Microbial manipulation of the rat dam changes bacterial colonization and alters properties of the gut in her offspring

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Submitted 10 January 2007; accepted in final form 20 October 2007

Fåk F, Ahrné S, Molin G, Jeppsson B, Weström B. Microbial manipulation of the rat dam changes bacterial colonization and alters properties of the gut in her offspring. Am J Physiol Gastrointest Liver Physiol 294: G148–G154, 2008.—The impact of an altered bacterial colonization on gut development has not been thoroughly studied, despite the increased risk of certain diseases with a disturbed microbiota after birth. This study was conducted to determine the effect of microbial manipulation, i.e., antibiotic treatment or Escherichia coli exposure, of the dam on bacterial colonization and gut development in the offspring. Pregnant rats were administered either broad-spectrum antibiotics 3 days before parturition or live nonpathogenic E. coli Culture Collection of University of Göteborg, Sweden type strain (CCUG 29300T) 1 wk before parturition and up to 14 days of lactation in the drinking water. Cecal bacterial levels, gut growth, intestinal permeability, digestive enzyme levels, and intestinal inflammation were studied in 2-wk-old rats. Pups from dams that were antibiotic-treated had higher densities of Enterobacteriaceae, which correlated with a decreased stomach growth and function, lower pancreatic protein levels, higher intestinal permeability, and increased plasma levels of the acute phase protein, haptoglobin, compared with pups from untreated mothers. Exposure of pregnant/lactating mothers to E. coli CCUG 29300T, also resulting in increased Enterobacteriaceae levels, gave in the offspring similar results on the stomach and an increased small intestinal growth compared with the control pups. Furthermore, E. coli pups showed increased mucosal disaccharidase activities, increased liver, spleen, and adrenal weights, as well as increased plasma concentrations of haptoglobin. These findings indicate that disturbing the normal bacterial colonization after birth, by increasing the densities of cecal Enterobacteriaceae, appears to have lasting effects on the postnatal microflora, which affects gut growth and function.

Rodents are born with an immature gastrointestinal (GI) system, which is relatively permeable to macromolecules due to a high endocytotic activity of the small intestinal enterocytes (25). With age, the high permeability declines until a more mature gut barrier is established at weaning (gut closure). During the early suckling period, the gut undergoes morphological as well as functional development with rapid growth of the intestine and an increased expression of digestive enzymes such as lactate. At weaning, crypt hyperplasia along with intestinal closure and an increased expression of maltase, sucrase, and pancreatic trypsin denote vast changes of the GI tract (25).

Studies with germ-free animals have revealed that bacteria are essential to the growth and development of the GI system. In animals without a microflora, the intestinal weight and surface area are decreased (3). Additionally, the intestinal villi are thinner, and the enterocytes show an abnormal shape. However, the cecum can be almost eight times larger in germ-free animals compared with conventionally raised animals due to mucus and fiber accumulation and subsequent water retention (23). Additionally, pancreas protein content is increased in germ-free animals, reflecting higher amounts of some digestive enzymes (19).

An aberrant gut microflora may be just as detrimental for the individual as having no microflora at all. Diarrhea, inflammatory bowel diseases, pancreatitis, and even allergies have been shown to be related to the bacterial flora (22, 24, 34). The effect of antibiotics during pregnancy on the newborn has not been extensively studied, but one report showed that by destabilizing the maternal digestive microflora, the newborn rat pups had significantly lower numbers of intestinal staphylococci and lactobacilli up to 5 days after birth (5). However, the impact of the microfloral alteration on the GI system was not studied nor whether the changes in bacterial numbers persisted (5).

The present study was designed to elucidate the effects of the maternal microflora on bacterial colonization and GI development of the neonatal rat. We hypothesized that disturbing the normal colonization sequence by administering antibiotics in the drinking water to the dam during late pregnancy would have an effect on GI growth and maturation of her offspring. Thus, just before the normal weaning process starts, at 2 wk of age, rat pups from antibiotic-treated mothers were compared with pups from untreated mothers. Numbers of two important members of the gut microflora, Enterobacteriaceae and lactobacilli, in the gut of both pups and mothers were analyzed, as

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well as gut organ growth, small intestinal permeability, and disaccharidase activities, as well as pancreas enzyme activities and intestinal inflammation. After establishing that the antibiotic treatment of the dams elevated Enterobacteriaceae levels in the pups and influenced the GI system, we exposed additional pregnant and lactating dams to Escherichia coli Culture Collection of University of Göteborg, Sweden type strain (CCUG 29300T), a major member of the Enterobacteriaceae family, to further investigate this aspect.

