Progress report

The demonstration and function of antibodies in the gastrointestinal tract

The presence of antibody activity in intestinal secretions has been known for 50 years since Davies\(^1\) demonstrated specific antibodies in the faeces of patients suffering from dysentery. It is now well documented that the gut of germ-free animals\(^2\) and the human neonate\(^3\) contains very few plasma cells, and after bacterial contamination the normal complement of plasma cells appears rapidly. These studies have been reviewed recently\(^2\). This germ-free condition is associated with absence of immunoglobulin A in the intestinal secretions and the immunoglobulin appears as a response to colonization. The importance of IgA in secretions has been emphasized in reviews by Tomasi and Bienenstock\(^4\), by Tomasi and DeCoteau\(^5\), and by Heremans\(^6\). Work on salivary IgA has been extensively reviewed by Brandtzaeg, Fjellanger, and Gjeruldsen\(^7\).

It is the purpose of this review to present recent information on gastrointestinal antibody production, particularly in the stomach and small intestine, with emphasis on the probable functions of the antibody. There are many technical difficulties in the detection and measurement of antibodies in gastrointestinal secretions. If the gastroenterologist is to maintain a critical understanding of the many relevant papers in this immunological field, then he must appreciate some of the technical problems which are reviewed here. Furthermore, antibody responses within the bowel are important in the study of oral vaccination and some of the relevant experimental work is discussed. The immunological changes in inflammatory bowel disease have been reviewed by Kraft and Kirsner\(^8\), and the bowel abnormalities in IgA deficiency are discussed by Bull and Tomasi\(^9\). These are not dealt with in the present review.

Origin of Intestinal Immunoglobulin

Immunoglobulin may reach the lumen of the gut either by transudation from serum, or as a result of local production and release. There is evidence from studies in animals\(^10,11\) and man\(^12\) that exudation into the gut is a most important route in the catabolism of serum globulins. Currently, however, interest is focused mainly on the local production of immunoglobulin in the tissues of the gut itself. Tissue culture studies show that radioactively labelled amino acids are incorporated into IgG, A, and M by tissues taken from various levels of the gastrointestinal tract\(^13,14\). Immunofluorescent studies, using fluorescein-labelled antisera specific for the various immunoglobulin classes, have shown the presence of immunoglobulin-containing cells in all gut tissues examined\(^15-20\). The immunoglobulin-containing cells are
lymphoid cells, and when examined by conventional microscopy mostly have the appearance of mature plasma cells, although cells which appear to be lymphocytes are also seen to contain immunoglobulin. Immunofluorescence also shows the presence of interstitial immunoglobulin (mainly IgG), and IgA staining is seen lining the basement membrane, between the epithelial cells and, with certain antisera, in the apical regions of the epithelial cells.

In normal gut tissues, the great majority of fluorescein-staining cells contain IgA. IgG is present in small numbers of cells, and IgM is intermediate. In addition, cells containing IgD and IgE have been described.

The importance of local production of immunoglobulin is emphasized by studies on the transport of immunoglobulins into secretions. There is evidence that very small amounts of IgA may be transported into the saliva of hypogammaglobulinaemic patients following infusion of fresh plasma, but the bulk of evidence from infusion studies indicates that although a small amount of IgA may be transferred from serum, this makes a very small contribution to the total amount of IgA in saliva. Although South, Cooper, Wollheim, Hong, and Good did not demonstrate the transfer of infused IgG and IgM into saliva in hypogammaglobulinaemias, there is evidence that IgM may be preferentially transported into saliva, while IgG may appear in saliva in amounts related to the level in serum.

Further evidence for the local production of immunoglobulin in the gut comes from studies discussed below which show dissociation of the antibody response in secretions from that in the serum, in terms of specificity, time course, and immunoglobulin class.

Special Characteristics of Intestinal Immunoglobulins

STRUCTURE

There has been particular interest in secreted immunoglobulins since the observations that the amounts of IgA relative to the other immunoglobulins is much greater than in serum, and this IgA in secretions was in a different form to the IgA in serum. IgA in human secretions has been shown to have a higher molecular weight than serum IgA, and to possess an additional component, known as the secretory piece. This is a glycoprotein, molecular weight about 76000, which appears to be joined to two molecules of IgA by covalent and non-covalent forces, giving a molecule with an electron microscopic appearance of a double 'Y'. The secretory piece can be dissociated from the IgA molecules by reduction and alkylation, and the resulting IgA molecules closely resemble serum IgA, but are mainly of a form of the IgA subclass IgA₂, which is unusual among immunoglobulins in having no disulphide bonds linking the heavy and light chains. The significance of this is not yet known.

