Journal of Surgical Research **117,** 262–271 (2004)
doi:10.1016/j.jss.2003.11.021

Probiotics Partly Reverse Increased Bacterial Translocation after Simultaneous Liver Resection and Colonic Anastomosis in Rats

Daniel Seehofer, M.D.,*[,1] Nada Rayes, M.D.,* Reinhard Schiller, M.D.,† Martin Stockmann, M.D.,* Andrea R. Müller, Ph.D.,* Anja Schirmeier,* Frank Schaeper, M.D.,‡ Stefan G. Tullius, Ph.D.,* Stig Bengmark, Ph.D.,§ and Peter Neuhaus, Ph.D.*

**Department of General, Visceral, and Transplant Surgery, Charité, Campus Virchow, Humboldt University of Berlin, Berlin, Germany; †Department of Microbiology, Charité, Campus Virchow, Humboldt University of Berlin, Berlin, Germany; ‡Department of Pathology, Charité, Campus Virchow, Humboldt University of Berlin, Berlin, Germany; §Departments of Hepatology and Surgery, University College of London, Liver Institute, London, United Kingdom*

Submitted for publication July 2, 2003

Background. **Bacterial translocation is one important cause of nosocomial infections following major abdominal surgery. Oral administration of probiotics has been proposed to diminish bacterial translocation.**

Material and methods. **In total 68 rats were divided into seven groups: five of the groups received standard rat chow and were subjected to either sham-operation, 70% liver resection, colonic anastomosis, or a combination of 30 or 70% liver resection with synchronous colonic anastomosis, respectively. In two additional groups with synchronous operation, a combination of four different lactic acid bacteria and four fibers was administered two times daily pre- and postoperatively. Bacterial concentrations in cecum, mesenteric lymph nodes, liver, and spleen were analyzed and blood cultures were taken 48 h after operation. Furthermore, the following parameters were assessed: histological changes in the intestine, intestinal paracellular permeability (Ussing chamber), bursting pressure of the colonic anastomosis, and mitosis rate of the remnant liver.**

Results. **Bacterial translocation was observed in all rats, except in the sham group. Following liver resection, the highest bacterial concentrations were seen in liver and spleen, following colon anastomosis in the mesenteric lymph nodes. Bacterial translocation was increased in the animals with combined operation, in parallel to the extent of liver resection. In rats with colon anastomosis, bacterial concentration in the cecum was also higher than in the sham group. Application of probiotics significantly decreased bacterial concentration in the lymph nodes. In addition, animals with a high cecal concentration of lactobacilli had less translocation than the others. No histological changes were observed in the intestine. Paracellular permeability for ions, but not for the larger molecule lactulose, was increased in the colon in all groups with colon anastomosis. The bursting pressure of the colon anastomosis was not significantly different between the groups. Seventy percent liver resection led to a high rate of hepatocyte mitosis, whereas combination with colon anastomosis impaired the regeneration process.**

Conclusion. **Synchronous liver resection and colon anastomosis led to increased bacterial translocation compared to the single operations in the rat model. It is possible to diminish this process by oral administration of probiotics. Bacterial overgrowth in the cecum and impaired hepatic regeneration, but not histological changes or alterations of paracellular permeability, are potential pathogenic mechanisms for translocation in this setting.**

Key Words: **bacterial translocation; liver resection; colonic anastomosis; probiotics; liver regeneration; intestinal permeability.**

INTRODUCTION

Despite advances in intensive care medicine, antibiotic therapy, and surgical technique, nosocomial bacterial infections still represent an important problem. They cause an increase of morbidity and mortality resulting in prolonged hospital stay and additional costs [1]. Patients undergoing major abdominal sur-

[1] To whom correspondence and reprint requests should be addressed at Department of General, Visceral, and Transplant Surgery, Charité, Campus Virchow, Augustenburger Platz 1, D-13353 Berlin, Germany. Fax: 030-450 552900. E-mail: daniel.seehofer@charite.de.

0022-4804/04 $30.00

gery especially often have several risk factors for infections in addition to the surgical stress [2]. Following major liver resection, an infection rate of 34% has been reported [3]. Some authors report an increased morbidity in the case of synchronous liver and colon resection, caused by pneumonia [4] or sepsis [5]. In a study including 165 patients, operative mortality was 1% in the case of liver resection alone and 17% in the case of simultaneous resection. Multivariate analysis revealed that synchronous operation was a significant factor for higher postoperative mortality [6].

One important pathogenetic mechanism for nosocomial infections is translocation of bacteria, most frequently *Escherichia coli* and Enterococci, from the gut into mesenteric lymph nodes, liver, spleen, and blood or other sterile organs [7, 8, 9]. The main reasons for bacterial translocation are intestinal bacterial overgrowth, impaired local immune function, and increased intestinal permeability.

Bacterial translocation has been demonstrated in various experimental models [10, 11], including the surgical model of liver resection [12]. Efforts to reduce that process using antibiotics have paradoxically revealed a higher rate of translocation [13]. Also dietary variations influence the intestinal barrier: for example, total parenteral nutrition and elemental diets induce bacterial translocation, presumably by impairing the endoluminal nutrition of enterocytes [14, 15, 16, 17]. In contrast, enteral nutrition with normal rat chow prevented this phenomenon. Thus far, most experimental studies have focused on the effect of dietary manipulation on the permeability of the small bowel and used enteral formulas which were mainly absorbed in the ileum [18, 19]. New dietary concepts including prebiotics (fibers) and probiotics (lactic acid bacteria) try to prevent translocation in the large bowel because of its high bacterial count of 10^9–10^{11} bacteria/g feces, representing the major reservoir for bacterial translocation.

Pre- and probiotics act synergistically in the large bowel. Prebiotics are broken down by probiotics to omega-fatty acids which stabilize the intestinal barrier [3]. They also lead to an increase of stool mass, a reduction of pressure in the large bowel, and a positive trophic effect by increasing the DNA synthesis of enterocytes and their absorptive function. Probiotics have antimicrobial activity, stimulate the host's immune system, activate macrophages in the liver and peritoneum, and improve the intestinal immune function [20]. Recent experimental studies could demonstrate a synergistic effect if several probiotic strains were combined [21]. A University of Lund based microbiology team performed an extensive screening of the human gut and isolated 355 various strains of lactic acid bacteria [22]. The ability of these strains to bind to porcine mucin, to express cell surface hydrophobicity, and to bind to collagen, fibronectin, and other extracellular matrix proteins were extensively studied. Based on these studies, four strains (two *lactobacilli*, one *pediococcus*, and one *leuconostoc*) were able to survive exposure to the gastrointestinal environment. All of the strains also produced antimicrobial substances. They were combined with four fibers (betaglucan, inulin, pectin, and resistant starch), known for their strong bioactivities, to form a synbiotic composition.

In this experimental study, we analyzed the incidence and severity of bacterial translocation following single and synchronous liver resection and colon anastomosis in the rat. After that, we studied the impact of the selected synbiotic composition on bacterial translocation. In addition, composition of the cecal flora, liver regeneration, paracellular permeability, and histological structure of the jejunum and colon were assessed to detect potential pathogenic mechanisms for bacterial translocation.