MATERIALS AND METHODS

Animals and Experimental Procedure

Sprague-Dawley rats (Taconic, Ry, Denmark) were used, and the dams were chosen to be of similar age and weight to minimize differences in their gut microflora. The dams were housed separately from 1 wk before parturition in polycarbonate cages on a good laboratory practice chopped aspen wood bedding (Beekay bedding; Scanbur BK AB, Sollentuna, Sweden) with free access to a breeding diet (RM1; Special Diets Services, Essex, England, containing 22.5% protein, 5% fat, vitamins, minerals, and the rest carbohydrates) and tap water in our animal facility under a controlled environment (21 ± 1°C, 50% ± 10% relative humidity, 12:12-h light-dark cycle). After birth (the day of birth was assigned as day 0), each litter was restricted to a number of 8–13 pups and held with its respective dam for 2 wk.

The local Ethical Review Committee for Animal Experiments approved the study.

In the antibiotic study, from 3 to 4 days before the expected day of delivery and up to day 0 (birth) or day 1, a mixture of the broad-spectrum antibiotics (0.5 mg/ml metronidazole, 2.5 mg/ml neomycin, and 0.09 mg/ml polymixin B) was administered in the drinking water (31). The mean water consumption of the dams (n = 3) was ~20 ml/day, whereas the control dams (n = 3) given water without antibiotics had a consumption of ~40 ml/day.

In the bacterial exposure study, E. coli CCUG 29300T was administered in the drinking water at 1.8 × 10⁸ colony-forming units (CFU)/per ml, from 1 wk before expected parturition and continuing during the suckling period until 2 wk of age. Both treated (n = 2) and control dams given water without bacteria (n = 2) consumed an average of ~40 ml of water per day.

Bacterial Preparation

E. coli CCUG 29300T was grown in Luria Broth medium (10 g/l tryptone, 5 g/l yeast extract, and 5 g/l NaCl) in a shaking water bath at 37°C for 20 h. The cells were harvested by centrifugation at 3,000 g for 5 min, resuspended in freezing buffer (3.6 mM K₂HPO₄, 1.3 mM KH₂PO₄, 2.0 mM sodium citrate, 1.0 mM MgSO₄, 12% glycerol), and kept at −70°C until feeding. At each day of administration, new bacterial suspensions were thawed, washed with saline, and centrifuged. The cell pellet was dissolved in tap water and added to the dams’ water bottles. Viable count was performed on the water bottles containing bacteria, which gave a final concentration of 1.8 × 10⁸ CFU/ml.

Procedure at Death

At the end of the experiment, when the pups were 2 wk of age, they were separated from their mothers and gavaged by use of a Teflon stomach tube with a marker solution containing 1.25 mg/g body wt BSA and 0.25 mg/g body wt bovine immunoglobulin (BlgG) (both Sigma-Aldrich, St. Louis, MO). After 3 h, the pups were anesthetized with a mixture of 0.5 mg/g body wt ketamin (Ketalar; Parke-Davis, Solna, Sweden) and 0.4 mg/g body wt azaperon (Stresnil; Janssen-Cilag, Wien, Austria) in 0.15 M NaCl. The bowel and chest was cut open, and blood samples were taken by heart puncture into tubes containing 1.5 mg of EDTA and 20,000 IU aprotinin (Trasylol; Bayer, Leverkusen, Germany) and ice-chilled. The pancreas was then carefully dissected, rinsed in ice-cold saline, weighed, and immediately frozen. After this, the small intestine (divided into 2 halves of equal length, proximal and distal small intestine) and stomach were dissected, flushed with ice-cold saline, weighed, and frozen. The contents of the stomach were collected and saved on ice. After dissection of the spleen, thymus, liver, and adrenals, their weights were recorded. Finally, the cecum with contents was removed, weighed, and frozen in freezing buffer (3.6 mM K₂HPO₄, 1.3 mM KH₂PO₄, 2.0 mM sodium citrate, 1.0 mM MgSO₄, 12% glycerol) at −70°C.

