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Low Intestinal Lactase Activity in Offspring from Ethanol-Treated Mothers

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Abstract. Some aspects of small intestine maturation have been studied in the newborns from chronic ethanol-treated pregnant rats (25% ethanol in drinking fluid) immediately after birth (before suckling) and after 30 days of life. Litters delivered by mothers fed ad libitum with a standard diet diluted 50% with cellulose were used as a nutritional control. At birth, pups from ethanol-treated mothers showed significant decreases in total intestinal length and thickness, low total lactase activity and low somatostatin intestinal content. The intestinal alterations of these neonatal parameters are not present in newborns from mothers on fiber-diluted diet. From delivery, pups from different experimental groups were nursed by normal lactating dams. At 30 days of age neither of those parameters differed among the groups. We propose that the low levels of total lactase activity in newborns from alcoholic mothers, that are a consequence of a lower intestinal mucosa content, are a direct effect of ethanol in utero on the fetal gastrointestinal system.

Introduction

Negative effects of ethanol in utero on the development of the fetus and the offspring (fetal alcohol syndrome, FAS) have been extensively studied in clinical and experimental conditions [1–3]. These effects range from physical, physiological and behavioral abnormalities, to elevated postnatal mortality rates in the offspring from alcoholic mothers in both humans [4, 5] and experimental animals [6, 7]. However, gastrointestinal alterations have not been described as yet in spite of the fact that postnatal development is known to be very much dependent on adequate nutritional status, including both the availability of appropriate food and gastrointestinal capabilities [8].

It is well known that ethanol ingestion produces a wide range of gastrointestinal ef-

fects in adult mammals [9–12], but it is difficult to extrapolate them to similar effects found in the intestinal tract of fetuses from alcoholic mothers. Moreover, this fetal damage could also be directly or indirectly produced by the physiological changes in the mother caused by ethanol intake.

In this work we study the effects of ethanol ingestion by pregnant rats on physical and some functional parameters in the intestinal tract of newborns, such as lactase activity and somatostatin content.

Material and Methods

Animals

The experiments were performed on the offspring of female Wistar rats weighing 150–160 g from our own colony, maintained under automatically controlled temperature (22–23 °C) and 12-hour light-dark cycles. Animals were placed in plastic cages distributed in groups of three rats per cage and were fed ad libitum with Purina Chow rat diet (UAR-Panlab, Barcelona, Spain).

Four weeks before pregnancy, the animals were divided into three experimental groups and subjected to different nutritional treatments [13]: ethanol-treated rats received 10, 15, 20 and 25% (w/v) of ethanol in the drinking fluid on successive weeks before fecundation (pregestational period). After mating with nontreated males (day 0 of pregnancy), pregnant rats were kept on 25% ethanol in drinking water until delivery. This dose of ethanol produced an ethanol ingestion that corresponds to 30–35% of the whole caloric intake, and makes the rats isocaloric with control rats fed ad libitum [13]. Fiber-treated rats received ad libitum the standard control diet diluted 50% with cellulose and water as drinking fluid. Total caloric intake of these animals was 75% of the age-matched controls, and it was found that the daily caloric intake of these animals was similar to the caloric intake from solid diet of ethanol-treated rats [13]. Control rats were handled in the same way as the other experimental groups, fed ad libitum with the normal standard diet and water as drinking fluid.

Handling of Newborns

All rats were allowed to deliver spontaneously. No differences in the length of the gestational period were observed between the experimental groups. Pups from mothers of the same group were removed from their mothers, pooled, and distributed in litters of 8–10 pups. They were either kept in a thermostatically controlled chamber (37 °C) until sacrifice (newborn rats) or placed until weaning with normal rat nurses, delivered within the 6 h prior to the experimental animals' mothers.

From birth and during the successive postnatal days, the individual mortality of the pups was evaluated as the percentage of deaths per litter.