MATERIALS AND METHODS

Male Sprague Dawley rats (Winkelmann, Bochum, Germany) weighing 250–350 g were used for all experiments. The rats were housed in single cages and had free access to standard rat chow and water. Animals were kept at a 12-h day and night cycle at a constant room temperature. All experiments were performed in accordance with the German legislation on the protection of animals

Experimental design. In total 68 rats were randomized to the following groups: Sham operation (group 1, SHA, $n = 8$), 70% liver resection (group 2, LR70, $n = 8$), right-sided colonic anastomosis (group 3, CA, $n = 8$), 30% liver resection plus right-sided colonic anastomosis (group 4, LR30/CA, $n = 11$), 30% liver resection plus right-sided colonic anastomosis with perioperative application of probiotics (group 5, LR30/CA/P, $n = 11$), 70% liver resection plus right-sided colonic anastomosis (group 6, LR70/CA), and 70% liver resection plus right-sided colonic anastomosis with perioperative application of probiotics (group 7, LR70/CA/P, $n = 11$).

A combination of four different probiotic strains and four different fibers was used for the experiments: Rats in the treatment groups each received 10^9 *Pediacoccus pentoseceus* 5-33:3, *Lactococcus raffinolactis* 32-77:1, *Lactobacillus paracasei* subspecies paracasei 19, and *Lactobacillus plantarum* 2362, as well as 0.2 g betaglukan, inulin, pectin, and resistant starch in 3 ml drinking water two times daily by gavage starting 3 days prior operation until harvesting at the second postoperative day. Animals which were randomized to the other groups received 3 ml drinking water by gavage following the same procedure.

Operations. All rats were operated by the same surgeon under sterile conditions in isoflurane inhalation anesthesia. Preoperatively all rats received 10 ml of sterile isotonic saline subcutaneously to prevent dehydration. Afterwards a midline abdominal incision was done. In the sham-operation group, the liver was only separated from its ligaments. Resection of 70% of the liver was performed by removal of the middle and left lateral liver lobe by placing two Vicryl 3/0 ligatures at the respective vascular pedicle as described in detail previously [23]. A 30% liver resection was performed by resection of the left lateral lobe only.

For colonic anastomosis the right colon was placed on sterile swabs to avoid contamination of the abdominal cavity. The right colon was transected and a seromuscular end-to-end anastomosis was performed by using one layer of interrupted 6/0 polypropylene sutures (Prolene, Ethicon, UK) using 10 to 12 stitches per animal. After completion, the anastomosis and the surrounding peritoneum were swabbed with pv-iodine and afterward the right lower quadrant of

the abdomen was flushed with 15 ml sterile isotonic saline. After the procedures, the abdomen was closed in two layers with continuous 4/0 Vicryl suture. Postoperatively all rats received a subcutaneous injection of metamizol for pain relief. In addition, metamizol was added to the drinking water.

Microbiological assessment. Forty-eight hours after the first operation the midline incision was reopened under sterile conditions and a swab from the peritoneal cavity was done. Afterward 1 cc of blood each was taken from the portal vein and the inferior caval vein. The three compartments of the mesenteric lymph nodes [24], the right and caudate liver lobe, the spleen, and the cecum were harvested and weighed. Blood cultures were cultivated immediately in commercially available pediatric blood culture bottles (BactAlert PF, BioMerieux, Lyon, France) and incubated at 37°C for a maximum of 6 days, when they were classified as negative in the case of an absence of bacterial growth. The mesenteric lymph nodes were homogenized in 200 μl of sterile soy bouillon and 100 μl was cultivated on blood and McConkey agar, respectively. Liver and spleen were homogenized in 1 ml of soy bouillon and 100 μl was cultivated on blood and McConkey agar plates. The cecum was homogenized in 10 ml of soy bouillon. Afterwards a serial dilution was performed and 10 μl of the 10^3-, 10^4-, and 10^5-fold dilutant was cultivated on blood, McConkey, and sodium acid plates, respectively. Blood agar plates were incubated for 48 h in 95% O_2 and 5% CO_2; all other plates were incubated for 48 h in 100% O_2. All cultures were analyzed after 24 and 48 h. Differentiation of Gram-negative bacteria was performed by using routine clinical methods.

Intestinal permeability. For measurement of paracellular permeability, 2-cm segments from jejunum (10 cm behind Treitz) and from ascending colon were removed during the second operation, opened along the mesenteric border, and washed. The colon was stripped from the underlying muscle layer; the jejunum was used unstripped due to its fragility. Both were mounted in a small Ussing chamber with an exposed area of 0.28 cm^2. The mucosal and serosal surfaces were bathed independently in a bath solution containing the following (mmol/l): Na (140), Cl (123.8), K (5.4), Ca (1.2), Mg (1.2), HPO_4 (2.4), H_2PO_4 (0.6), HCO_3 (21), glucose (10), glutamine (2.5), mannose (10), and OH-butyrate (90.5) [25]. The bath solution was oxygenated with 95% oxygen and 5% carbogen in a gas-lift recirculation system and maintained at 37°C by water-jacketed reservoirs. After a 30-min equilibration period, measurements were started. The potential difference (PD) was measured with a voltage clamp and calomel electrodes. Electrical continuity between the mucosal and serosal media was maintained with 4% agar bridges. All experiments were performed under short-circuit conditions in which an external feedback circuit of the voltage clamp continuously maintained the PD at 0 through the application of a current that directly opposed the tissue current. The current was applied through Ag/AgCl electrodes that were in contact with the mucosal and serosal media via agar bridges. The short-circuit current (I_{sc}) magnitude was obtained directly from the voltage clamp. The transmural resistance (R^t) was calculated using Ohm's law after passing a 4 μA current through the membrane and measuring the PD. The resistance measurements are expressed as (Ohms $\times$ cm^2).

^{3}H-lactulose-flux measurements were performed to analyze paracellular permeability for larger molecules [26]. ^{3}H-lactulose (20 mmol/l; Biotrend, Cologne, Germany) was added to the mucosal side. Samples were taken from the mucosal and serosal side and radioactivity was counted by a Tri-Carb 2100TR Liquid Scintillation Analyzer (Packard, Meriden, CT, USA). Fluxes (J) were calculated by the standard formula described by Schultz and Zalusky [27].

Bursting pressure. The colonic anastomosis in the respective groups was freed from adhesions and a 5-cm segment of the colon including the anastomosis was removed. The distal end of the segment was ligated (Vicryl 2/0) and from the proximal end a 16-G catheter was inserted and secured. The segment was placed inside a glass jar filled with isotonic saline and air was pumped into the segment, increasing the intraluminal pressure 10 mmHg every 10 s. The intraluminal pressure at which air leakage from the anastomosis was noted was recorded as bursting pressure.

Histology. For histomorphological analysis the right liver lobe, a 3-cm segment of the jejunum 10 cm distal to the ligament of Treitz and the anastomotic segment were fixed in formalin, embedded in paraffin, and routinely stained with hematoxylin and eosin.

In the small bowel, mucosal integrity and inflammatory infiltrations were evaluated [28]. The proportion of crypt and villus height as a parameter for mucosal atrophy was quantified using morphometry in at least 50 crypts and villi per slide. The large bowel was examined for microinsufficiencies of the anastomosis and inflammatory infiltrates. In the liver, degree and location of fat in the hepatocytes were determined. To evaluate liver regeneration, the number of mitoses in 50 visual fields per slide was assessed.

