Non-immunological defence mechanisms of the gut

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Abstract
Non-immunological defence mechanisms represent an important line of intestinal defence in addition to humoral and cellular immunity. This review summarises the evidence for the role of the non-immunological defence system. Protective factors that have been amply documented are gastric juice, intestinal motility, and intestinal flora. Components of pancreatic juice, lysozyme, and epithelial cell turnover may also be involved. Special attention is given to gastric acid, infection with Helicobacter pylori, and hypochlorhydria and their association with infectious diarrhoea. Epidemic hypochlorhydria is discussed since this increases sensitivity to intestinal infections in third world countries.

It is generally accepted that humoral and cellular immunity plays a key role in the defence mechanisms of the gut. In addition, the non-immunological defence system (Table I) represents an important and often first line of defence. It probably affords sufficient protection, at least in people living in developed countries with good hygiene. This could explain observations that infectious bacterial disorders of the gastrointestinal tract are not more frequently observed in patients with humoral and cell mediated immune deficiency. In some instances, patients totally lacking secretory immunoglobulins maintain a perfectly functioning gut.

The acidity of the stomach, the motility of the intestine, the antibacterial effects of pancreatic enzymes, the normal intestinal flora, lysozyme, and the intestinal secretions are all effective antimicrobial factors that contribute to the non-specific host defence system. Furthermore, normal epithelial cell turnover and intestinal motility act together to purge the intestinal tract of harmful micro-organisms.

Changes in the non-immunological defence factors can lead to increased susceptibility of the host to infections. For example, cholera and salmonella infections are more common in achlorhydric patients, and slowing down the intestinal motility with belladonna alkaloids prolongs symptomatic shigellosis.

Gastric acid
The roles of gastric acid in health and disease are manifold. Firstly, it facilitates digestion of dietary nutrients and augments dietary iron and calcium absorption. Secondly, it acts as a major non-immunological defence factor against exogenous pathogenic micro-organisms, and also suppresses colonisation of the proximal bowel by the oropharyngeal and faecal flora. The following sections will concentrate on the bactericidal activity of gastric acid, clinical consequences of hypochlorhydria, the effect of gastrointestinal infections on gastric acid secretion, the possible effect of Helicobacter pylori on acid secretion, the gastric acid barrier in malnutrition, and the epidemiology of hypochlorhydria.

BACTERIAL PROPERTIES OF GASTRIC JUICE
The low pH of the gastric juice (pH < 3.0) is responsible for its bactericidal properties. Exogenous bacteria introduced into the stomach when the pH is less than 3.0 are usually destroyed within 15 minutes. This bactericidal activity is retained up to pH 4.0.

There are a number of studies that show the bactericidal activity of gastric juice in vivo. For example, intragastric bacterial counts, which are normally < 10^5 organisms per ml, rise to > 10^5 organisms per ml when acidity is reduced. Neutralisation of acid by pretreatment with 2 g sodium bicarbonate reduced the infective dose of Vibrio cholerae 10,000 fold in healthy volunteers. Infections with cholera were more likely to occur in dogs if the bacteria were given together with bicarbonate. Similarly, the frequency of enteric multiplication of the vaccine strain of Shigella flexneri increases threefold with sodium bicarbonate neutralisation of gastric juice. With Campylobacter jejuni, a higher incidence of illness was observed in a group of healthy volunteers when the organisms were ingested with sodium bicarbonate. The use of H2 receptor antagonists and proton pump inhibitors (omeprazole) leads to increased intragastric bacterial counts. The small bowel colonisation after cimetidine therapy is also considered to be related to reduced gastric acidity due to the drug. Gastric alkaliisation of critically ill patients with Mylanta II was found to be associated with intragastric bacterial and fungal colonisation.

CLINICAL CONSEQUENCES OF HYPOCHLORHYDRIA
One possible serious consequence of achlorhydria or hypochlorhydria was suggested by the eminent British gastroenterologist Sir Arthur Hurst in 1934, when he mentioned the possibility that 'bacillary and amoebic dysentery
occur much more commonly in people with achlorhydria and hypochlorhydria. He further speculated that the same might be true for typhoid fever and cholerā. "This observation has been confirmed by a series of studies showing hypochlorhydria as a predisposing factor for the growth of the organisms responsible for typhoid and non-typhoid salmonellosis, bacillary dysentery, giardiasis, and cholerā and of other infectious agents responsible for gastroenteritis. Another report suggests that Clostridium difficile infection (pseudomembranous colitis) may also be associated with hypochlorhydria."

