Recolonization and colonization resistance of the large bowel after three methods of preoperative preparation of the gastrointestinal tract for elective colorectal surgery

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SUMMARY

The impact of three current types of preoperative large bowel preparation on the microbial flora and the colonization resistance (CR) was investigated in 15 volunteers. In the first group a whole gut irrigation was performed without administration of antibiotics (group WGI). In the second group 0·5 g/l metronidazole and 1 g/l neomycin was added to the irrigation fluid (group WGI + AB). A whole gut irrigation with prior oral administration of 1 l mannitol 10 % was performed in the third group. The antibiotic prophylaxis in this group consisted of two doses of 80 mg gentamicin i.v. and 500 mg metronidazole orally 24 h after lavage (group Mann + AB). One hour after the mechanical cleansing procedure was finished all volunteers were orally contaminated with one dose of an *Escherichia coli* test strain. The aerobic faecal reduction due to the cleansing procedure was 2–3 logs, while for the anaerobes it was 4–5 logs. The anaerobic flora in group WGI recovered within 24 h, while the aerobes showed a transient 'overgrowth' for the period of 2 days. The overgrowth of aerobes in group WGI + AB was observed for more than a week and the total numbers of aerobes started gradually to decline after the anaerobic flora had reached pretreatment levels at day three or four. Despite the normal numbers of anaerobes present 24 h after treatment, overgrowth of *E. coli* was seen in the group Mann + AB, probably due to residual mannitol left in the intestinal tract. The test strain of *E. coli* was excreted for a period of 1 week by the volunteers in the groups WGI and Mann + AB, but it was isolated for more than 10 weeks in the group WGI + AB. It is thought that all three methods of preoperative large bowel preparation decreased the CR of the gastrointestinal tract because of a disturbance of the interaction between aerobic and anaerobic microorganisms and alterations of the colonic wall. The anaerobic microflora, however, appeared to be primarily responsible for the maintenance of the CR. Antimicrobial prophylaxis should consist of a high dose, short term, systemic antibiotic regimen, not only because an adequate serum level of an appropriate drug at the time of operation substantially decreases the incidence of postoperative septic complications but also because a systemic regimen scarcely influences the

CR of the gastrointestinal tract. β-Aspartylglycine appeared to be a specific but not very sensitive marker for decreased CR.

INTRODUCTION

Colorectal surgery is associated with a high incidence of postoperative sepsis; incidences exceeding 50 % have been reported (Willis, Jones & Reilly, 1981). The obvious reason is the unavoidable heavy contamination of the field of operation by intestinal microorganisms. To prevent spillage of intestinal contents and to minimize contamination, preoperative regimens for elective colorectal surgery include a procedure for reducing the faecal mass. In the classical preparation of the colon for elective colorectal surgery the patients were given low or non-residue diets, enemas and purgatives. Hewitt et al. (1973) reported an alternative method for preoperative mechanical cleansing of the large bowel by whole gut irrigation (WGI). Osmotic cathartics, such as mannitol have also been advocated either alone or in combination with WGI. However, although impeccable mechanical cleansing of the gut may be achieved, these procedures have little or no effect on the numbers of bacteria in the residual contents of the lumen or associated with the colonic wall (Arabi et al. 1978). This is corroborated by the observation that mechanical cleansing alone scarcely reduces the postoperative sepsis rate (Clarke et al. 1977). Therefore, most regimens for elective colorectal surgery include the perioperative prophylactic use of antibiotics.

