yu, R. K. 1978. Cerebral, cerebellar and brain stem gangliosides in mice susceptible to audiogenic seizures. J. Neurochem. 31:21–27.
46. Shrader, R. E., and Zeman, F. J. 1969. Effect of maternal protein deprivation on morphological and enzymatic development of neonatal rat tissues. J. Nutr. 99:401–412.
47. Sigh, S. P., and Snyder, A. K. 1982. Ethanol ingestion during pregnancy. Effects on pregnant rats and their offspring. J. Nutr. 112:98–103.
48. Skoza, L., and Mohes, S. 1976. Stable TBA chromophore with dimethyl sulfoxide (Application to sialic acid in analytical de-o-acylation). Biochem. J. 159:457–462.
49. Soto, E. F., Bohner, L. S., and Calvino, M. C. 1966. Chemical composition of myelin and other subcellular fractions isolated from bovine white matter. J. Neurochem. 13:989–998.
50. Spence, M. W., and Wolfe, L. S. 1967. Gangliosides in developing rat brain. Isolation and composition of subcellular membranes enriched in gangliosides. Canad. J. Biochem. 45:671–688.
51. Streissguth, A. P., Landesman-Dwyer, S., Martin, J. C., and Smith, D. W. 1980. Teratogenic effects of alcohol in humans and laboratory animals. 209:353–361.
52. Suzuki K. 1970. Formation and turnover of myelin gangliosides. J. Neurochem. 17:209–213.
53. Suzuki, K., Poduslo, S. E., and Norton, W. T. 1967. Gangliosides in the myelin fraction of developing rats. Biochim. Biophys. Acta. 144:375–381.
54. Svennerholm, L. 1957. Quantitative estimation of sialic acids II. A colorimetric resorcinol- hydrochloric acid method. Biochim. Biophys. Acta. 24:604–611.
55. Tze, W. J., and Lee, M. 1975. Adverse effects of maternal alcohol consumption on pregnancy and foetal growth in rats. Nature. 257:479–480.
56. Vanier, M. T., Holm, M., Chman, R., and Svennerholm, L. 1971. Developmental profiles of gangliosides in human and rat brain. J. Neurochem. 18:581–592.
57. Vaswani, K. K., and Sharma, M. 1985. Effect of neonatal undernutrition on rat brain gangliosides. Internat. J. Vit. Nutr. Res. 55:323–329.
58. Vrbaski, S. R., Injac, B. G., and Ristic, M. 1984. Phospholipid and ganglioside composition in rat brain after chronic intake of ethanol. J. Neurochem. 42:1235–1239.
59. Warren, L. 1959. Thiobarbituric assay of sialic acids. J. Biol. Chem. 234:1971–1975.
60. Weigandt, H. 1971. Glycosphinogolipids, Pages 249–289, *in* Paoletti, R. and Kritchevsky, D. (eds) Advances in Lipid Research. Vol. 9 Academic Press, New York and London.
61. Woodson, P. M., and Ritchey, S. J. 1979. Effect of maternal alcohol consumption on fetal and brain cell number and cell size. Nutr. Rep. Inter. 20:225–228.
62. Yamakawa, T., and Nagai, V. 1978. Glycolipids at the cell surface and their biological functions. TIBS. 3: 128–131.
63. Zamenhof, F. S., Van Marthens, E., and Margolis, F. 1968. DNA (cell number) and protein in neonatal brain: Alteration by maternal protein restriction. Science. 160:322–323.
64. Zanetta, J. P., Federico, A. A., and Vincendon, G. 1980. Glycosidases and cerebellar ontogenesis in the rat. J. Neurochem. 34:831–834.