It has also been found that reduction in acid secretion in atrophic gastritis allows bacterial colonisation of the stomach, which is most extreme in achlorhydria patients with pernicious anaemia. The bacterial flora in the stomach of patients with pernicious anaemia consist of enterobacteriaceae, enterococci, streptococci, staphylococci, lactobacilli, and less frequently obligatory anaerobes, such as clostridia and bacteroides. An association of hypochlorhydria after a course of omeprazole therapy and recurrent enteritis has recently been reported. Gastric resection and/or vagotomy frequently leads to the colonisation of the stomach and small intestine by faecal types of bacteria. During epidemics of cholerā in Israel and Italy, the attack rates were significantly higher in subjects with hypochlorhydria due to surgical resection of the stomach and chronic ingestion of antacids.

It is widely, though not unanimously, assumed that there is an increased risk of gastric cancer and premalignant changes in pernicious anaemia and after partial gastrectomy. The increased risk of cancer is probably related to overgrowth of nitrate reducing bacteria producing nitrates and N-nitrosamine compounds, which have already been shown to be carcinogenic. A number of recent studies also demonstrated that reduction in gastric acid output caused by different types of drugs, including H$_2$ receptor antagonists, usually results in significant bacterial overgrowth and intragastric biochemical changes, including the production of nitrite and nitrosamine compounds.

**EFFECT OF GASTROINTESTINAL INFECTIONS ON GASTRIC ACID SECRETION**

It is known that bacterial, viral, and parasitic infections suppress gastric acid production in man and in animals. In fact, bacterial infections, including salmonellosis, tuberculosis, and bronchopneumonia, are associated with suppression of histamine stimulated acid secretion. Normochlorhydria was restored upon eradication of infection in most of the cases. Recently, H pylori has been associated with hypochlorhydria and gastritis. This organism will be discussed in the next section in greater detail. There is little information on the effect of parasitic infections on gastric acid output. In Brazil, a decreased basal and stimulated acid output has been observed in patients with Chagas disease. Decreased basal and stimulated acid output was also noted in patients with schistosomiasis or giardia infections. Impaired gastric acid secretion was seen in people infected with ancylostoma or Diphyllolothrium latum.

The mechanism of the suppression of acid secretion in infection is not well understood. It has frequently been thought to be related to direct morphological effects of the infection on the gastric mucosa. On the other hand, suppression of acid secretion was found to be more marked when there was fever, which indicates that fever rather than infection per se may be responsible for suppression. A transient hypochlorhydria has been shown in people kept in a heated cabinet with a rise of body temperature from 37° to 39°C. The hypochlorhydria observed in infection might be modulated through the stress induced release of endogenous prostaglandins (PGE$_2$), which has been found to suppress acid secretion.

**H PYLORI AND GASTRIC ACID SECRETION**

There is increasing interest in the role of H pylori in the pathogenesis of peptic ulcer and gastritis. H pylori is found in 90–100% of patients with duodenal ulcers, 70% with gastric ulcers, and 6–31% of children complaining of vomiting and upper abdominal pain. The role of H pylori in these disorders is indicated by the observation that its eradication in ulcer disease reduces the chance of subsequent relapse. Hunt et al recently reported the effect of H pylori on acid production, using 14C-aminopyrin uptake by isolated guinea pig parietal cells. H pylori caused a reduction in basal acid secretion of about 80%, and histamine stimulated acid secretion was reduced to 50% within 15 minutes of inoculation with H pylori. There are also reports describing H pylori associated gastric hypochlorhydria in man. Although a striking association between the presence of H pylori and hypochlorhydria exists, the question of cause and effect is still obscure. In 1983, Marshall, serving as his own volunteer, rendered himself temporarily hypochlorhydric with cimetidine and then swallowed cells of H pylori cultured from a patient with gastritis. Although gastritis and H pylori were detected in his follow up tissue specimens, a definite association of the organism with hypochlorhydria could not be established, as gastric acid secretion before and after challenge was not monitored in that experiment. The possible association of H pylori with the high prevalence of hypochlorhydria in poor countries is further alluded to. Because of poor environmental conditions in developing countries, people are very often exposed to enteric pathogens such as V cholerae, Shigella, and Escherichia coli. Low gastric acid production has been found to be associated with a high risk of cholera and E coli diarrhoea in those communities. It is not unlikely that H pylori associated hypochlorhydria leads to increased susceptibility to enteric pathogens. A longitudinal prospective study is thus warranted to investigate the association of H pylori with the incidence of hypochlorhydria and of infectious diarrhoea.
GASTRIC ACID BARRIER IN MALNUTRITION
The gastric acid barrier was found to be impaired in malnourished children and in protein depleted experimental animals.70-72 The effect of malnutrition on gastric acidity and gastric bacterial colonisation was studied in 35 severely malnourished Bangladeshi children by Gilman et al.73 Gastric acid output, both basal and after betazole stimulation, was found to be significantly lower in malnourished children than in the better nourished counterparts. Hypochlorhydria was accompanied with Gram negative bacterial colonisation in 81% of the malnourished children, whereas none of 20 better nourished children had Gram negative colonisation of their gastric juice. After three weeks of nutritional rehabilitation, gastric acid secretion still remained depressed in the malnourished children. The factors responsible for the decreased acid output in those children remain unclear. Although anaemia has been postulated to be associated with reduced gastric acid output,74 the study in Bangladeshi children did not show any correlation between acid output and haematocrit. However, the low gastric acid output may be related to chronic gastritis. In other studies, evidence for this was found in biopsy specimens from the gastric fundus of malnourished children.70-72 No data are available on parietal cell numbers and their changes upon rehabilitation in malnourished children. The prolonged depression in acid concentration observed in the study with Bangladeshi children suggests that nutritional support for a period longer than three weeks is required to ensure full recovery of acid secretion.