Attempts to reduce the intestinal microflora by oral administration of nonabsorbable antibiotics began more than 40 years ago (Garlock & Seley, 1939). Most of these early antimicrobial prophylactic regimens made use of nonabsorbable agents active against only the aerobic flora. The results varied considerably but only marginal success in reducing the postoperative sepsis rate was achieved (Barker et al. 1971; Brass et al. 1976). Ecological and epidemiological studies have now shown that nonsporing obligate anaerobic bacteria (OAB) play an important role in postoperative sepsis after colorectal surgery. This not only reflects the dominate position of OAB in the intestinal flora, but mixed infections with aerobes and anaerobes have been shown to have an enhanced virulence (Onderdonk, Bartlett & Louie, 1976; McGowan & Gorbach, 1981). Furthermore, it became clear that to 'sterilize' the colon in vivo by short-term use of oral antibiotics is virtually impossible and that the protection offered by antimicrobial prophylactic regimens is due to adequate tissue levels of suitable antibiotics at the time of contamination (Keighly & Burdon, 1979). The prophylactic use of antibiotics for colorectal surgery has as a result of these findings shifted from antibiotics active against aerobes given orally to parenterally administered antibiotics mainly active against OAB.

Although from the onset of antibiotic therapy the effect of antibiotics on the colonic microflora has been recognized (Weinstein, 1947), it is only recently that this effect and especially its consequences for the resistance against colonization and overgrowth by potentially pathogenic microorganisms have seriously been considered. Van der Waay (Van der Waay, Berghuis-de Vries & Lekkerkerk-van der Wees, 1971) introduced the concept of colonization resistance (CR) to describe this phenomenon.

In animals CR can be assessed experimentally by oral contamination with a known dose of a specific bacterial challenge strain. The concentration reached by this strain in the faeces expressed as the number of colony forming units (c.f.u.) per gram faeces and the excretion time can be used as parameters to measure the CR. Welling & Groen (1978) reported that in mice β-aspartylglycine occurs in faeces and faecal contents when the CR is diminished and this finding appeared also to apply to man (Welling, 1979). It might be possible to use this parameter to monitor the CR in patients.

Mechanical cleansing of the bowel removes most of the bacterial mass in the colon. In combination with perioperative use of antibiotics this certainly influences the stability of the residual mucosa-associated colonic microflora, and therefore is most likely to affect the CR. Thus, mechanical cleansing of the colon with or without perioperative antimicrobial prophylaxis might actually enhance perioperative colonization of patients with (potentially) pathogenic bacteria and in consequence may influence the perioperative nosocomial infection rate.

Evaluation of the influence of the mechanical cleansing with or without antimicrobial prophylaxis on the CR is for obvious reasons not possible in patients by direct challenge contamination with potentially pathogenic bacteria. Therefore we embarked upon a study with well-informed healthy volunteers to investigate the impact of three different current methods of preoperative bowel preparation on the faecal flora and the CR of the gastrointestinal tract including an assessment of the time required for the intestinal microflora to return to normal after treatment. One of the factors determining the CR is the volatile fatty acid (VFA) production by OAB (Byrne & Dankers, 1979). Levels of VFA and β-aspartylglycine were measured in all faecal samples to determine if these methods could be used to monitor CR in colorectal surgery patients in clinical trials.

MATERIALS AND METHODS

Bacterial test strain and contamination dose

A neomycin and gentamicin (MIC > 100 mg/l) resistant strain of *Escherichia coli* was used to investigate the CR of the gastrointestinal tract. After oral contamination with doses as high as 10^{11} c.f.u. suspended in milk and taken on an empty stomach by three volunteers the *E. coli* test strain could not be cultured from their faeces in a period of one week after ingestion of the challenge dose. Subsequently, a challenge dose of 10^6 c.f.u. was used in the experiments to test the impairment of the CR.

Volunteers

After permission from the ethical committees of the hospital and medical faculty had been obtained, 15 healthy volunteers (10 males and 5 females with a mean age of 23·4 years) were divided at random into three groups of 5 volunteers and each group underwent one of the following three currently used methods of large bowel preparation:

Group WGI

In this group of volunteers whole gut irrigation was performed; with this method an isotonic irrigation fluid (approximately 15 l) was introduced through a masogastric tube into the stomach at a rate of 50–75 ml per min until the rectal effluent passed was clear. In this group no antibiotics were administered.

Group WGI + AB

The second group was prepared by means of whole gut irrigation. However, after the first 5 l of irrigation fluid had been introduced, 0·5 g/l metronidazole and 1 g/l neomycin were added to the remaining irrigation fluid.