Sacrifice and Tissue Processing

Pups were sacrificed by decapitation at birth or at 30 days of age. The whole intestine (from the piloric end to the ileal-cecal region) was removed. Its length was measured and the first 50% was selected as the duodenal and jejunal region [14]. For newborn rats, small intestinal segments as the same level of the duodenum tract were sampled, and used for histological procedures. In all the cases, the piece of intestine was thoroughly rinsed with ice-cold 0.9% NaCl and opened longitudinally; afterwards the mucosa was gently dried with a damp piece of cellulose paper and weighed. In the case of 30-day-old pups, the intestinal mucosa was scraped with a glass slide. Aliquots of this mucosa (for 30-day-old rats) or of the complete duodenum plus jejunum (for newborn rats) were homogenized in ice-cold 0.9% NaCl with a micropotter (8 mm in diameter), and frozen at −20 °C until protein and lactase activity were determined. Other aliquots were used for somatostatin extraction according to Ghirlanda et al. [15]. Briefly, immediately after dissection, mucosa or total intestine samples were boiled for 5 min in 1 *N* acetic acid in order to destroy the proteolytic enzymes and coagulate the bulk of the proteins and homogenized (1–2 min) with a motor-driven Teflon pestle. The homogenates were centrifuged at 3,000 rpm for 30 min at 4 °C and the resultant supernatants were stored at −70 °C until somatostatin was determined. Some samples of the newborn intestine were dried for measurement of total water content.

Biochemical Determinations

Proteins were determined according to Lowry et al. [16] using bovine serum albumin as standard. Lac-

Table 1. Effect of maternal alcohol ingestion during pregnancy on general parameters of offspring in the rat

Maternal treatment during pregnancy	Control	Ethanol	Fiber-diluted diet
Litter size, n			
At birth	11.26 ± 0.49	8.32 ± 0.49***	8.88 ± 0.49***
Pup body weight, g			
At birth	5.60 ± 0.09	4.55 ± 0.07***	5.58 ± 0.12
On day 30th	91.66 ± 2.23	91.75 ± 2.70	98.14 ± 2.19
Postnatal mortality, %			
At birth	0.38 ± 0.22	2.33 ± 1.21	1.48 ± 0.48
On day 1st	0	20.87 ± 4.10***,+++	1.60 ± 0.63
On day 30th	0	0	0

Litter size at birth before pup number re-adjustment, pup body weight at birth and at 30 days of extrauterine life, and percentage of pups per litter which died on different postnatal days. Values are mean ± SEM of all the pups from 5–7 litters/group and comparisons are versus controls (*) and rats on fiber-diluted diets (+) with Student's t test. ***,+++ $p < 0.001$.

tase activity (EC 3.2.1.2.3) was determined following Dahlqvist's [17, 18] method and was expressed as units of activity (micromoles of glucose liberated per minute). Somatostatin concentration was determined in tissue extracts by a radioimmunoassay method [19] with a sensitivity limit of 10 pg/ml. A 0.01 *M* phosphate buffer, pH 7.4, containing 0.15 *M* NaCl, 0.05 *M* disodium ethylenediaminetetraacetic acid (EDTA), 0.1 % (w/v) bovine serum albumin and 100 kallikrein inhibitor Units (KIU)/ml or aprotinin (Transylol) was used in the assay system. The possibility that substances present in the tissue extracts might interfere with antibody-antigen binding and thus give rise to erroneous results was discarded by assaying serial dilutions of extracts and comparing the resulting changes in hormonal immunoreactivity with those of the diluted standards. In addition, known standard amounts of each hormone were added to varying amounts of the extracts and serial dilutions and again assayed in order to determine if this exogenously added hormonal immunoreactivity could be reliably measured in the presence of the tissue extracts. Incubation tubes were prepared in triplicate and the following were added to assay tubes in sequence: 200 µl of sample or standard containing 0–320 pg of cyclic somatostatin; 200 µl of ^{125}I-Tyr^{11}-somatostatin (5,000 cpm, equivalent to 5–10 pg); and 400 µl of appropriately diluted antibody (final dilution usually 1:20,000). After brief vortexing, the tubes were incubated at 4 °C for 48 h; separation of bound and free hormone was carried out by adding 500 µl dextran-coated charcoal (dextran: Norit A, 0.25% w/v, Pharmacia T70, Uppsala, Sweden; charocoal: Norit A, 0.25% w/v, Serva, Feinbiochemica, Heidelberg). The dilution curve for rabbit tissue extracts was parallel to the standard curve. The intra-assay and inter-assay coefficients of variation were 7.8 and 9.2%, respectively.