EPIDEMIOLOGY OF HYPOCHLORHYDRIA
The prevalence of hypochlorhydria varies widely, but is high in third world countries. A reduced acid output has been shown in most malnourished children in Indonesia,76 Nigeria,77 South Africa,78 and Bangladesh.79 Relative hypochlorhydria has been demonstrated to occur in the first week of life and in people over the age of 60.76 This might explain the increased incidence of small bowel colonisation in infants7 and the increased risk for salmonella infection in the elderly.76-79

Nalin et al76 reported hypochlorhydria in 41% of Bangladeshi patients convalescing from cholera. A high gastric pH (>4-0) has also been observed in people of countries in Africa, South and Central America, and possibly southern and eastern Europe.78 In contrast, an acidic and sterile stomach is normal in the United States and north western Europe. These observations may suggest nutritional factors, environmental contamination, and a possible association with an infectious agent as the cause of hypochlorhydria in third world countries. Several reports have described spontaneous outbreaks of hypochlorhydria, sometimes resulting in complete anacidity, in previously healthy people with normal gastric acid secretion76 or patients with Zollinger-Ellison syndrome.76-78 Two reports previously described epidemic outbreaks of hypochlorhydria associated with gastritis, where recent evidence suggests an association with

H pylori. Ramsey et al76 reported an outbreak of hypochlorhydria in 17/35 volunteers participating in a study of food stimulated gastric acid secretion. Gledhill et al76 observed four cases of hypochlorhydria in five previously healthy volunteers participating in a study involving intragastric pH measurements. The clustering of cases in both the studies suggests a possible infective cause. Both of the studies were conducted before 1983, when Marshall and Warren76 had not yet described the curved bacillus H pylori (previously called C pyloridis). A retrospective examination of the gastric mucosal biopsies obtained from the volunteers participating in the study conducted by Gledhill et al, however, did detect H pylori. A rising antibody titre against H pylori was also demonstrated in the convalescent gastritis sera obtained from the subjects participating in the investigation by Ramsey and his group.76 Both observations suggest the association of H pylori with hypochlorhydria.

Intestinal factors in the defence mechanisms of the gut

EPITHELIAL CELL TURNOVER
The mammalian intestine is covered by a universal epithelium that can either absorb or secrete. An increase in the rate of epithelial surface renewal may occur as a result of bacterial, viral, or parasitic infections. The luminal loss of epithelial cells, connective tissue stroma, and lymphocytes with exuded fluid tends to dilute, dissolve, or wash away the various infective agents adhering to the mucosa and between the cells. This loss of intestinal surface cells acts together with interdigestive motor activity (MMC) to cleanse the intestine and thus prevent stasis and bacterial colonisation.