Group Mann + AB

In the last group the administration of 1 l of 10 % mannitol solution as an osmotic cathartic was followed by a whole gut irrigation with approximately 8 l of irrigation fluid. The next day, approximately 24 h *after* the irrigation procedure an antibiotic prophylactic regimen was given consisting of two doses of 80 mg gentamicin i.v. and 500 mg metronidazole orally at 9.00 h and 17.00 h.

All three groups were orally challenged 1 h after the whole gut irrigation was finished with a contaminating dose containing 10^6 c.f.u. of the test strain suspended in a glass of milk. After the bowel preparation the volunteers were only allowed to drink clear fluids till the next day, when a breakfast rich in dietary fibres was served.

Microbiological methods and techniques

Faecal samples were obtained from each volunteer: one before, one at the start and one at the end of the bowel preparation. Faecal specimens were examined daily during the first 7 days after treatment, and once a week thereafter.

Liquid faecal samples were aspirated with a syringe. After expelling all air bubbles, the syringe was closed with a rubber top. Solid faecal specimens were transported under aerobic conditions. All faeces samples were immediately transported to the laboratory, placed in an anaerobic glove box and processed within 1 h.

By using a turrax mixer 1 part of faeces was thoroughly mixed with 9 parts of prereduced anaerobic sterilized diluent (saline + 0·05 % cysteine HCl). The homogenates were serially diluted tenfold (10^1–10^6) and aliquots of 0·036 ml were spread over the surface of plates using a spiral plater (Spiral system model B, Lameris, Utrecht, The Netherlands).

The aerobic bacterial microflora was presumptively identified by the use of selective and differential media. The dilutions 10^1–10^4 were spread on the following plates: 5 % sheepblood agar (Oxoid CM55); Endo-agar (BBL 11199) and MacConkey agar (Oxoid CM7). The plates were incubated for 48 h under atmospheric conditions at 37 °C before the colonies were counted. For detection and enumeration of the challenge strains 0·036 ml of the 10^1–10^4 dilutions and 0·1 ml of the undiluted sample were spread over 5 % sheepblood agar and MacConkey agar containing 100 mg/l neomycin.

For the anaerobic bacteriology a modified Wensinck medium (Wensinck & van Ruseler-Embden, 1971) was used, in which whole blood was replaced by Fildes'

extract (Oxoid SR 46). The 10^1–10^6 dilutions were spread over this medium as well as over the following selective and differential media: a Wensinck medium containing kanamycin (7·5 mg/l) and vancomycin (100 mg/l) and a Wensinck medium containing bile and aesculin agar. These plates were incubated anaerobically at 37 °C and the colonies counted after 72 h and 7 days of incubation.

Isolates were identified on the basis of morphology, biochemical reactions (Minitek® system), antibiotic resistance and analysis of volatile fatty acids by gas chromatography.

The concentrations of microorganisms were expressed as the logarithm to the base ten of colony forming units per g or ml faeces (^{10}log c.f.u./g).

Identification of β-aspartylglycine

By using a turrax mixer 1 part of faeces was thoroughly mixed with 2 parts of distilled water. The homogenized samples were centrifuged at 2000 **g** for at least 30 min. The supernatants were freeze dried and stored at −25 °C until transportation. The determination of β-aspartylglycine was performed at the biochemical laboratory of the University of Groningen as described by Welling & Groen (1978).

Analysis of volatile fatty acids

The volatile fatty acids (VFA) were measured in the faeces or rectal effluent by gas liquid chromatography (g.l.c.). The g.l.c. system consisted of a Packard Becker 433 gas chromatograph equipped with a dual column system and flame ionization detectors in conjunction with a digital processor. The glass columns (1 × 2 mm) were packed with Carbopack-C/0·3 %, Carbowax 20M/0·1 % H_3PO_4.

Faeces were diluted 1:10 in anaerobic dilution fluid and rectal effluents were used undiluted. These samples were prepared for gas chromatography by cation-exchange chromatography as described by van den Bogaard, Hazen & van Boven (1986).

Statistical evaluation

The significance of differences was assessed by the student t test.