Histological Procedures

Samples of the duodenum were immersed in Bouin's fixative, and embebbed in paraffin. Transversal sections of 2–3 µm were obtained and stained with hematoxylin-eosin. Photomicrographs were observed for general features of epithelial cells.

Statistics

Values are expressed as means ± SEM and statistical comparisons between the three experimental groups were done with Student's t test.

Table 2. Effect of alcohol ingestion during pregnancy on intestinal parameters of offspring

Maternal treatment during pregnancy		Control	Ethanol	Fiber-diluted diet
At birth				
Total length	cm	23.6 ± 0.3	19.2 ± 0.5***,+++	22.2 ± 0.3
	cm/g body weight	3.96 ± 0.04	3.95 ± 0.06	3.92 ± 0.06
Thickness	mg/cm	5.7 ± 0.1	4.9 ± 0.2**,+	5.6 ± 0.2
	mg/cm/g body weight	0.96 ± 0.03	0.97 ± 0.03	0.98 ± 0.03
Protein, mg/100 mg		7.2 ± 1.0	7.8 ± 0.9	8.0 ± 0.6
Water, %		79.1 ± 1.2	78.9 ± 1.6	76.2 ± 0.8
On day 30				
Total length	cm	82.3 ± 2.8	95.4 ± 3.1**	90.0 ± 7.7
	cm/g body weight	0.95 ± 0.07	1.15 ± 0.06*	1.11 ± 0.07
Thickness	mg/cm	25.3 ± 3.5	23.1 ± 2.2	22.7 ± 2.2
	mg/cm/g body weight	1.20 ± 0.14	0.97 ± 0.12	0.92 ± 0.11
Protein, mg/100 mg		8.8 ± 1.0	8.6 ± 0.5	9.0 ± 1.1

Values at birth correspond to all the pups from 5–7 litters, whereas those on day 30 correspond to 5–7 pups from 5–7 different litters. They are expressed as mean ± SEM and statistical comparisons are versus controls (*) and rats on fiber-diluted diets (+) with Student's t test. +,* $p < 0.05$, ** $p < 0.01$; ***,+++ $p < 0.001$.

Results

As shown in table 1, at delivery the litter size from ethanol-treated mothers was similar to that of those on the fiber-diluted diet, and in both groups, values were smaller than in the control group. Compared with the control group, at birth, body weight was lower in newborns from ethanol-treated mothers but not from mothers on the fiber-diluted diet (table I). At 30 days of age, there were no differences between the three groups in body weight values. A high mortality index (% of deaths in each litter) in the 24 h following delivery is observed in the pups from the ethanol-treated group when compared to both control and fiber-diluted diet groups (table 1).

At birth, the intestinal length and thickness were significantly smaller in newborn rats from ethanol-treated mothers, but there were no differences when expressed per body weight (table 2). Protein and water content did not differ between the groups. At 30 days all these parameters were similar in the three groups studied except for the total intestinal length in the offspring from ethanol-treated rats, which was longer than in controls even when it was related to body weight (table 2).

Pups from the ethanol-treated mothers were born with significantly lower lactase activity than controls, even when expressed

per total small intestine (fig. 1), per unit length of the intestine or per gram of body weight (table 3). Otherwise, at delivery the lactase activity expressed per intestinal weight and per total intestinal protein did not show any statistical difference versus control values. In the pups from mothers fed tho fiber-diluted diet, neither of these parameters was statistically different from those of control animals (table 3). Also at birth, histological studies of epithelial cells from selected duodenal villi of experimental litters showed an important thickness reduction in brush-border membrane of offspring from ethanol-treated mothers versus the fiber-diluted diet and control groups (fig. 2).