After completion of the necropsy, blood samples were centrifuged (3,000 g for 15 min at 4°C), and plasma was removed and stored at −70°C until further analysis. The stomach content was mixed in 0.5 ml of 0.9% NaCl and centrifuged (3,000 g for 15 min at 4°C), after which the pH was measured.

Fecal samples collected freshly from the mothers at three time points, the day before start of antibiotic or bacterial treatments, at parturition, and after 2 wk at the end of experiment (n = 2–6 at each time point and treatment group), were frozen in freezing buffer and stored at −70°C until analysis.

Analyses

The cecal and fecal samples. The cecum with its content or fecal samples was thawed and homogenized in the freezing medium using a sterile pipette. After vortexing the samples, serial dilutions were made in dilution medium (9 mg/ml NaCl, 1 mg/ml peptone, 0.2 mg/ml cysteine, 1 ml Tween/1,000 ml distilled water) and spread on violet red bile glucose (VRBG) and Rogosa agar plates, respectively (Oxoid; Basingstoke, Hampshire, England). After incubating VRBG plates for 24 h aerobically and Rogosa plates for 48 h anaerobically (AnaeroGen, Oxoid), the number of colonies was estimated and calculated as CFU per gram cecum with content. Colonies found growing on VRBG agar plates were considered to be enterobacteria belonging to the family Enterobacteriaceae, whereas colonies found on Rogosa agar plates were considered to be lactobacilli (Lactobacillus-like bacteria).

Randomly amplified polymorphic DNA. Colonies from VRBG plates were randomly picked from E. coli pups and control pups and washed twice in sterile water. By shaking with glass beads (2 mm) for 45 min at 4°C, the cells were disintegrated, and DNA was recovered. The samples were centrifuged at 20,817 g for 5 min, after which the supernatant (1 μl) was used for PCR. The reaction mixture contained PCR buffer (Boehringer Mannheim Scandinavia, Bromma, Sweden), 0.2 mmol/l each nucleotide (Perkin-Elmer, Branchburg, NJ), Taq DNA polymerase (Boehringer Mannheim Scandinavia), and 1 mg/ml primer (5′-ACG CGC CCT-3′, synthesized by Scandinavian Gene Synthesis, Köping, Sweden). The PCR reaction was performed in a Perkin-Elmer thermal cycler with the following temperature profile: four cycles consisting of 94°C for 45 s, 30°C for 120 s, 72°C for 60 s, followed by 26 cycles consisting of 94°C for 5 s, 36°C for 30 s, 72°C for 30 s (1 s extension per cycle). The PCR session was terminated at 72°C for 10 min, followed by cooling to 4°C.

Gel electrophoresis was run by applying 20 μl of the PCR product on a horizontal 1.5% agarose gel for 2.5 h at 80 V in TB buffer (89 mM boric acid, 2.5 mmol/l EDTA, pH 8.3) with a DNA molecular weight standard (0.5 μg, Type VI Boehringer Mannheim Scandinvia). The gel was stained in ethidium bromide for 15 min, followed by washing for 2 × 10 min. Bands were visualized at 302 nm with a UV transilluminator (UVP, San Gabriel, CA), and pattern from E. coli pups and control pups were compared with the band pattern from the E. coli CCUG 29300T given to the dams.

The pancreas. The pancreas protein content was determined according to the method of Lowry et al. (20), modified for 96-well microplates. Briefly, the pancreata were homogenized in ice-cold 0.2 mol/l Tris-HCl buffer + 0.05 mol/l CaCl₂ (pH 7.8) using a glass–glass homogenizer, followed by centrifugation at 15,000 g for 20 min at 4°C.
4°C. The protein concentration was determined in the supernatant by reading the absorbance of the samples at 690 nm using a plate reader and BSA as the standard. To estimate the trypsin amount, the pancreatic supernatants were activated with enterokinase and thereafter incubated with the substrate Bz-Arg-pNA (Sigma-Aldrich), and the absorbance change was then measured at 405 nm (11). The amount of enzyme causing transformation of 1.0 μmol of substrate per minute at 25°C was defined as 1 unit (U).