RESULTS

Reduction of the aerobic and anaerobic faecal microflora

The reductions in the aerobic and anaerobic faecal microflora produced by the three different types of large bowel preparation are summarized in Table 1. In both groups not receiving antibiotics during the irrigation procedure (WGI and Mann+AB) a reduction of the aerobic faecal microflora of 2 logs was achieved, while in the group WGI+AB the reduction was 3 logs.

The anaerobic faecal flora showed a reduction of 4–5 logs in all three groups. Differences observed between the groups were not statistically significant.

Recolonization

The quantitative and qualitative composition of the faecal microflora in the days following the three types of bowel preparation are presented in Fig. 1. In all three groups a temporary increase in the numbers of aerobic colonic microorganisms was

Table 1. *Comparison of the reductions of the aerobic and anaerobic faecal microflora in volunteers, due to three different types of large bowel preparation*

| | Log_{10} c.f.u./g faeces (means±s.d., $n = 5$) | | |
	Before treatment	After treatment	Reduction
Aerobes			
WGI	7·18±1·42**	5·10±0·69	2·08±1·30
WGI+AB	7·40±0·58	4·40±0·58	2·99±0·74
Mann+AB	7·18±0·61	4·98±0·85	2·19±1·09
Anaerobes			
WGI	10·43±0·52	6·74±0·90	3·69±0·94
WGI+AB	10·48±0·42	6·01±0·54	4·47±0·80
Mann+AB	10·77±0·35 ·	6·03±0·35	4·74±0·31

observed as compared to the pretreatment counts. In group WGI this 'overgrowth' disappeared within 2 days after irrigation. The anaerobic flora seemed hardly affected quantitatively. The short duration, high dose antibiotic regimen in group WGI+AB had a marked and long lasting effect on the faecal flora. In particular the aerobic microflora showed 'overgrowth'. During the first 3 days the numbers of isolated aerobes increased, followed by a gradual decline. The total number of anaerobes, however, reached normal values within 3–4 days.

In the group Mann+AB an overgrowth of *E. coli* was observed which reached high levels on the first day and declined gradually to pretreatment levels over a period of 1 week. The anaerobic flora appeared to be scarcely affected and reached normal numbers within 24 h.

Colonization resistance

No neomycin resistant bacteria were cultured from any faecal sample taken before contamination with the challenge strains.

Figure 2 shows the excretion of the *E. coli* test strain in the three groups. The neomycin-resistant *E. coli* could be cultured from the faeces in high numbers after each regimen of large bowel preparation.

In group WGI the excretion of the *E. coli* test strain ($6·3 \pm 1·6$ log c.f.u./g faeces during the first 4 days) started to diminish from the fourth day on. The excretion pattern in group Mann+AB was similar to that of group WGI. After an initial 'take' during the first week ($6·5 \pm 0·9$ c.f.u./g faeces during the first 5 days) the neomycin resistant *E. coli* disappeared in the following 4 weeks.

The impact on the CR in group WGI+AB appeared to be the most profound. The neomycin-resistant *E. coli* was seen for a period of at least 6 weeks. Cultures of subsequent faecal samples showed that it took 12 weeks before the stools of all volunteers in this group were free of the neomycin-resistant *E. coli*. During the first 5 days the challenge strain was recovered in enhanced concentrations of $8·2 \pm 0·9$ ^{10}log c.f.u./g faeces. Thereafter a decline was seen to a mean baseline concentration of $4·8 \pm 1·3$ ^{10}log c.f.u./g faeces in the fifth week after challenge and a total disappearance after 12 weeks.