Statistical analysis. All values are given as mean and standard error of mean. For comparison of continuous variables between groups the Mann–Whitney t test was used or the Kruskal–Wallis test if more than two groups were compared. The χ^2 test was applied for comparison of noncontinuous variables. Differences were considered significant if P was less than 0.05. All statistical analyses were performed by using SPSS 10.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

All animals of groups 1 to 5 survived until the second operation; in contrast three rats of group 6 (LR70/CA) and two rats of group 7 (LR70/CA/probiotics) died before. Reason of death was intraabdominal bleeding in one animal of each group, and anastomotic leakage with peritonitis in one animal of group 6. The remaining two had no signs of technical failure, especially no intraabdominal or gastrointestinal bleeding, no anastomotic leakage, and no inflow or outflow obstruction of the hepatic vessels.

Cecal Flora

The concentrations of different bacteria in the cecum are given in Table 1 for all groups. The total bacterial concentration was significantly increased after colonic anastomosis, and it was further increased by the extent of simultaneous liver resection, although this was not statistically significant. In parallel the concentration of Gram-negative bacteria and enterococci increased significantly after colonic anastomosis without and especially with simultaneous liver resection. In the sham operation group a high concentration of lactobacilli was found. In contrast, colonic anastomosis especially but also liver resection resulted in a disturbance of the cecal flora with significant decrease of lactobacillus concentrations. The concentration of lactobacilli could be significantly increased by exogenous application of probiotics (groups 5 and 7). A sufficient concentration of lactobacilli could not be reached in every single animal, but significantly more animals of groups 5 and 7 had lactobacillus concentrations of more than 1 $\times$ 10^8 than in the groups without exogenous application (groups 4 and 6). Since in the groups without exogenous application of probiotics some animals still had high lactobacillus concentrations, the animals were classified according to their lactobacillus concen-

TABLE 1

Bacterial Concentrations in the Cecum

	Total bacteria ($\times 10^9$ CFU/g)	Gram-negative ($\times 10^9$ CFU/g)	Enterococci ($\times 10^9$ CFU/g)	Lactobacilli ($\times 10^9$ CFU/g)
Sham	1.7 ± 0.5‡	0.1 ± 0.1*	0.2 ± 0.1*	1.7 ± 0.3*
LR70	1.7 ± 0.3‡	0.4 ± 0.2*	0.1 ± 0.1*	1.2 ± 0.4*
CA	3.1 ± 0.7	2.2 ± 0.6	0.6 ± 0.2	0.2 ± 0.1
LR30/CA	5.0 ± 0.8	3.4 ± 0.6	1.3 ± 0.3	0.2 ± 0.1
LR30/CA/Probiotics	4.5 ± 0.9	3.6 ± 0.9	0.5 ± 0.1†	0.4 ± 0.1†
LR70/CA	7.0 ± 2.0	5.6 ± 1.8	1.3 ± 0.4	0.1 ± 0.1
LR70/CA/Probiotics	6.1 ± 1.7	4.4 ± 1.5	1.1 ± 0.5	0.5 ± 0.3†

* $P < 0.05$ *versus* all other groups.
† $P < 0.05$ *versus* respective LR/CA group without lactobacilli.
‡ $P < 0.05$ *versus* all LR/CA groups by Mann Whitney *U* test.

tration in the cecum. The threshold was assumed as 1×10^8 CFU lactobacilli per gram stool (Fig. 1).

Organ Cultures

The number of animals with bacterial translocation for the different organs and groups are listed in Table 2. In the sham-operated group enteric bacteria were found in the mesenteric lymph nodes of two of eight animals (25%); in contrast, cultures of MLN were positive in all animals of the other groups. No animal in the sham group was found to have bacteria in the liver, whereas 37.5% of the liver-resected animals and 62.5% of the animals with colonic anastomosis had positive bacterial cultures of liver tissue. In animals with simultaneous liver resection and colonic anastomosis 63 and 86% had positive liver cultures after 30 and 70%

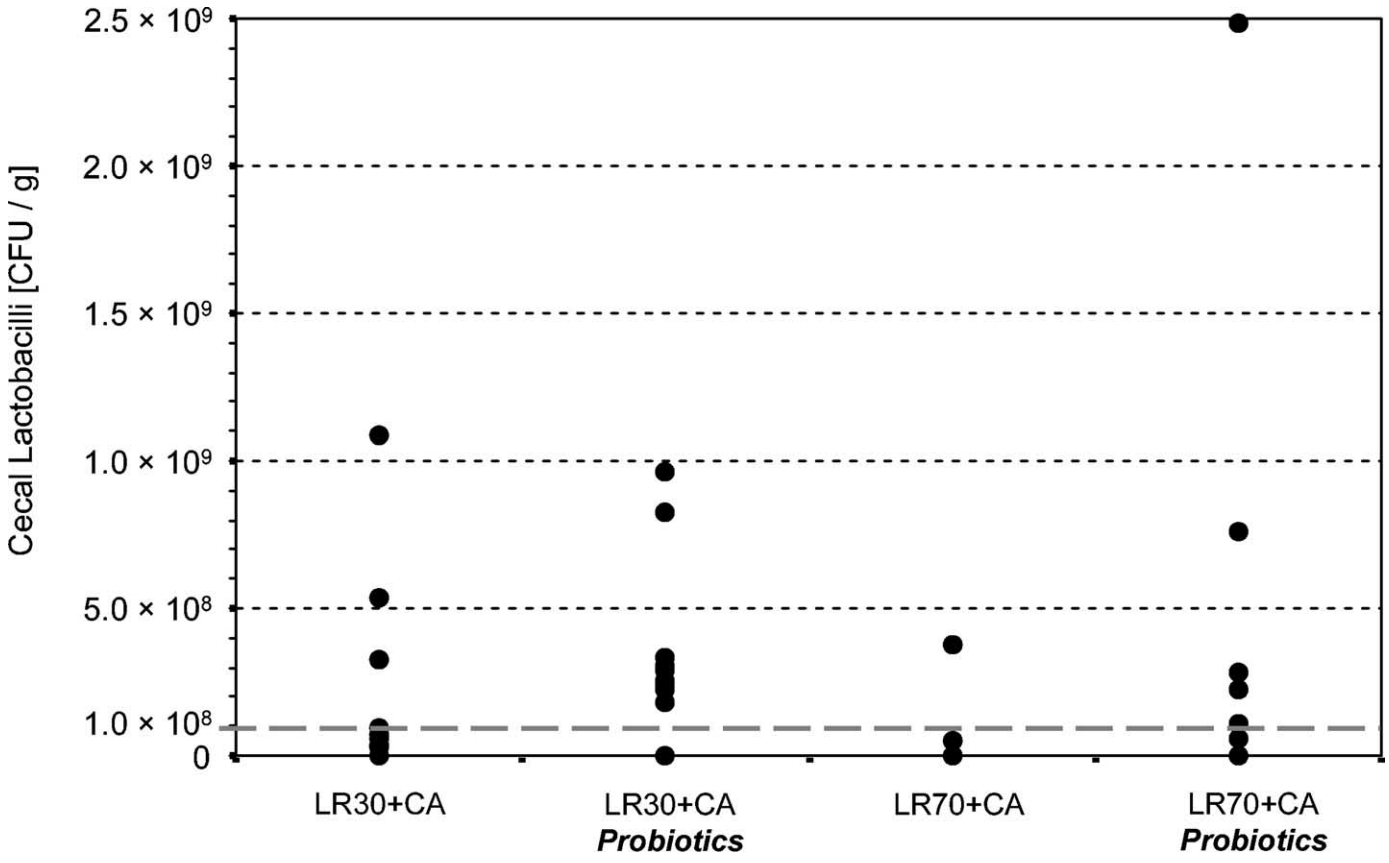

FIG. 1. Cecal concentration of lactobacilli in the single animals of the groups with synchronous operation and with or without exogenous application of probiotics.