**The small intestine.** After homogenizing the proximal small intestine in 9 volumes of ice-cold NaCl using a knife homogenizer, the protein amount was determined as described above. In addition, the Dahlqvist method (7) was used to measure the intestinal disaccharidase activities. The substrates lactose, maltose, and sucrose were incubated with the intestinal homogenates for 1 h, after which the reaction was stopped with a glucose oxidase reagent (Sigma-Aldrich), and the amount of generated glucose was determined. Glucose (0.05–1.0 mg/ml) was used as standard. The disaccharidase activities were estimated by reading the absorbance at 450 nm.

**The blood.** The intestinal macromolecular permeability was determined by measuring the concentrations of the marker molecules BSA and BlgG in blood samples taken 3 h after gavage by electroimmunoassay (rocket electrophoresis; Refs. 2, 17) using specific antisera for BSA (rabbit anti-cow albumin, Dako A/S, Glostrup, Denmark) and BlgG (rabbit anti-BlgG, Dako). Purified BSA and BlgG (Sigma-Aldrich) were used as standards.

The concentration of the plasma acute phase protein, haptoglobin, was analyzed using a commercially available kit (Phase Range Haptoglobin Assay; Tridelta Development, Maynooth, Ireland) according to the manufacturer’s instructions. In short, plasma was incubated with hemoglobin, which bound to any haptoglobin present in the samples leading to preservation of peroxidase activity of the haptoglobin. A colorimetric reaction showing the peroxidase activity in the samples was then compared with a haptoglobin standard (0–2 mg/ml). Absorbance was measured at 630 nm. The assay sensitivity was reported to be 0.05 mg/ml haptoglobin.

**Calculations**

Student’s t-test was performed (unpaired, 2-tailed) on all of the results, where P values <0.05 were considered significant. Exact P values are reported unless below 0.001. The antibiotic group (n = 11) was compared with the controls (n = 11) run in parallel, whereas the E. coli pups (n = 16) were compared with their respective control pups (n = 12). The effect of antibiotic or E. coli CCUG 29300T treatments on the dams’ fecal flora was estimated by comparing bacterial numbers of treated dams with control dams at 1 wk before treatment, at the day of parturition, and at the final day of the experiment.

To compensate for body weight differences, all organ parameters are given per gram of body weight, giving a relative organ weight that could be compared between groups.

**RESULTS**

**Effects of Microbial Manipulation on the Microflora of Dams**

No diarrhea was noticed among the antibiotic-treated mothers, but the stool consistency became softer during the treatment, an effect that did not persist after 2 wk. At the end of the 3- to 4-day treatment period, antibiotics significantly reduced the numbers of lactobacilli in the fecal samples, although 2 wk later, the numbers of lactobacilli were restored (Fig. 1A). The fecal numbers of Enterobacteriaceae were not altered by the antibiotic treatment, although an insignificant increase (P = 0.46) was found at the end of the treatment at parturition (Fig. 1B).

Exposure to live E. coli CCUG 29300T via the drinking water during 3 wk did not appear to affect the stool consistency of the mothers. At day of parturition as well as 2 wk later, fecal samples from E. coli dams showed a higher amount of Enterobacteriaceae, although not significantly due to a low number of female samples. The numbers of lactobacilli did not change due to the E. coli CCUG 29300T exposure (Fig. 1A).

Exposure to live E. coli CCUG 29300T of rat dams had any impact on the body weight of their pups compared with pups of the untreated control mothers (Table 1). Two pups out of 13 in the antibiotic group died at the age of 9 days for unknown reasons, whereas there was 100% survival in the E. coli and control groups.

**Effects on the Cecum and Microflora of the Pups**

At death, when the pups were 2 wk old, no difference in the weight of the cecum, including its contents, was found between
any of the groups (Table 1). The number of Enterobacteriaceae, however, was significantly higher in cecal samples from pups from the antibiotic-treated dams compared with pups from untreated dams, whereas in the numbers of lactobacilli, no differences were found (Fig. 2). In the E. coli CCUG 29300T exposure study, higher numbers of Enterobacteriaceae was found in the E. coli pups compared with the control pups, whereas no difference was found with regard to the lactobacilli numbers (Fig. 2). The randomly amplified polymorphic DNA analysis showed that the E. coli CCUG 29300T given to the mothers was not recovered in the pups’ cecal flora.