In accordance with the minimum value of lactase activity reached in the control group (0.43 μmol glucose/min/total intestine), it was possible to select two populations of newborns from ethanol-treated mothers (E-I and E-II) that indicate two levels of intestinal affection. Newborns of E-I and E-II presented respectively mean values of total lactase activity of 0.31 and 0.63 μmol glucose/min/total intestine (40 and 80%, respectively, versus controls), but the other individual parameters studied at birth (body

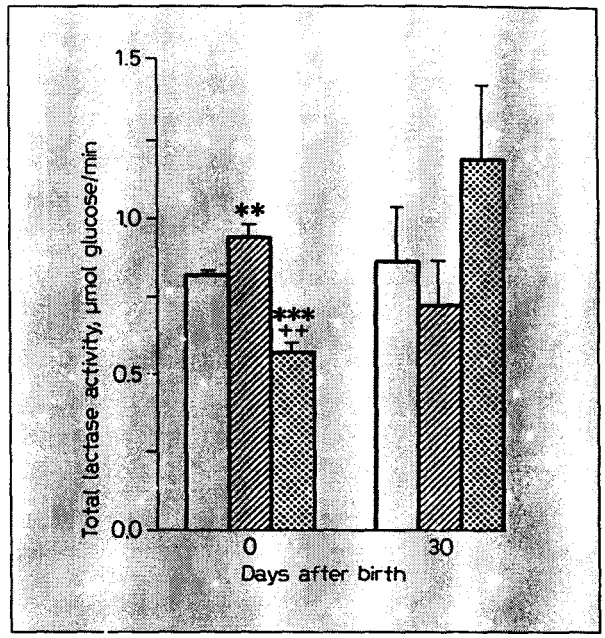

Fig. 1. Intestinal total lactase activity in newborn (day 0) and in 30-day-old rats from mothers treated with ethanol (▒), fiber-diluted diet (▨) or standard control diet (□) during pregnancy. Total lactase activity is expressed in units of enzyme activity (one unit is the amount of enzyme that releases 1 μmol of glucose/min) per total duodenum and jejunum (day 0) or per total mucosa of duodenum and jejunum (day 30). Values are means ± SEM of 5–7 litters/group and comparisons are made versus controls (*) and versus pups from fiber-treated mothers (+) with Student's t test. $^{++,**}p < 0.01$; $^{***}p < 0.001$.

Table 3. Intestinal lactase activity at birth in newborns from mothers treated with ethanol, fiber-diluted diet or standard control diet during pregnancy

	Control	Ethanol	Fiber-diluted diet
mU/g body weight	144 ± 8	109 ± 6$^{***,+++}$	163 ± 8
mU/cm intestine	36.7 ± 1.7	27.8 ± 1.7$^{***,+++}$	40.9 ± 2.0
mU/mg intestine	13.2 ± 0.6	11.7 ± 0.6^{+++}	14.5 ± 0.4
mU/mg protein	178 ± 9	158 ± 9^{+}	191 ± 10

Values are expressed in milli-units of enzyme activity (1 unit is the amount of enzyme that releases 1 μmol of glucose/min) and are means ± SEM of 40–42 pups/group from six different litters/group. Comparisons are made versus controls (*) and versus pups from fiber-treated mothers (+) with Student's t test. $^{+}p < 0.05$; $^{***,+++}p < 0.001$.