β-Aspartylglycine in the faeces

In group WGI, β-aspartylglycine was not detected in the faeces, while in group Mann+AB β-aspartylglycine was present in low levels in the faeces of two

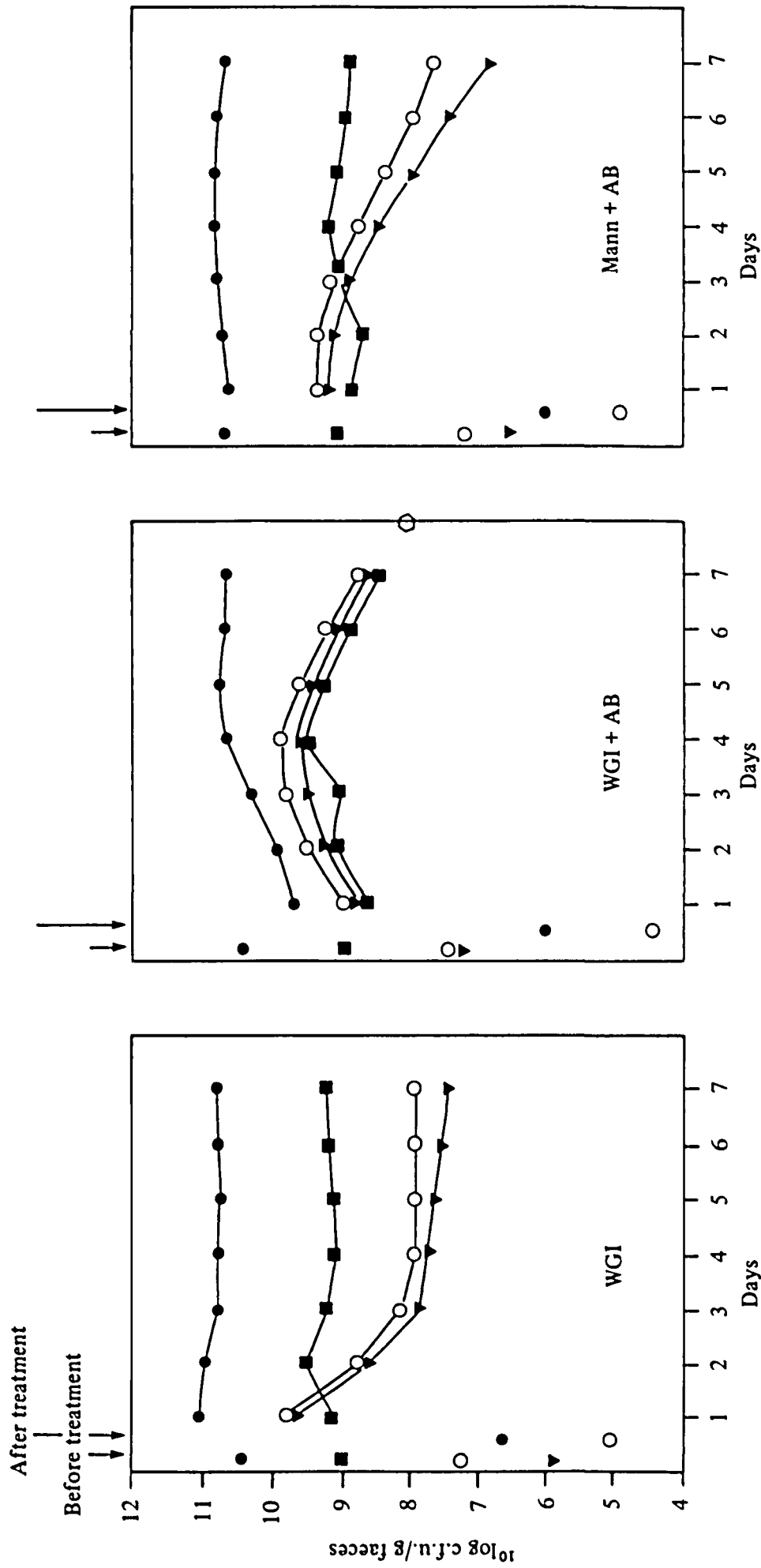Fig. 1. Recolonization of the faecal microflora after three different types of large bowel preparation in volunteers. ●, Total anaerobes; ○, total aerobes; ▼ *E. coli*; ■, *Bacteroides fragiles*.
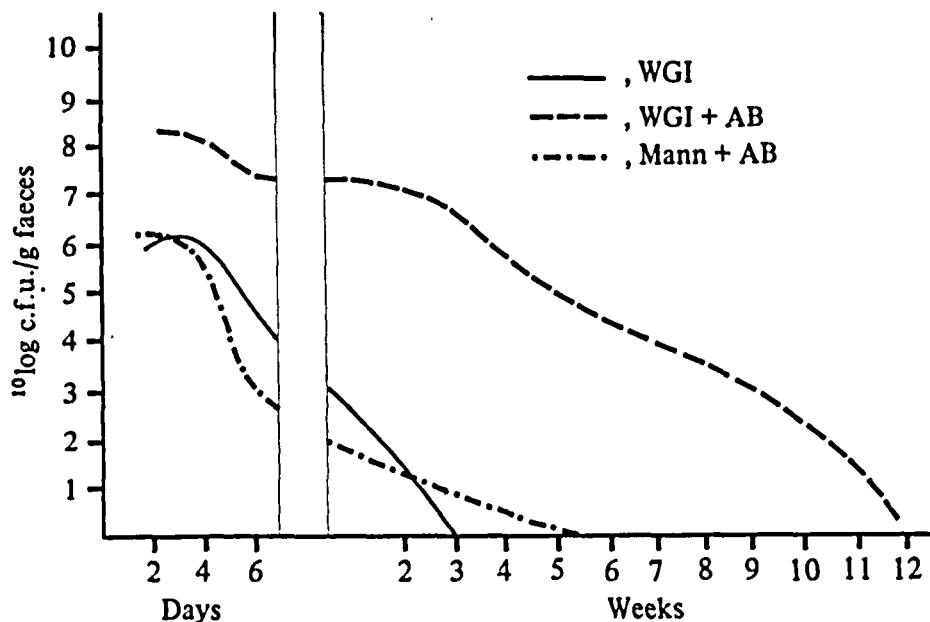
Fig. 2. Excretion of a neomycin- and gentamicin-resistant *E. coli* challenge test strain
in the faeces after three different types of large bowel preparation.