Effects on the Stomach of the Pups

The weight of the stomach tissue was significantly lower in the pups born from the antibiotic-treated mothers compared with the control pups. In addition, the pH of the stomach contents was significantly higher in the antibiotic group (Table 1). Similarly, in the E. coli exposure CCUG 29300T study, the stomach pH was found to be significantly higher in the E. coli pups than in the control pups, but the stomach weight did not differ between groups (Table 1).

Table 1. Body weight, weight of stomach, SI (proximal and distal halves), cecum including its contents, liver, adrenals, thymus, and spleen, and pH of stomach contents from 14-day-old rats born from either antibiotic-treated or Escherichia coli-exposed dams or from untreated dams (controls)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body Weight, g</th>
<th>Stomach Weight, mg/g body wt</th>
<th>Stomach pH</th>
<th>Proximal SI, mg/g body wt</th>
<th>Distal SI, mg/g body wt</th>
<th>Cecum, mg/g body wt</th>
<th>Liver, mg/g body wt</th>
<th>Adrenals, mg/g body wt</th>
<th>Thymus, mg/g body wt</th>
<th>Spleen, mg/g body wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotic group (n = 11)</td>
<td>28.8 (2.9)</td>
<td>6.4 (0.5)</td>
<td>4.3 (0.8)</td>
<td>14.7 (1.3)</td>
<td>13.1 (2.0)</td>
<td>3.0 (0.5)</td>
<td>32.5 (3.0)</td>
<td>0.2 (0.05)</td>
<td>4.9 (0.6)</td>
<td>3.9 (0.4)</td>
</tr>
<tr>
<td>Significance</td>
<td>NS</td>
<td>P = 0.005</td>
<td>P = 0.02</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>P = 0.007</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Controls (n = 11)</td>
<td>27.6 (1.6)</td>
<td>7.0 (0.3)</td>
<td>3.6 (0.4)</td>
<td>14.7 (1.5)</td>
<td>13.9 (1.4)</td>
<td>3.2 (0.5)</td>
<td>29.5 (1.4)</td>
<td>0.2 (0.1)</td>
<td>4.8 (0.5)</td>
<td>4.1 (0.5)</td>
</tr>
<tr>
<td>E. coli group (n = 16)</td>
<td>30.0 (1.4)</td>
<td>7.3 (0.5)</td>
<td>5.0 (0.5)</td>
<td>18.3 (1.0)</td>
<td>15.8 (1.0)</td>
<td>3.6 (0.5)</td>
<td>31.0 (1.5)</td>
<td>0.21 (0.1)</td>
<td>4.5 (0.5)</td>
<td>4.6 (0.4)</td>
</tr>
<tr>
<td>Significance</td>
<td>NS</td>
<td>NS</td>
<td>P = 0.01</td>
<td>P &lt; 0.001</td>
<td>P = 0.001</td>
<td>NS</td>
<td>P = 0.0001</td>
<td>P = 0.04</td>
<td>NS</td>
<td>P = 0.03</td>
</tr>
<tr>
<td>Controls (n = 12)</td>
<td>30.4 (1.3)</td>
<td>6.9 (0.6)</td>
<td>4.4 (0.8)</td>
<td>15.2 (1.4)</td>
<td>14.2 (1.4)</td>
<td>3.6 (0.5)</td>
<td>28.3 (1.6)</td>
<td>0.15 (0.1)</td>
<td>4.4 (0.4)</td>
<td>4.4 (0.3)</td>
</tr>
</tbody>
</table>

Values are expressed as means (SD). Significant differences were found between the antibiotic group or the E. coli group and their respective control groups. NS, not significant; SI, small intestine.

Effects on the Small Intestine of the Pups

Antibiotic treatment of the dams did not significantly affect the weight of the small intestine of the pups (Table 1). The small intestinal protein content and the intestinal lactase and maltase activities did not differ between groups, although the sucrase activity was significantly higher in the antibiotic group compared with the control group (Table 3).