INTESTINAL MOTILITY
One important host mechanism limiting the proliferation of organisms in the small intestine is gut motility. In humans, the well defined phase III activity of the interdigestive motor complex (MMC) occurs every 84–112 minutes and migrates down the upper intestinal tract with a speed of about 6–8 cm/minute. The caudate moving band of intense contraction during phase III has been called the 'intestinal housekeeper.'76-78 The contraction moves digestive contents rapidly down the small intestine. At the port of entry, gastric acid destroys most of the organisms from the outside environment. In the upper intestine this 'housekeeper' mechanism gets rid of microorganisms that managed to escape the gastric acid barrier, and thus prevents stagnation and bacterial overgrowth. The other important role of this movement is to maintain an appropriate distribution of the endogenous enteric microflora and to prevent migration of organisms from the colon.76-78

Altered intestinal motility might have adverse effects on the normal ecology of the intestine. In conditions associated with increased motility, a reduction of the normal gut flora could occur. A
considerable reduction of the anaerobic bacterial flora has indeed been observed in some cholera patients. This reduction, however, is likely to reflect not only a specific effect on gut motility but much more the high flow rate of the gut contents in this disease condition. On the other hand, decreased motility might lead to stasis and create an ideal culture medium for the development of bacterial overgrowth in the small intestine. The presence of such overgrowth in diabetic patients could in fact be related to delayed intestinal motility. On the other hand, motility disturbances have been reported in the small bowel overgrowth syndrome, which may be the result rather than the cause of increased bacterial growth. The mechanism is still not clear. However, humoral factors may play a role.

No thorough studies have yet been performed on the effect of enteric infection on intestinal motility in man. Studies in animal models have provided some information, for example that bacterial enterotoxins may produce abnormal patterns of smooth muscle motor activity. In rabbits, cholera toxin has been found to produce an abnormal electrical pattern called the migrating action potential (MAPC), which contains bursts of intense activity. Experiments on invasive bacteria in the same animal model showed a different pattern of electrical activity characterised by repetitive bursts of action potentials. The authors suggested that MAPC is characteristic of toxigenic bacteria, and repetitive bursts of invasive bacteria. The finding suggests that the disturbed motility observed in infectious diarrhoea is an important factor in its pathogenesis.

PANCREATIC JUICE AND BILE
Pancreatic exocrine insufficiency has been documented in protein calorie malnutrition in animals and in man. Patients with pancreatic insufficiency were reported to have more severe and prolonged episodes of acute diarrhoeal illness.

The basis of the possible anti-infective role of the exocrine pancreas has been elucidated in a series of studies in animals by Gyr and Felsenfeld. In protein depleted animals with exocrine pancreatic insufficiency, a challenge with V cholerae produced a longer lasting diarrhoea and more prolonged excretion of the organism than in pair-fed controls. Oral administration of pancreatic extract to the protein-depleted animals was found to modify the course of the diarrhoea by reducing the severity and duration. These studies thus suggest an involvement of exocrine pancreatic secretion in the local defence against cholera under conditions of protein deficiency.

In vitro experiments by the same group showed a bactericidal activity of pancreatic lipase, which damaged the cell wall and the cytoplasm of V cholerae. Earlier, Dutta and Oza showed that extracts of the intestinal fluid of adult rabbits degraded cholera toxins. Antibacterial activity of the canine pancreatic fluid against E Coli, Shigella spp, Salmonella spp, and Klebsiella pneumoniae has been observed by Rubinstein et al (Table II). Human duodenopancreatic secretions were also found to contain a factor that enhances the bactericidal activity of cloquinol and certain other drugs, including chloroquinodole, chloramphenicol, and trimethoprim. The observation has recently been confirmed by Bassi et al, who also demonstrated bacteriostatic properties of pure pancreatic juice (PPJ). In his study the minimum inhibitory concentration (MIC) of the antibiotic mezlocillin against E coli was found to be four times less when PPJ was added, suggesting the ability of PPJ to enhance the activity of this antibiotic. The factors or substances present in PPJ associated with the antibacterial properties are not yet fully elucidated. Mett’s investigations suggest a heat stable substance with a molecular weight of less than 8000 Da.

The association of exocrine pancreatic insufficiency and bacterial overgrowth in the proximal bowel has been observed in children with protein energy malnutrition. The effect of oral pancreatic enzymes on the intestinal flora has also been studied by Gyr et al in protein deficient monkeys. The study demonstrated that pancreatic extracts hastened the restoration of the normal intestinal flora in protein depleted monkeys to their pre-dietary state. The mechanism of the restoration of intestinal ecology by the pancreatic extract is still unknown.

The biological effects of bile salts on infection have not been thoroughly investigated. Although in vitro studies demonstrated that deconjugated bile acids exert a potential inhibitory effect on micro-organisms, in vivo studies in support of this observation are lacking. IgA in bile is considered to be involved in eliminating harmful antigens from the circulation by the disposal of IgA antigen complexes into the gut lumen. But further work is needed to elucidate the role of bile salts in local defence against enteric pathogens.