TABLE 2

Number of Animals with Bacterial Translocation to Different Organs in the Experimental Groups

	MLN	Liver	Spleen	Blood cultures
Sham	2/8	0/8	1/8	0/8
LR70	8/8*	3/8	3/8	1/8
CA	8/8*	5/8*	5/8*	1/8
LR30/CA	11/11*	7/11*	6/11	1/11
LR30/CA/Probiotics	11/11*	8/11*	6/11	4/11*
LR70/CA	7/7*	7/7*†	6/7*	6/7*†‡
LR70/CA/Probiotics	8/8*	7/8*†	7/8*†	6/8*†‡

* $P < 0.05$ *versus* sham group.
† $P < 0.05$ *versus* LR70 group.
‡ $P < 0.05$ *versus* CA group, by χ^2 test, differences between the synchronous groups 4 to 7 were not statistically significant.

resection, respectively. The bacterial concentrations in different organs of animals in groups 1 to 7 are given in Table 3. Bacterial translocation was low after 70% liver resection alone; it was higher (although not significantly) after colonic anastomosis and simultaneous minor (30%) liver resection and colonic anastomosis. In contrast it increased significantly after simultaneous major (70%) liver resection in MLN, liver, and spleen. Exogenous application of probiotics resulted in significantly lower bacterial concentrations in the MLN in both major and minor simultaneous liver resection. In the liver and spleen bacterial concentrations were lower in the case of application of probiotics, but the differences were not statistically significant, with the exception of 30% liver resection and colonic anastomosis.

The differences were more pronounced if animals with simultaneous minor or major liver resection were divided according to their cecal lactobacillus concentrations (see above). The concentration of enteric bacteria in the mesenteric lymph nodes (Fig. 2), liver, and spleen (Fig. 3) were significantly lower in animals with high lactobacillus concentrations ($>10^8$ CFU/g) compared to animals with low concentrations or complete disappearance of lactobacilli.

Blood Cultures

None of the sham-operated animals revealed positive blood cultures. After 70% liver resection one of eight animals (12.5%) had positive blood cultures and also one of eight animals had positive blood cultures after colonic anastomosis (Table 2). Three animals of 11 (27%) with minor liver resection and colonic anastomosis with low lactobacillus concentrations ($<10^8$ CFU/g) had positive systemic blood cultures; this percentage was similar in animals with high lactobacillus concentrations (Fig. 4). In contrast, after major liver resection and colonic anastomosis 100% of the animals with low lactobacillus concentrations were found to have positive blood cultures; this percentage was significantly lower (50%) in animals with high cecal lactobacillus concentrations (Fig. 4).

Differentiation of Translocating Bacteria

Most of the bacteria isolated in blood or organ cultures were Gram-negative enteric bacteria, which were predominantly identified as *E. coli* strains. The second most frequently isolated bacteria were enterococci. In every single animal the respective bacteria isolated in organ and/or blood cultures could also been demonstrated in the cecal cultures. The predominant translocating strain was in almost all animals also the dominant cecal (mostly Gram-negative) strain. In the lymph node/organ cultures of 19 of 61 animals only one translocating strain could be demonstrated; in 35 of 61 (57%) two different strains were found. In 49 of 54 translocating animals *E. coli* was found in the lymph node/organ cultures. Other detected bacteria were enterococcus species (in $n = 13$ animals), proteus species ($n = 9$), citrobacter species ($n = 4$), enterobacter species ($n = 2$), other Gram-negative bacteria ($n = 5$), and streptococci ($n = 3$).

In 10 animals only one bacterial strain was found in the blood cultures; in 9 animals two different bacteria were isolated. Eleven of 19 animals with positive blood cultures revealed *E. coli*. Other isolated bacteria were enterococcus species ($n = 5$ animals), proteus species ($n = 3$), other Gram-negative bacteria ($n = 4$), and streptococci ($n = 1$).

Paracellular Permeability

Transepithelial resistance as a parameter for passive transport of ions was significantly diminished in the colon in all groups with colon resection (Table 4). In contrast, the small bowel only showed impairment of resistance in the group with 70% liver resection (Table 5). Administration of pre- and probiotics did not reverse the decrease in resistance. The lactulose flux was not increased in any of the groups, not in the colon nor in the ileum.

Bursting Pressure

The mean bursting pressure in group 3 (CA) was 40.3 ± 4.1 mmHg; in group 4 (LR30/CA) mean bursting pressure was 32.2 ± 4.3 mmHg and in group 5 (CA30/CA/probiotics) mean bursting pressure was 37.3 ± 4.1 mmHg. The lowest bursting pressure was observed in group 6 (LR70/CA) with 24.9 ± 3.1, which was markedly lower than in group 7 (LR70/CA/probiotics: 39.6 ± 5.3 mmHg), but all differences were not statistically significant.

TABLE 3

Bacterial Concentrations in the Mesenteric Lymph Nodes, Liver, and Spleen

	Mesenteric lymph nodes [CFU/g]	Liver [CFU/g]	Spleen [CFU/g]
Sham	121 ± 96*	0 ± 0*	10 ± 10*
LR70	460 ± 223	$1.83 \pm 1.77 \times 10^4$	$2.68 \pm 2.44 \times 10^4$
CA	3612 ± 2717	113 ± 80	$2.89 \pm 2.67 \times 10^4$
LR30/CA	4520 ± 1273	$8.69 \pm 7.51 \times 10^4$	$8.91 \pm 8.62 \times 10^4$
LR30/CA/Probiotics	1489 ± 445†	1591 ± 760†	1480 ± 1050
LR70/CA	48,498 ± 14,204*	$8.50 \pm 5.46 \times 10^{6}$*	$4.95 \pm 4.16 \times 10^{6}$*
LR70/CA/Probiotics	14,589 ± 7680†	$11.23 \pm 9.55 \times 10^6$	$4.00 \pm 3.71 \times 10^6$

* $P < 0.05$ *versus* all other groups.
† $P < 0.05$ *versus* respective LR + CA group without lactobacilli.
‡ $P < 0.05$ *versus* all other groups except LR70 + CA + Lactobac., by Mann Whitney *U* test.

Histomorphology

There were no relevant differences regarding the morphometrical relation of villous and crypt height in the small bowel between the groups, and no mucosal atrophy or villous damage was detected. In parallel, the mucosa of the large bowel and the anastomosis were histologically intact in all groups.

In the groups with liver resection, there was a marked fatty infiltration of the hepatocytes in the remnant liver with a periportal distribution that increased with the degree of liver resection but no inflammatory infiltrates. Regarding liver regeneration, no mitoses were seen in the rats with sham operation or colon anastomosis (Fig. 5). Following 30% liver resection and colon anastomosis, there was a median number of mitoses which was not altered by addition of probiotics. Seventy percent of liver resection caused a marked induction of mitosis, which was reduced in the case of simultaneous colon anastomosis. In group 7 with probiotics, the number of mitoses was similar to that of 70% liver resection alone. These differences were not statistically significant.