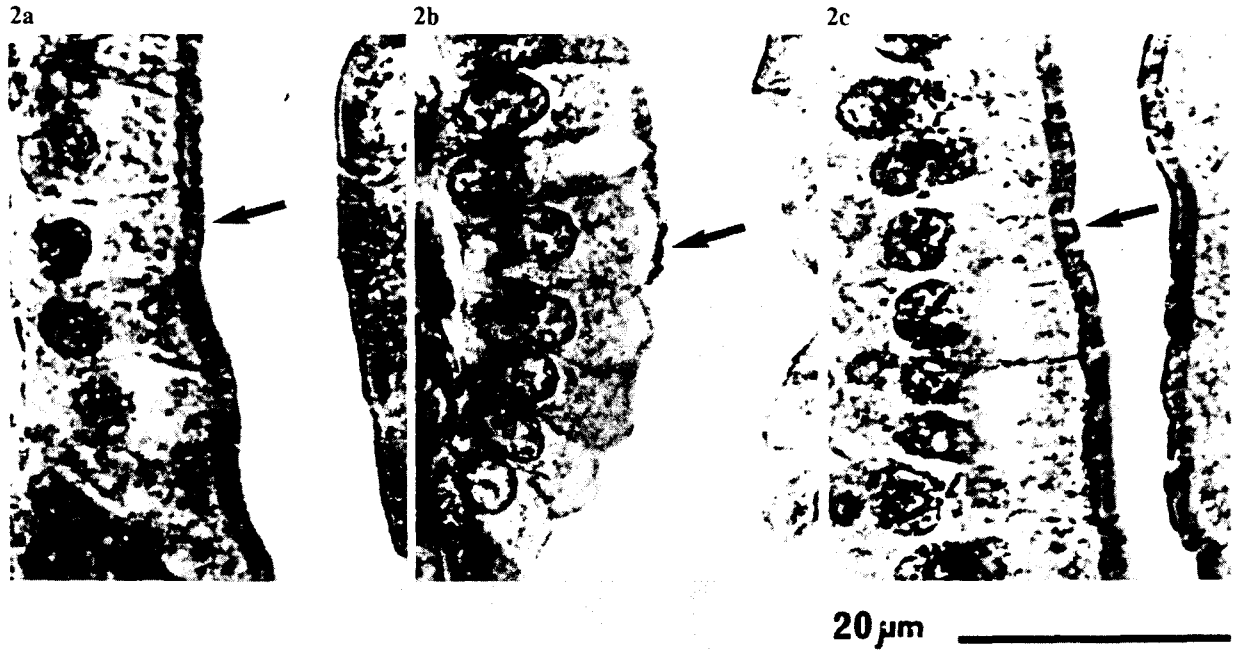

Fig. 2. Intestine epithelial cells of newborn (day 0) from mothers on (**a**) the standard control diet, (**b**) ethanol and (**c**) fiber-diluted diet. Pictures correspond to transversal sections of duodenal villi of histological preparations selected at random from different experimental pups. Arrows show apical membrane of the duodenal enterocytes (brush-border membrane).

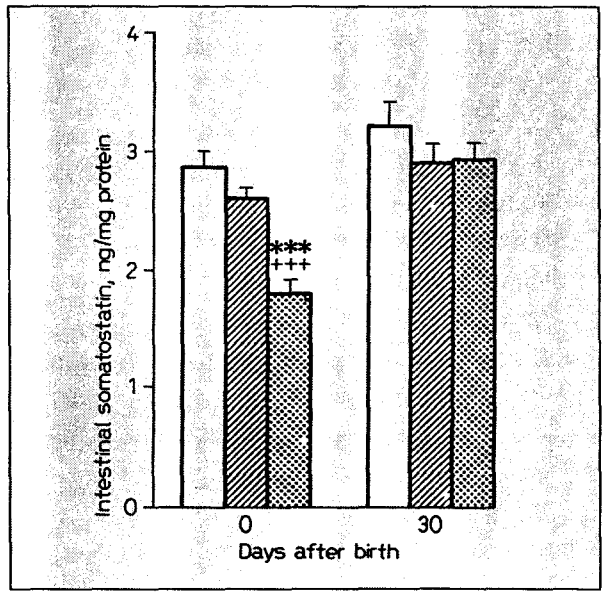

Fig. 3. Somatostatin concentration in the intestine of newborns (day 0) and of 30-day-old rats from mothers treated with ethanol (▒), fiber-diluted diet (▨) or standard control diet (□) during pregnancy. Values correspond to somatostatin concentration in small intestine (day 0) or intestinal mucosa (day 30) and are mean ± SEM of 5–7 litters/group. Comparisons are versus controls (*) and versus pups from fiber-treated mothers (+) with Student's t-test. $^{***,+++}$ $p < 0.001$.

weight, intestinal length and thickness) were not significantly different between both groups (data not shown). According to the same criterion but using other expressions of lactase activity (units/cm intestine and units/g body weight), we always selected 24% of the newborns that presented disaccharidase values much lower than the minimum control level.

The intestinal somatostatin concentration in the newborn rats from ethanol-treated mothers exhibits significant reduction at birth, but reaches the control values on the 30th day after birth (fig. 3).