The E. coli CCUG 29300T exposure of the mothers did, however, affect their pups’ intestinal growth. Both the small intestinal weight (both proximal and distal part) and the protein content of the proximal part were significantly increased in the E. coli pups compared with the control pups (Table 1 and Table 3). Also, the activities of the disaccharidases lactase, maltase, and sucrase were significantly higher in E. coli pups (Table 3).

The plasma level of the macromolecular markers at 3 h after gavage was significantly higher in the antibiotic group of pups with regard to B1G (Fig. 3), whereas no significant difference was found for BSA [antibiotic group: 14.7 (2.1); control group: 14.5 (3.7)]. In the E. coli group, no significant differences were
observed for either BIgG (Fig. 3) or BSA [E. coli group: 12.6 (2.4); control group: 13.6 (4.0)] between the groups.

Effects on the Pancreas and the Liver of the Pups

No difference was found in the pancreas weight between the antibiotic and the control groups. The pancreatic protein content, however, was significantly lower in the antibiotic group compared with the control group, whereas no differences were found for the trypsin content (Table 2). The E. coli CCUG 29300T exposure study showed a significant increase in the pancreas weight in the E. coli group compared with the control group. No significant differences were found in the protein or trypsin content of the pancreas between groups (Table 2). The pancreatic protein content (Table 2). The pancreatic protein content, however, was significantly lower in the antibiotic group compared with the control group, whereas no differences were found for the trypsin content (Table 2). The E. coli CCUG 29300T exposure study showed a significant increase in the pancreas weight in the E. coli group compared with the control group. No significant differences were found in the protein or trypsin content of the pancreas between groups (Table 2). The pancreatic protein content and disaccharidase activities (Table 2) are shown.

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Table 2. Effect of antibiotic treatment or E. coli exposure of rat dams compared with control dams on the pups’ pancreas weight and protein and trypsin content

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight, mg/g body wt</th>
<th>Protein, mg/g body wt</th>
<th>Trypsin, U/g body wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotic group (n = 11)</td>
<td>3.7 (0.2)</td>
<td>146 (24)</td>
<td>10.4 (2.2)</td>
</tr>
<tr>
<td>Significance</td>
<td>NS</td>
<td>P &lt; 0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Controls (n = 11)</td>
<td>3.5 (0.3)</td>
<td>214 (17)</td>
<td>13.1 (3.4)</td>
</tr>
<tr>
<td>E. coli group (n = 16)</td>
<td>3.1 (0.4)</td>
<td>181 (40)</td>
<td>13.3 (3.4)</td>
</tr>
<tr>
<td>Significance</td>
<td>P = 0.003</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Controls (n = 12)</td>
<td>2.6 (0.5)</td>
<td>222 (133)</td>
<td>13.1 (4.1)</td>
</tr>
</tbody>
</table>

Values are expressed as means (SD). Significant differences were found between the antibiotic group or the E. coli group and their respective control groups. U, units.

DISCUSSION

Effect of Microbial Manipulation on the Bacterial Flora of the Dam and Her Offspring

Microbial manipulations by antibiotic treatment or E. coli CCUG 29300T exposure of pregnant and lactating rats led to transient changes in their gut microflora that also affected their offspring with increased numbers of cecal Enterobacteriaceae at 2 wk of age.

Treating the dams with broad-spectrum antibiotics resulted in an increase in the Enterobacteriaceae levels and significantly reduced the numbers of fecal lactobacilli at parturition, but after 2 wk, these numbers had normalized. Similarly, exposure to E. coli CCUG 29300T via the drinking water led to increases in the levels of fecal Enterobacteriaceae, whereas no significant effect was found in the lactobacilli numbers.

The resulting microflora in the offspring from the antibiotic-treated mothers showed an increase in the numbers of Enterobacteriaceae but no effect on the lactobacilli compared with the offspring from control dams. It is plausible that the overgrowth of antibiotic-resistant bacteria, i.e., Enterobacteriaceae, in the dams’ microflora favored the colonization of Enterobacteriaceae in the offspring. In a study with penicillin-treated, lactating mice, Enterococci, coliforms, and clostridia were found in the offspring’s ceca, whereas lactobacilli did not colonize pups of treated mothers (27). It is interesting to note that although the rat dams restored their numbers of lactobacilli and Enterobacteriaceae after 2 wk, their pups had remaining increased Enterobacteriaceae levels at 2 wk of age, indicating that the microflora disturbances induced after birth were main-