DISCUSSION

In the present rat model, a low bacterial translocation to the mesenteric lymph nodes was observed even in some animals of the sham-operated group. As all cultures from the intraabdominal swabs taken before harvesting the organs were negative, bacterial contamination was not responsible for these results. The surgical stress per se together with the laparotomy can therefore induce a limited bacterial translocation, which does not disseminate to other organs. However, other experimental studies found no translocation in sham-operated animals [12]. Following 70% liver resection, mainly translocation to the liver occurred without severe disturbance of the cecal flora compared to the control group. In parallel another study demon-

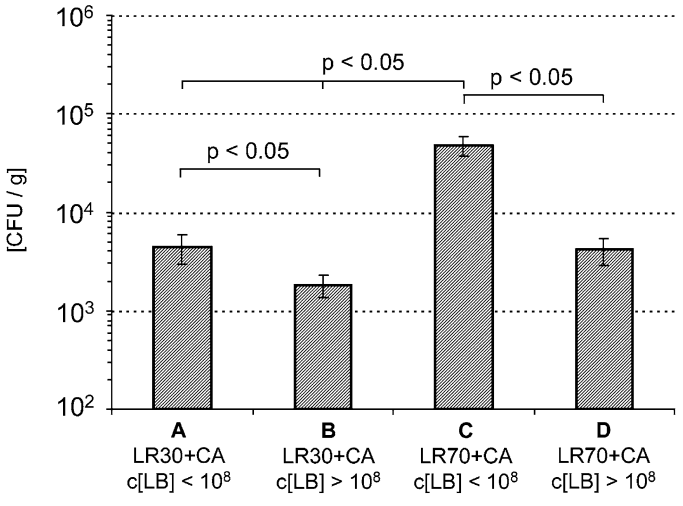

FIG. 2. Concentration of enterobacteria in the mesenteric lymph nodes depending on the cecal concentration of lactobacilli.

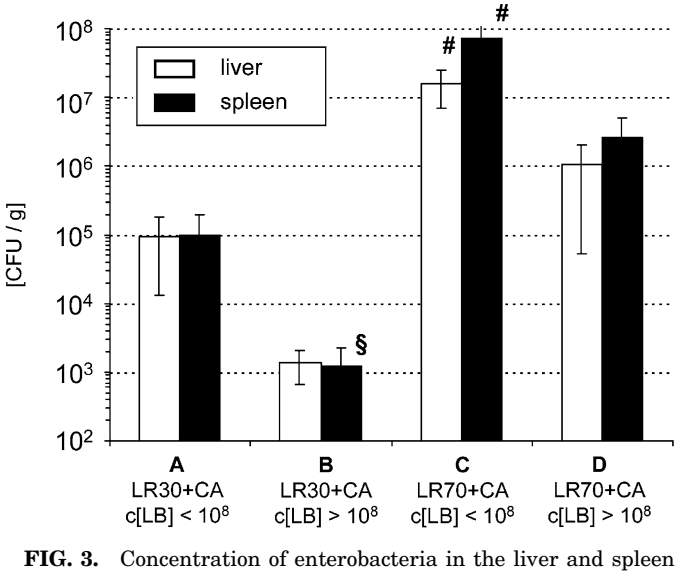

FIG. 3. Concentration of enterobacteria in the liver and spleen depending on the cecal concentration of lactobacilli (#$P < 0.05$ *versus* groups A, B, D; §$P < 0.05$ *versus* group A).

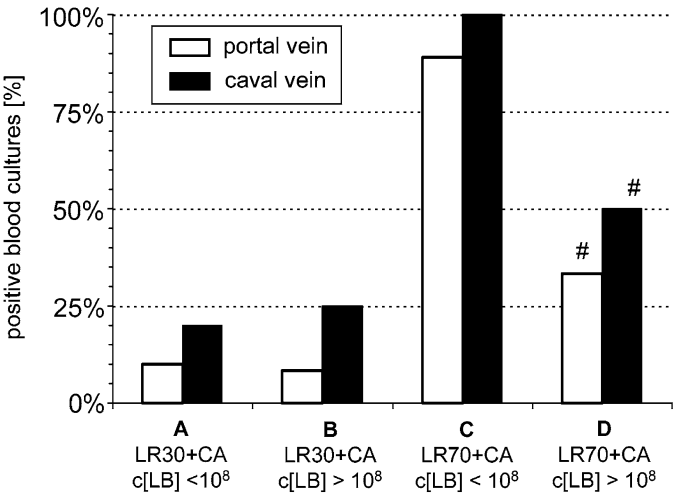

FIG. 4. Positive blood cultures depending on the cecal concentration of lactobacilli (#$P < 0.05$ *versus* group C by χ^2 test).

strated bacterial translocation to mesenteric lymph nodes, liver, and spleen in all rats 24 and 48 h after 70% liver resection without changes in the cecal flora [29]. Therefore, not bacterial overgrowth but impaired function of the hepatic reticulo-endothelial system in combination with reduced bactericidal bile flow and impaired synthesis of acute phase proteins by the liver seem to be responsible for translocation in the rat liver resection model. Further arguments confirming this hypothesis are given by the significantly diminished clearance of intravenously administered *E. coli* after 70% liver resection [30].

Colonic anastomosis caused high bacterial concentrations in mesenteric lymph nodes and spleen as well as a high coecal concentration of enteropathogenic bacteria. In clinical studies, 38 to 59% of patients with intestinal obstruction had bacteria in the mesenteric lymph nodes [31]. Following induction of colitis in rats, a reduction of lactobacilli and an increase of Gram-negative bacteria with consecutive bacterial translocation in lymph nodes, liver, and spleen occurred in more than 50% of the animals [10, 32]. Thus, bacterial antagonism and colonization resistance presumably are important pathogenetic factors.

A combination of liver resection and colon anastomosis worsened the disturbance of the cecal microflora and increased the bacterial translocation to all organs in parallel to the extent of liver resection. These findings might explain the higher infection rates and mortality after synchronous liver and bowel resection in men compared to single procedures [6]. On the other hand, a simultaneous colon operation impaired regeneration of the remnant liver after liver resection in rats [33]. Presumably, both mechanisms, bacterial overgrowth and impaired bacterial clearance of the liver, act synergistically in this model.

Whereas often only the number of translocating animals is used in translocation studies [12, 29], in the present model additionally the bacterial concentration in different organs has been analyzed. This was due to the facts that even after minor surgical trauma most animals were found to have bacterial translocation to the mesenteric lymph nodes and that in the groups with the synchronous procedures bacterial translocation to other organs was detected in most animals due to the immense surgical trauma. Therefore bacterial concentration was additionally used to evaluate differences after major trauma and the influence of therapeutic interventions. This was done by using standardized methods as described elsewhere [20, 34].