Discussion

The late prenatal period is a critical and vulnerable fetal phase that has a genetic program for the appearance of different enzymes in the small intestine [8, 20, 21]. These determine the acquisition of the digestive function, which is clearly necessary for postnatal feeding and survival. Our findings in rats show a general delay at birth in intestinal parameters of newborn rats from ethanol-treated mothers, accounted by a significant decrease in intestinal thickness, total lactase activity, brush-border mucosa atrophy and intestinal somatostatin content. The fact that these alterations are not present in newborn rats from fiber-treated mothers (pair-fed with the ethanol-treated mothers) indicates a direct effect of ethanol on the intestinal development of the fetus, which was not previously described.

It is well known that the direct contact of ethanol on fetal mucosa, after transplacental passage, is manifested by high concentrations of ethanol-^{14}C found in this tissue and in the amniotic fluid during autoradiographic studies [22, 23]. Also, the accumulation of elevated ethanol levels in the amniotic fluid during chronic ethanol ingestion by pregnant rats has been reported [24, 25] along with the constant and generalized drainage of this fluid through the fetal intestine in mammals [26]. These reported findings, together with the present observations, would indicate that ethanol reaching the fetus after maternal ingestion could affect its normal intestinal development and its adaptation to postnatal nutrition. These events could agree with an ethanol-caused impairment in mucosal protein synthesis, which has been described in adult animals in in vitro studies [27], and could be related to the diminished intestinal thickness and indirectly to the low total lactase activity found in our experimental animals.

The indirect effects of ethanol on the prenatal development of the intestine due to its effects on fetal hormone levels and/or on the fetal and maternal nutritional status must also be taken into account. In this sense, it is well known that fetal somatostatin emerges in the rat at 16–17 days of gestation in the pancreas and duodenum [28]. Although the specific effects of somatostatin on the growth of the intestinal epithelium is not well established, an elevated number of endocrine cells has been found in this fetal tissue. This is not found in adult animals, and this fact could suggest a specific and transitory function of gastrointestinal peptides on intestinal growth [29]. Our results for somatostatin concentration are preliminary data in an unexplored field of research, because it is not well established yet how the intestinal somatostatin content is affected by oral ethanol ingestion in adult animals. Although it is impossible, at present, to estimate the contribution of different factors to the reduction of the somatostatin mucosal levels in newborns from ethanol-treated pregnant rats, the fact that they were clearly lower than the values in controls could suggest their association with the atrophic changes of the intestinal mucosa.

In infants, a relation between the severity of lactase deficiency and the degree of mucosal damage has been demonstrated [30]. Gastrointestinal mucosal injury at birth may result in dangerous diarrheas on nursing due to lactose intolerance [31]. Subsequent neonatal malnutrition, dehydration and shock followed by bacterial infection and intestinal perforation (necrotizing enterocolitis), provoke fairly high rates of neonatal infant mor-

tality [32]. In the clinical studies there are no data at present on viral or bacterial infections in infants born to alcoholic mothers, although a clinical case with necrotizing enterocolitis of unknown etiology has been described [33]. The cause of the well-known high rates of mortality in newborn babies from alcoholic mothers both in humans and in experimental animals during the first days of life [5, 34–36] has not been found yet. In our study, the dissections of the abdominal cavity of the ethanol-treated mothers' pups which died during the first 24 h of life (after suckling), show a large bacterial proliferation, high air retention and intestinal bursting. Although further studies are needed, these results should be taken into account in evaluating prenatal ethanol effects on postnatal mortality.

The morphological and physiological intestinal adaptations of the newborn rats from ethanol-treated mothers made after the natural mortality selection and during the subsequent postnatal development (see especially the length of the intestine of the 30-day-old rats from the ethanol-treated group; table 2) suggest an adaptative response by these pups that would allow them to attain the control values of body weight at the end of the weaning period (table 1).

Reports on humans and experimental animals affected by the fetal alcohol syndrome describe a general postnatal growth retardation [37–39], which most of the cases do not totally compensate. There is scarcely any information about the gastroenteropathies in the fetal alcohol syndrome [40], but the cases which have been diagnosed with the fetal alcohol syndrome and a specific gastrointestinal disease show the best growth gain during periods of an adequate dietary supply [40]. This finding should be taken into consideration in future therapeutic research on the fetal alcohol syndrome because the postnatal development of these infants could depend in part on the gastrointestinal injury presented and contribute to an inadequate postnatal dietary supply.

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