volunteers on the second day after challenge and in the faeces from one of these
also on the third and fourth day. In group WGI+AB β-aspartylglycine was
present in the faeces of two volunteers on the first day after challenge and in high
levels in the faeces of all volunteers on the second day, disappearing gradually
during the third and fourth days. On the fifth day after challenge, faecal samples
of all groups were free from β-aspartylglycine.

Volatile fatty acids in the faeces

After an inital wash-out as a consequence of the mechanical cleansing procedure
faecal samples of group Mann+AB showed 24 h after irrigation increased levels
(> 160 mmol/l) of VFA in the faeces, but they returned to normal levels
(80–150 mmol/l) within 4 days. In group WGI+AB the levels of VFA remained
low during the first few days after the bowel preparation and reached normal levels
after 5 days. In group WGI the levels of VFA returned to normal within 48 h after
irrigation.

DISCUSSION

According to the literature (Bornside & Cohn, 1969; Gorbach *et al.* 1970; Nichols
et al. 1972) mechanical cleansing procedures without the administration of
antibiotics do not have a profound impact on the colonic microflora. After
conventional mechanical cleansing procedures an immediate decrease of the
aerobic flora of 1–2 logs is found but is of relatively short duration. After 12–18 h
bacterial concentrations have returned to pretreatment levels. After whole gut
irrigation no reduction of the microflora was seen, either aerobic or anaerobic at
the time of operation (Stock *et al.* 1977; Huk *et al.* 1980; Hollender *et al.* 1980).
In group WGI the relatively low and only temporary reductions were mainly
caused by dilution. The total numbers of the anaerobic flora returned to normal
within 24 h, although, the concentration of their metabolites, the VFA, in the stools
took approximately 48 h to do so. As VFA are an indicator for the metabolic