Perioperative administration of a synbiotic combination caused a reduction of the total cecal bacterial count especially of Gram-negative bacteria and enterococci and an increase of lactobacilli. At the same time, bacterial translocation in mesenteric lymph nodes and partly in other organs was significantly diminished. Likewise lactobacilli have been shown to reduce bacterial translocation in other experimental studies. In a model of acute pancreatitis 14/20 rats developed bacterial translocation to mesenteric lymph nodes compared to only 5/20 rats receiving probiotics (lactobacillus plantarum) [35]. Moreover administration of lactobacillus plantarum reduced the cecal concentration of enterobacteria and prevented bacterial translocation to the liver in a rat model of D-galactosamin-induced acute liver failure. In addition hepatocellular necrosis and inflammatory infiltrates in the liver were prevented by administration of probiotics [20, 29]. Even prebiotics alone (methyl-cellulose) could reduce bacterial translocation to mesenteric lymph nodes and blood after 70 and 90% liver resection in rats [36]. Up to now, no data were available on combined administration of pre- and probiotics.

The time point for microbiological analysis of 48 h after primary surgery was chosen for several reasons: first, after oral administration the positive effect of the lactobacillus needs some time, so that 24 to 48 h after surgery is the interval most frequently used in lactobacillus studies. It has been shown in the literature that 48 h after surgery the effect of lactobacilli is more pronounced than after 24 h [20]. Moreover animals undergoing minor interventions were shown to have systemic translocation within the first 24 h after surgery, and thereafter, only in the MLN. In contrast major surgery led to systemic translocation until day 2 [37]. Therefore 48 h was selected as the point of best discrimination between different surgical interventions and lactobacillus application.

Animals with synchronous operations and low fecal lactobacillus concentrations tended to have a lower bursting pressure of the anastomosis, although the differences were not statistically significant. This might be due to the relatively early examination of the

TABLE 4

Results of Measurements in the Ussing Chamber in the Colon

	R (Ωcm^2) SEM (n-1)	Isc ($\mu A/cm^2$) SEM (n-1)	JLAC [$\mu mol(hcm^2)$] SEM (n-1)	R t test	Isc t test	JLAC t test
Sham	151.80 ± 6.55	55.44 ± 8.51	134.78 ± 8.63			
LR70	144.44 ± 9.95	51.84 ± 8.12	127.64 ± 18.28	0.53	0.77	0.70
CA	111.89 ± 10.25*	33.32 ± 5.46	180.68 ± 41.14	0.00*	0.06	0.22
LR 30/CA	130.54 ± 4.74*	44.16 ± 5.96	125.48 ± 10.55	0.02*	0.30	0.50
LR 30/CA/P	115.97 ± 17.36*	49.75 ± 19.34	157.58 ± 14.74	0.04*	0.76	0.17
LR 70/CA	116.03 ± 11.13*	55.32 ± 5.83	177.69 ± 33.19	0.01*	0.99	0.21
LR 70/CA/P	107.03 ± 10.68*	49.41 ± 5.55	182.94 ± 32.36	0.00*	0.59	0.12

Note. R = transepithelial resistance; SEM = standard error of mean; Isc = short circuit current; JLAC = Laktulose- Flux; t test = P values.
* $P < 0.05$ *versus* sham operation.

anastomosis, because this time point was selected for the microbiological analysis. After a longer interval of 3 to 7 days after surgery, Colucci *et al*. achieved a significantly higher bursting pressure of a colon anastomosis using lactobacilli and fiber compared to controls [38]. Also Demetriades *et al*. reported a higher bursting pressure after 7 days of enteral administration of fiber and glutamine compared to application of glucose and electrolyte solution in the rat [39].

Transepithelial resistance reflecting passive transport of ions was decreased in the groups with colon resection, but only in the colon. The flux of the disaccharide lactulose was not increased. These results implicate that the paracellular route of bacterial translocation is unlikely because only ions but not larger molecules pass through the epithelium. In addition, administration of pre- and probiotics did decrease bacterial translocation, but did not alter the resistance. Previous studies have shown corresponding results. 50% proximal small bowel resection in mice induced bacterial translocation to mesenteric lymph nodes but transepithelial resistance in the Ussing chamber was not altered and no increased flux of small or large molecules was noted. The colon was not analyzed in this study [40]. Therefore a transcellular passage via macrophages or enterocytes is most likely for bacterial translocation.

Gross histological changes of the intestine and colon at the time of the microbiological analyses could be excluded in all groups. Therefore, bacterial translocation in the present model is not caused by visible changes in mucosal structure, which is in accordance with most other studies: bacterial translocation occurred after administration of endotoxin [41], elementary diet [16], and D-galactosamin induced liver failure [29] without histological alterations of the intestinal mucosa. In contrast, some authors could demonstrate mucosal atrophy with shortening of the villus height, i.e., in newborn rats fed with parenteral nutrition [15] or following 90% liver resection [12]. This was not found in the present study, maybe due to the relatively late investigation time (48 h after surgery) and the high intestinal turn over rate in rats.

A high rate of mitosis was observed in the regenerating liver 48 h following liver resection. The rate was higher after 70% than after 30% liver resection. In combination with colon anastomosis, fewer mitoses were observed. Other experimental studies have also shown an association between liver regeneration and manipulation of the gut. In a mouse model Nelson *et al*. found a significantly lower amount of proliferating cell-nuclear antigen as parameter for liver regeneration after simultaneous liver and small bowel resection compared to liver resection alone [42]. Likewise Miyazaki *et al*. showed that hepatic DNA and protein synthesis was reduced in rats with simultaneous colon

TABLE 5

Results of Measurements in the Ussing Chamber in the Jejunum

	R (Ωcm^2) SEM (n-1)	Isc ($\mu A/cm^2$) SEM (n-1)	JLAC [$\mu mol(hcm^2)$] SEM (n-1)	R t test	Isc t test	JLAC t test
Sham	37.26 ± 3.67	109.51 ± 17.53	158.80 ± 21.04			
LR 70	26.78* ± 1.63	160.44 ± 24.45	189.62 ± 36.77	0.03*	0.10	0.45
CA	40.23 ± 4.92	94.48 ± 14.57	311.36 ± 78.02	0.63	0.54	0.05
LR 30/CA	33.18 ± 4.35	148.24 ± 19.70	242.77 ± 44.57	0.48	0.16	0.10
LR 30/CA/P	41.23 ± 8.49	110.96 ± 20.55	198.67 ± 17.96	0.66	0.96	0.18
LR 70/CA	29.90 ± 2.20	125.71 ± 15.27	226.71 ± 41.28	0.10	0.49	0.17
LR 70/Ca/P	37.99 ± 3.76	128.88 ± 19.91	208.34 ± 20.48	0.90	0.50	0.15

Note. R = transepithelial resistance; SEM = standard error of mean; Isc = short circuit current; JLAC = Laktulose- Flux, t test = P values.
* $P < 0.05$ *versus* sham operation.