LYSOZYME
Lysozyme is an enzyme which has been identified in the digestive system in the ductal cells of

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<th>Mean no of bacteria, log_10 ml at different incubation times:</th>
<th>0 h</th>
<th>2 h</th>
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<tr>
<td>Escherichia coli (24 strains)</td>
<td>PF</td>
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<tr>
<td>Shigella sp</td>
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<td>Salmonella enteritidis</td>
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SD in all instances ≤6-4×10² and therefore not included in table. PF=pancreatic fluid; C=control.

*Adapted from Rubinstein et al 1985.
**Difference of more than 2 log unit is considered significant.
salivary glands, in the absorbing epithelium of the intestinal tract, and in pancreatic fluid. The enzyme has been reported to increase in activity in a variety of infections. It is well known to lyse bacteria, and might therefore play a role in mucosal defence against invading organisms. However, further work is required to investigate its mechanism of action and its influence on specific organisms responsible for gastrointestinal infection.

**INTESTINAL FLORA**

**Luminal flora**

The intestinal flora is an enormously complex ecosystem and consists of both aerobic and anaerobic micro-organisms. Bacteriological studies indicate that the small intestinal flora is relatively sparse, containing less than 10^5 cfu per ml, while in the colon an individual harbours more than 400 species. The majority of them are anaerobic – that is, they do not grow in the presence of oxygen. Motility, immunological factors, and intestinal secretions are all considered to play a role in maintaining the normal gut microflora. An augmentation of the intestinal microflora was observed in vervet monkeys after pancreatic duct ligature. On the other hand, as discussed above, pancreatic extracts helped to restore the normal flora in protein-deficient monkeys.

The role of the normal intestinal flora as an extremely important host defence mechanism is now beginning to be appreciated. In normal situations, an individual's intestinal flora is highly effective in resisting colonisation by potentially pathogenic invaders. The indigenous flora produces a variety of antimicrobial substances, including colicins and short chain fatty acids, which are potentially bactericidal and bacteriostatic and are therefore considered to inhibit the growth of invading organisms.

There is considerable evidence for an increased susceptibility to infection in patients with reduced bacterial flora.** Diarrhoea associated with the use of antibiotics is common, and is likely to be due to alterations of the normal indigenous flora. In experimental mice, the infective dose of *Salmonella typhimurium* was found to be reduced 10000 fold when the microflora was eradicated by administration of streptomycin. The protective role of the intestinal flora in humans is also suggested by the increased frequency of salmonella infections among Swedish tourists who used prophylactic antibiotics compared with those who took no prophylaxis.

The endogenous gut flora also play an important role in maintaining the histological structure of the gut. In fact, it has been demonstrated in the germ-free animal that the 'normal' histology of the gut mucosa is determined by the presence of the bacterial flora. Without the resident flora the intestinal wall has been found to be thinner and to contain only few lymphocytes.

**FLORA IN THE EPITHELIUM**

Besides the organisms distributed along the intestinal lumen, a unique population of bacteria exists in the epithelium, consisting of organisms quite different from those colonising the lumen. Electron microscopic studies show that they are firmly adherent to the mucus layer overlying the microvilli of the crypts of the distal small bowel. This flora includes mainly *Lactobacillus*, *Bacteroides* and *Clostridium* spp, but present knowledge is incomplete. The role of the epithelial flora in defense is also not yet clear, but it seems possible that it interferes with the colonisation and multiplication of pathogenic bacteria by competing for nutrients.

**Conclusion**

This review has summarised evidence for the importance of the non-immunological defence system of the gut and discussed the various mechanisms that may be involved. The important defence factors whose efficacy has been amply documented are gastric acidity, intestinal motility, and the intestinal flora. Components of pancreatic juice, lysozyme, and epithelial cell turnover are also likely to be involved, but definite proof is still lacking. Special attention has been paid to gastric acid as a defence factor, the occurrence of hypochlorhydria, and its possible association with *H pylori*. Epidemic hypochlorhydria may be one of the factors that increases sensitivity to infectious diarrhea in third world countries. Further investigations are necessary to elucidate the efficacy and relative importance of the non-immunological defence system.

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Iatrogenic gastritis causes with acid function.

In pernicious anaemia, the role of Campylobacter pylori infection and gastroduodenal diseases.

Endotoxaemia: Campylobacter jejuni infection and prostacyclin analogue on gastric emptying.

I. Development of pernicious anaemia.

Clean and efficient.
Non-immunological defence mechanisms of the gut


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