activity of OAB in the colon and may also play a role in maintaining the equilibrium of microorganisms in the colon, this may explain the temporary overgrowth of aerobic species. All five volunteers became colonized with the challenge *E. coli* strain. This disturbance of the CR was not indicated by the presence of β-aspartylglycine in the faeces. The decrease in CR may have been caused by the removal of faecal mass and metabolic products such as VFA, but a direct effect of the lavage procedure on the mucosal wall could not be excluded. In group Mann + AB the same reduction caused by dilution of both the aerobic and anaerobic flora was observed. The total number of anaerobes returned to normal levels within 24 h. This was *before* the antibiotics were given to the volunteers in this group. However, in spite of the fact that the VFA levels were increased during the first 3 days after lavage, an overgrowth of the aerobic flora, mainly by *E. coli*, was observed in this group. It took more than a week before the total number of aerobes returned to normal. Both phenomena might have been the result of the fact that after lavage a certain amount of mannitol, a substrate for *E. coli* as well as most of the intestinal obligate anaerobes, was left in the colon. The mannitol levels in the rectal effluent collected at the end of the cleansing procedure, were still 17.5 ± 2.4 mg/l. The availability of this energy source might have enhanced the growth rate of *E. coli* as well as the metabolic activity of the obligate anaerobic flora. All five volunteers became colonized by the *E. coli* test strain and for a longer period than in group WGI. The fact that β-aspartylglycine could be detected after lavage in the faeces of two volunteers indicated also that the CR in this group seemed to have been more disturbed than in group WGI. It is possible that if, instead of mannitol, a non-fermentable osmotic cathartic, such as polyethylene glycol, had been used, this long-term overgrowth of *E. coli* and the prolonged colonization of the challenge strain could have been prevented. However, all osmotic cathartics cause a temporary mucosal oedema and no data are available on how this affects the CR. Differences between the WGI and Mann + AB groups seemed to have been mainly caused by the administration of mannitol prior to the whole gut irrigation because the most striking differences were observed before the antibiotics had been administered to the volunteers of group Mann + AB.

In group WGI + AB the considerable reduction of the anaerobic flora immediately after lavage must have been caused by the orally administered high dose regimen of antibiotics. It took 5 days before the anaerobic flora had returned to normal numbers. The aerobic flora showed an overgrowth after 24 h, and it did not start to decline until the anaerobic microflora had recovered to normal numbers.

The CR was more decreased in group WGI + AB than in both other groups. After colonization the elimination of the *E. coli* test strain took much longer in this group than in both other groups and even after 3 months two volunteers were still excreting the *E. coli* challenge strain. β-Aspartylglycine was detected in the faeces of all five volunteers. This difference must have been due to the administration of high doses of neomycine and metronidazole with the lavage fluid, resulting in high concentrations of these antibiotics in the colonic lumen. The presence of relatively high levels of neomycin and metronidazole in the colon must have disturbed the interaction between anaerobes and aerobes, resulting in a decrease of the CR. However, it has recently been shown that high oral doses of neomycin

cause pathophysiological changes in the mucosa of the colon (Longstreth & Newcomer, 1975). How far this might have influenced the decrease of the CR of the volunteers in this group can only be a matter of speculation.

Our observations lead to the conclusion that antimicrobial prophylaxis in elective colorectal surgery should consist of a high dose, short term systemic antibiotic regimen: a short high dose systemic regimen because an effective serum level of an appropriate antimicrobial drug at the time of operation decreases substantially the incidence of postoperative septic complications, and oral administration of (especially partly absorbable) antibiotics decreases the CR of the gastrointestinal tract much more profoundly. In spite of the fact that in group Mann + AB the metronidazole was given orally, this is not advised in patients, who are to undergo colorectal surgery, because of the probability of incomplete absorbtion of metronidazole in these patients. The use of osmotic cathartics, which can be fermented by the intestinal flora should be reconsidered. β-Aspartylglycine appeared to be a specific but not very sensitive indicator of lowered CR.

We did not find a clear correlation between the faecal VFA concentration and CR. However, normally VFA as well as β-aspartylglycine concentrations are measured in the faeces at the time of challenge with the test strain. This was not possible in this study, as they are rinsed away by the lavage procedure. Therefore, both methods seemed no good alternatives to challenge with a test strain to access in clinical trials the influence of preoperative preparations of the gastrointestinal tract of patients undergoing elective colorectal surgery.

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