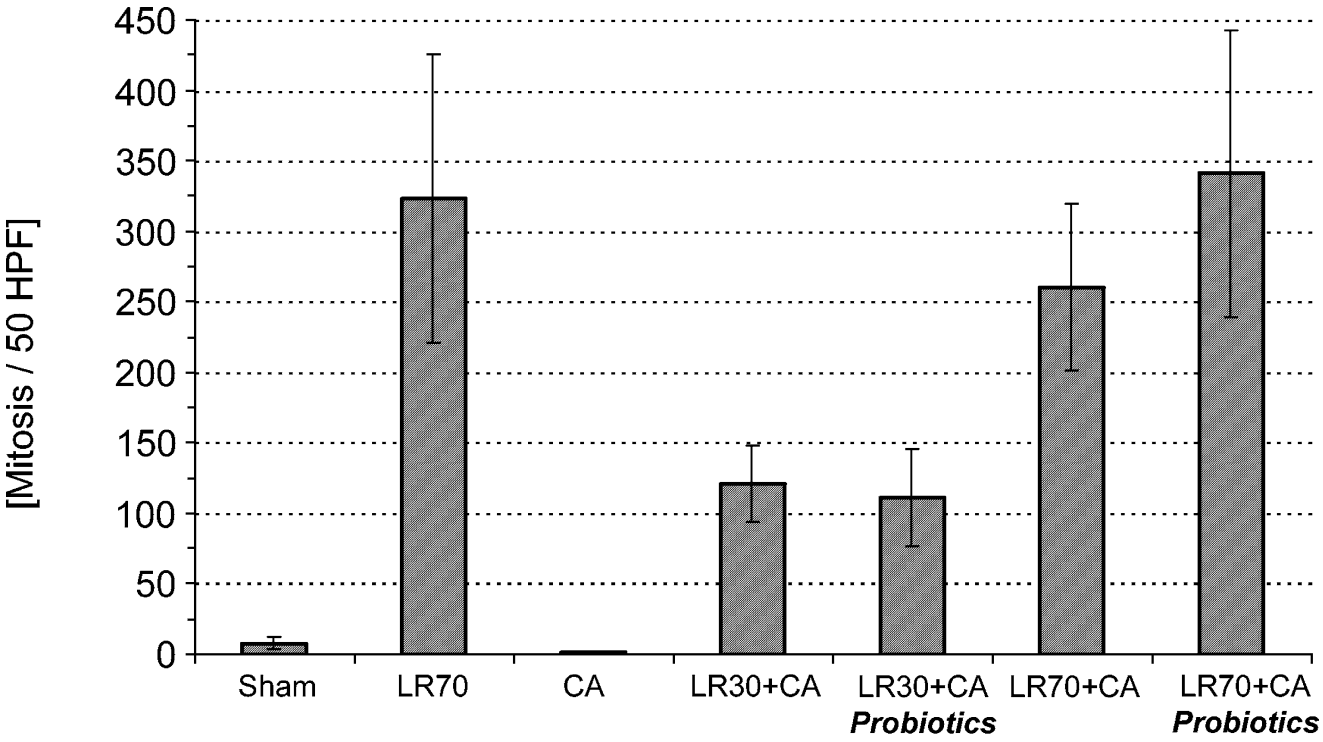

FIG. 5. Number of mitosis in the liver per 50 high power fields.

and liver resection. In this study also more anastomotic insufficiencies and higher portal and systemic endotoxinemia were observed after synchronous operations [33]. Both authors conclude that an increased bacterial translocation is responsible for the impaired hepatic regeneration. This is also in accordance with the present observations, where hepatic regeneration was impaired by increased bacterial translocation and partially improved by application of pre- and probiotics by means of preventing bacterial translocation.

In conclusion, synchronous liver resection and colon anastomosis led to potentiation of bacterial translocation compared to the single operations. It is possible to diminish this process by the simple means of oral administration of pre- and probiotics in the rat model used. Bacterial overgrowth in the cecum and impaired hepatic regeneration, but not histological changes or alterations of paracellular permeability, are potential mechanisms for translocation in this setting. The efficacy of the used probiotic combination in the human setting has to be proven in further studies.

ACKNOWLEDGMENTS

We thank Petra Wiedersatz and Heide Schernbeck for excellent technical assistance with the microbiological analyses, and Raes Ahmed for exceptional help in conducting the Ussing chamber experiments.

REFERENCES

1. Emori, T. G., and Gaynes, R. P. An overview of nosocomial infections, including the role of the microbiology laboratory. *Clin. Microbiol. Rev.* **6:** 428, 1993.
2. Wacha, H., Hau, T., Dittmer, R., and Ohmann, C. Risk factors associated with intraabdominal infections: A prospective multicenter study. *Langenbeck's Arch. Surg.* **384:** 24, 1999.
3. Bengmark, S. Aggressive peri- and intraoperative enteral nutrition—Strategy for the future. In S. A. Shikora, R. G. Martindale, and S. D. Schwaitzberg (Eds.), *Nutritional Considerations in the Intensive Care Unit—Science, Rationale and Practice*. Dubuque: American Society for Parenteral and Enteral Nutrition (ASPEN) and Kendall Hunt Publishing Co., 2002.
4. Elias, D., Detroz, B., Lasser, P., Plaud, B., and Jerbi, G. Is simultaneous hepatectomy and intestinal anastomosis safe? *Am. J. Surg.* **169:** 254, 1995.
5. Shinagawa, N., Hisada, M., and Ishihara, H. Postoperative infection control in patients with hepatic, biliary tract and pancreatic cancers. *Gan To Kagaku Ryoho* **19:** 173, 1992.
6. Bolton, J. S., and Fuhrman, G. M. Survival after resection of multiple bilobar hepatic metastases from colorectal carcinoma. *Ann. Surg.* **231:** 743, 2000.
7. Naaber, P., Smidt, I., Tamme, K., Liigant, A., Tapfer, H., Mikelsaar, M., and Talvik, R. Translocation of indigenous microflora in an experimental model of sepsis. *J. Med. Microbiol.* **49:** 431, 2000.
8. Tran, D. D., Cuesta, M. A., van Leeuwen, P. A., Nauta, J. J., and Wesdorp, R. I. Risk factors for multiple organ system failure and death in critically injured patients. *Surgery* **114:** 21, 1993.
9. Wilmore, D. W., Smith, R. J., O'Dwyer, S. T., Jacobs, D. O., Zeigler, T. R., and Wang, X. D. The gut: A central organ after surgical stress. *Surgery* **104:** 917, 1988.