Table 3. Effect of antibiotic treatment or E. coli exposure of rat dams compared with control dams on the pups’ small intestinal (proximal part) protein content and disaccharidase activities

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protein, mg/g body wt</th>
<th>Lactase, U/g body wt</th>
<th>Maltase, U/g body wt</th>
<th>Sucrase, U/g body wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotic group (n = 11)</td>
<td>145 (48)</td>
<td>126 (40)</td>
<td>66 (34)</td>
<td>2.7 (3.2)</td>
</tr>
<tr>
<td>Significance</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>P = 0.04</td>
</tr>
<tr>
<td>Controls (n = 11)</td>
<td>131 (31)</td>
<td>104 (13)</td>
<td>58 (14)</td>
<td>0.8 (0.8)</td>
</tr>
<tr>
<td>E. coli group (n = 16)</td>
<td>301 (83)</td>
<td>105 (15)</td>
<td>106 (14)</td>
<td>6.4 (3.3)</td>
</tr>
<tr>
<td>Significance</td>
<td>P &lt; 0.001</td>
<td>P = 0.01</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Controls (n = 12)</td>
<td>182 (49)</td>
<td>92 (13)</td>
<td>76 (12)</td>
<td>2.3 (2.5)</td>
</tr>
</tbody>
</table>

Values are expressed as means (SD). Significant differences were found between the antibiotic or the E. coli group and their respective control groups.
tained during the suckling period. In contrast, a recent study in rabbits showed that the bacteria colonizing during the suckling period had a greater impact on the cecal microbial communities than those colonizing at birth (1).

In the E. coli CCUG 29300T exposure study, also increased levels of Enterobacteriaceae were found in the 2-wk-old pups. However, the situation was slightly different since these pups were exposed to E. coli CCUG 29300T also during the suckling period, either directly via consumption of drinking water or indirectly via the mother. The DNA profile of the Enterobacteriaceae found in the pups was different from the E. coli strain consumed by the dams, indicating that the E. coli 29300T given to the mothers did not directly transfer to the pups but increased the numbers of other Enterobacteriaceae species transferred.

Increase in Enterobacteriaceae levels early in life appears occasionally and independently of any antibiotic regimen. According to Wang et al. (32), babies may become solely colonized with E. coli 1 wk after birth, which might not only influence their GI development, but also their health status in adulthood (32). Our findings, that elevated Enterobacteriaceae levels induced after birth persist during the suckling period, warrant further investigations concerning the significance of an early colonization of Enterobacteriaceae and their role in the pathogenesis of later disease.

Effects on Gut Organ Growth and Development in the Offspring

Disrupting the microbial colonization sequence by use of broad-spectrum antibiotics in pediatric care has been shown to modulate the expression of important GI developmental-related genes (28). In addition, there appears to be a correlation between antibiotic treatment after birth and development of allergy (24), but the influence of antibiotic therapy during the neonatal period on development of the digestive and immune function of the gut has not been investigated thoroughly.

The findings of the present study, using the neonatal rat model, show that an increased level of Enterobacteriaceae during the suckling period has an impact on the GI growth and development. The stomach weight was decreased in the antibiotic group of pups, and both the E. coli and the antibiotic groups had a decreased HCl secretion, reflecting a decreased growth and functional maturation of the stomach. This may weaken the animals’ defense against ingested pathogenic bacteria. Germ-free rats have been found to have decreased plasma levels of gastrin, and altered levels of gastrin might have been responsible for the stomach effects seen in the rat pups.

The small intestine was significantly heavier in the E. coli pups, whereas no difference was found in the antibiotic pups compared with the control group. This was correlated with an increase in the mucosal disaccharidase activities as well as increased protein content of the small intestine. Similarly, a stimulation of mucosal sucrase and lactase activities has been shown in suckling pigs by treatment with either an antibiotic or a probiotic (6), but it has also been observed that some probiotic bacteria possess endogenous lactase activity (26). Anyhow, it has been shown previously that the gut microflora can influence intestinal proliferation and enzyme expression, and our results confirm that Enterobacteriaceae can affect intestinal growth (15).