10. Fabia, R., Ar'Rajab, A., Johansson, M. L., Andersson, R., Willen, R., Jeppsson, B., Molin, G., and Bengmark, S. Impairment of bacterial flora in human ulcerative colitis and experimental colitis in the rat. *Digestion* **54:** 248, 1993.
11. Horton, J. W., and Walker, P. B. Oxygen radicals, lipid peroxidation, and permeability changes after intestinal ischemia and reperfusion. *J. Appl. Physiol.* **74:** 1515, 1993.
12. Wang, X., Andersson, R., Soltesz, V., Wang, L., and Bengmark, S. Effect of portal hypertension on bacterial translocation induced by major liver resection in rats. *Eur. J. Surg.* **159:** 343, 1993.
13. Berg, R. Promotion of the translocation of enteric bacteria from the gastrointestinal tract of mice by oral treatment with penicillin, clindamycin or metronidazole. *Infect. Immunol.* **33:** 854, 1981.
14. Spaeth, G., Gottwald, T., Specian, R. D., Mainous, M. R., Berg, R. D., and Deitch, E. A. Secretory immunoglobulin A, intestinal mucin, and mucosal permeability in nutritionally-induced bacterial translocation in rats. *Ann. Surg.* **220:** 798, 1994.
15. Steinwender, G., Schimpl, G., Sixl, B., Kerbler, S., Ratschek, M., Kilzer, S., Hollwarth, M. E., and Wenzl, H. H. Effect of early nutritional deprivation and diet on translocation of bacteria from the gastrointestinal tract in the newborn rat. *Pediatr. Res.* **39:** 415, 1996.
16. Deitch, E. A., Dazhong, X., Naruhn, M. B., Deitch, D. C., Qi, L., and Marino, A. A. Elemental diet and iv-TPN-induced bacterial translocation is associated with loss of intestinal mucosal barrier function against bacteria. *Ann. Surg.* **221:** 299, 1995.
17. Kayama, S., Mitsuyama, M., Sato, N., and Hatakeyama, K. Overgrowth and translocation of Escherichia coli from intestine during prolonged enteral feeding in rats. *J. Gastroenterol.* **35:** 15, 2000.
18. Van der Hulst, RRWJ, Van Kreel, B. K., Von Meyenfeldt, M. F., Brummer, R. J., Arends, J. W., Deutz, N. E., and Soeters, P. B. Glutamine and the preservation of gut integrity. *Lancet* **341:** 1363, 1993.
19. Liboshi, Y., Nezu, R., Kennedy, M., Fujii, M., Wasa, M., Fukuzawa, M., Kamata, S., Tagaki, Y., and Okada, A. Total parenteral nutrition decreases luminal mucous gel and increases permeability of small intestine. *J. Parenter. Enteral Nutr.* **18:** 346, 1994.
20. Adawi, D., Kasravi, F. B., Molin, G., and Jeppsson, B. Effect of lactobacillus supplementation with and without arginine on liver damage and bacterial translocation in an acute liver injury model in the rat. *Hepatology* **25:** 642, 1997.
21. Ouwehand, A. C., Isolauri, E., Kirjavainen, P. V., Tolkko, S., and Salminen, S. J. The mucus binding of Bifidobacterium lactis Bb12 is enhanced in the presence of Lactobacillus GG and Lact. delbrueckii subsp. bulgaricus. *Lett. Appl. Microbiol.* **30:** 10, 2000.
22. Ljungh, Å., Lan, J.-G. and Yamagisawa, N. Isolation, selection and characteristics of Lactobacillus paracasei ssp paracasei isolate F19. *Microb. Ecol. Health Dis.* **Suppl 3:** 4, 2002.
23. Minato, M., Houssin, D., Morin, J., Szekely, A. M., and Bismuth, H. Surgically induced acute hepatic failure in the rat. *Eur. Surg. Res.* **15:** 129, 1983.
24. Gautreaux, M. D., Deitch, E. A., and Berg, R. D. Bacterial translocation from the gastrointestinal tract to various segments of the mesenteric lymph node complex. *Infect. Immunol.* **62:** 2132, 1994.
25. Schulzke, J. D., Fromm, M., Bentzel, C. J., Zeitz, M., Menge, H., and Riecken, E. O. Ion transport in the experimental short bowel syndrome of the rat. *Gastroenterology* **102:** 497, 1992.
26. Stockmann, M., Fromm, M., Schmitz, H., Schmidt, W., Riecken, E. O., and Schulzke, J. D. Duodenal biopsies of HIV-infected patients with diarrhoea exhibit epithelial barrier defects but no active secretion. *AIDS* **12:** 43, 1998.
27. Schultz, S. G., and Zalusky, R. Ion transport in isolated rabbit ileum. I. Short circuit current and NA fluxes. *J. Gen. Physiol.* **47:** 567, 1964.
28. Orlando, M. D., Chandrasekhar, A., Bundz, S., Burt, E. T., Moorman, D. W., and Timberlake, G. A. The effect of peritoneal contamination on wound strength of small bowel and colonic anastomoses. *Ann. Surg.* **65:** 673, 1999.
29. Kasravi, A. B., Wang, L., Wang, X. D., Molin, G., Bengmark, S., and Jeppsson, B. Bacterial translocation in acute liver injury induced by D-galactosamine. *Hepatology* **23:** 97, 1996.
30. Gross, K., Katz, S., Dunn, S. P., Cikrit, D., Rosenthal, R., and Grosfeld, J. L. Bacterial clearance in the intact and regenerating liver. *J. Pediatr. Surg.* **20:** 320, 1985.
31. Sedman, P. C., Macfie, J., Sagar, P., Mitchell, C. J., May, J., Mancey-Jones, B., and Johnstone, D. The prevalence of gut translocation in humans. *Gastroenterology* **107:** 643, 1994.
32. Gardiner, K. R., Erwin, P. J., Anderson, N. H., Barr, J. G., Halliday, M. I., and Rowlands, B. J. Colonic bacteria and bacterial translocation in experimental colitis. *Br. J. Surg.* **80:** 512, 1993.
33. Miyazaki, M., Kohda, S., Itoh, H., Kaiho, T., Kimura, F., Ambiru, S., Hayashi, S., Gohchi, E., Takanashi, K., and Nagai, M. Inhibition of hepatic regeneration after 70% partial hepatectomy by simultaneous resection of the bowel in rats. *Eur. Surg. Res.* **27:** 396, 1995.
34. Sambol, J. T., Forsythe, R. M., and Deitch, E. A. Gut barrier failure. In W. W. Souba, and D. W. Wilmore (Eds.), *Surgical Research*. San Diego: Academic Press, 2001, Pp. 601–610.
35. Mangiante, G., Canepari, P., Colucci, G. L., Nicoli, N., Bassi, C., Marinello, P., Signoretto, C., and Bengmark, S. Lactobacillus plantarum reduces infection of pancreatic necrosis in experimental pancreatitis (Abstract). *Eur. Surg. Res.* **32:** 147, 2000.
36. Wang, X., Andersson, R., Soltesz, V., Guo, W., and Bengmark, S. Water-soluble ethylhydroxy cellulose prevents bacterial translocation induced by major liver resection in the rat. *Ann. Surg.* **217:** 155, 1993.
37. Wang, X., Andersson, R., Soltesz, V., and Bengmark, S. Bacterial translocation after major hepatectomy in patients and rats. *Arch. Surg.* **127:** 1101, 1992.
38. Colucci, G. L., Mangiante, G., Facci, E., Casaril, A., Ciola, M., Gottin, L., Colombari, R., and Bengmark, S. Administration of fibers and Lactobacillus plantarum improves healing of coloc anastomosis. *Eur. Surg. Res.* **32**(Suppl. 1): 1, 2000.
39. Demetriades, H., Botsios, D., Kazantzidou, D., Sakkas, L., Tsalis, K., Manos, K., and Dadoukis, I. Effect of early postperative enteral feeding on the healing of colonic anastomoses in rats. *Eur. Surg. Res.* **31:** 57, 1999.
40. O'Brien, D. P., Nelson, L. A., Kemp, C. J., Williams, J. L., Wang, Q., Erwin, C. E., Hasselgren, P. O., and Warner, B. W. Intestinal permeability and bacterial translocation are uncoupled after small bowel resection. *J. Pediatr. Surg.* **37:** 390, 2002.
41. Go, L. L., Healey, P. J., Watkins, S. C., Simmons, R. L., and Rowe, M. I. The effect of endotoxin on intestinal mucosal permeability to bacteria in vitro. *Arch. Surg.* **130:** 53, 1995.
42. Nelson, L. A., O'Brien, D. P., Kemp, C. J., Williams, J. L., Dunke-Jacobs, E., Erwin, C. R., and Warner, B. W. Intestinal and hepatic response to combined partial hepatectomy and small bowel resection in mice. *Am. J. Surg.* **183:** 435, 2002.