In addition, an increased intestinal marker permeability was found in the antibiotic pups whereas this was not seen in the E. coli pups. Whatever the mechanism behind this is, it remains clear that a decreased barrier function during early life can predestine to diseases later in life such as allergies (4, 24). In contrast, feeding probiotic lactobacilli have been shown to decrease intestinal permeability in the same model with suckling rat pups (10). Furthermore, a mix of probiotic strains have been found to be able to improve the barrier integrity in mice with colitis (21), and Lactobacillus paracasei exposure was able to counteract stress-induced elevated gut permeability in rats (9). Infants later developing allergies show an increased colonization of aerobic bacteria and a delayed colonization of anaerobic bacteria with a concurrent increased intestinal permeability, further supporting our findings above (4, 16).

The gut-associated organ, the exocrine pancreas, also appeared to have been influenced by the antibiotic treatment of the dams where the pups had lower total pancreatic protein content than the control group. The slightly heavier pancreata found in the E. coli pups was not reflected in any changes in the pancreatic content of protein or trypsin, suggesting that there was an increased growth but no functional maturation of the pancreas. The altered gut microflora might have influenced the degradation of pancreatic enzymes in the intestine, thereby possibly influencing the pancreatic enzyme production similar to what has been observed in germ-free rats (12, 19).

The liver was also affected, as both the antibiotic and the E. coli pups had heavier livers than the control pups. It appears likely that this was due to an enhanced fat deposition in the liver because of an inflammatory response to endotoxin exposure due to the increased levels of Enterobacteriaceae in the gut (8). This is most likely also true for the E. coli group, since exposure to lipopolysaccharide could possibly have initiated an inflammatory reaction leading to an increased liver weight. The increased weight of the spleen in the E. coli group of pups further implies that this might be the case. In addition, both the antibiotic and the E. coli groups showed increased levels of the acute phase protein, haptoglobin, at 14 days of age, which strongly supports that an inflammatory response triggered by the bacterial flora had indeed taken place (14). An aberrant colonization with an overgrowth of bacteria after birth could lead to an inflammatory cascade triggering damage of the small intestine and necrotizing enterocolitis in infants (13). Moreover, the adrenals showed an increased weight in the E. coli group, and it is possible that the physiological stress induced by the inflammation led to an increased production of adrenal stress hormones. Possibly, the local immune system in the gut was activated in a manner similar to what happens during weaning when activation of T cells occurs and proinflammatory cytokines increase, leading to an increase in growth of the small intestine (29, 31).

One could argue that the effects seen on GI organs in the antibiotic experiments were due to direct toxic effects of the antibiotics pre- or postnatally, since Brunel and Gouet (5) reported, in a similar experiment, that some antibiotics might cross the placental barrier and thus be found in the newborn. However, they claimed that the antibiotics used, ampicillin, did not transfer from the tissues to the intestinal lumen affecting the microflora. Furthermore, since similar effects on gut growth and maturation were found in the E. coli CCUG 29300T exposure study, it is likely also that the effects seen in the
antibiotic study were, in fact, caused by effects on the bacterial colonization.

In summary, disturbing the maternal microflora had vast effects on her offspring, with elevated cecal numbers of opportunistic Enterobacteriaceae, leading to intestinal inflammation, altered GI properties, and decreased barrier properties. The mechanism behind the effects remains to be further elucidated, but we can conclude that the inflammation was probably not responsible per se for the stimulated growth of the GI tract in the E. coli pups since the antibiotic pups showed no such growth increase of the intestine despite their elevated haptoglobin levels.

It was intriguing that only a threefold increase in the levels of Enterobacteriaceae could have such vast effects on gut growth and development in the laboratory (specific pathogen-free) rats. This is, to our knowledge, the first study reporting effects on postnatal GI development by increasing the levels of a single family of bacteria. More studies are needed to investigate the influence of the mother’s intestinal microflora on the newborn as well as the effects of antibiotic treatment during the neonatal period (28) and how this affects the health in adulthood.

ACKNOWLEDGMENTS

We thank Inger Mattson and Camilla Björklöv for expert technical assistance.

GRANTS

The Royal Physiographic Society in Lund is greatly acknowledged for financial support.

REFERENCES