Antisera can be raised against the intact secretory IgA molecule, and these antisera detect the presence of an additional determinant, absent from serum IgA, and due to the presence of the secretory piece. Antisera against the secretory piece can also be prepared by immunizing animals with free secretory piece, and by suitable absorptions a serum can be prepared which will react only with free secretory piece, and not with secretory piece bound to IgA. Using this antiserum it has been shown that normal secretions (saliva and colostrum) contain significant amounts of free secretory piece. Secretions deficient in IgA also contain free secretory piece. Using fluorescent
labelled antisera to the secretory piece, fluorescence is localized to surface epithelial cells, goblet cells, and duct-lining epithelial cells of glands. Since the secretory piece does not appear to exist in the lamina propria of the gut, where IgA-containing lymphoid cells are seen, it has been suggested that IgA, synthesized by these cells in the lamina propria, moves across the basement membrane and complexes with the secretory piece before entering the gut lumen, possibly by transport through the epithelial cells. However, it is extremely difficult to produce antisera specific for secretory piece which do not cross-react with lactoferrin. It is likely that an IgA dimer is formed before the secretory piece is incorporated, and that the addition of the secretory piece stabilizes the structure of the dimer.

**THE EFFECTS OF PEPsin AND TRYPSIN ON IMMUNOGLOBULINS**

The presence of the secretory piece appears to alter the susceptibility of the IgA molecule to enzymatic digestion, and so may be important in preserving the function of IgA antibody in the gut. Secretory IgA is resistant to digestion by trypsin. When it is dissociated from secretory piece, the free IgA loses this resistance. The effects of digestion can be assessed by gel filtration, which shows that in the case of secretory IgA there is little breakdown into low molecular weight fragments. It can also be assessed by studying antigen-binding by methods involving radiolabelled antigen. These methods show that the antigen-binding capacity of IgA and IgG is not greatly affected by tryptic digestion. However, in the case of IgG, the antigen is bound by a fragment of the immunoglobulin molecule, whereas the secretory IgA antibody appears to remain intact. Further evidence for the trypsin resistance of secretory IgA comes from observations that the ability of secretory IgA to agglutinate bacteria is not affected by tryptic digestion, and since it has been shown that polymer IgA is a considerably more efficient haemagglutinating antibody than monomer IgA, it seems likely that secretory IgA remains in its dimeric form following trypsin digestion, otherwise its agglutinating ability would be reduced.

Secretory IgA also has a greater resistance to peptic digestion than do IgA myeloma proteins despite an earlier report which indicated otherwise. It is interesting that when IgA myeloma proteins were studied, the IgA subclass which possesses disulphide bonds between heavy and light chains was shown to be considerably more resistant to peptic digestion than the IgA_2_ proteins which do not have these disulphide bonds. Since, as already mentioned, the great majority of secretory IgA is of IgA_2_ type, there is a strong indication that secretory piece provides protection against peptic digestion. It should be pointed out that in these studies peptic digestion was carried out at pH values around 4. When secretory IgA is exposed to pepsin at pH 2 or to acid gastric juice, degradation is very rapid indeed, so it seems unlikely that even secretory IgA could survive in the lumen of the normal, acid-secreting stomach.

**CONTRIBUTION OF THE GASTROINTESTINAL TRACT TO SERUM IMMUNOGLOBULINS**

The immunoglobulin-producing cells of the gut probably make a significant contribution to the serum immunoglobulins. IgA synthesized by isolated perfused segments of human gut appears in part in the secretions, and in part
in the venous effluent perfusion fluid\textsuperscript{42}. Studies in the dog show that IgA appears in the efferent lymph from the intestine\textsuperscript{43}. Furthermore, secretory IgA can be detected in the serum of normal individuals, and in higher concentrations in disease states, particularly those in which mucosal abnormalities are present, such as coeliac disease and ulcerative colitis, and can be detected in the serum of normal individuals, and in IgA

The secretory IgA in serum 

juice\textsuperscript{49}. Achlorhydric exocrine IgA

Although the levels in the serum of normal individuals are low, it has been demonstrated that the precipitation of immunoglobulin by acid-pepsin\textsuperscript{41} which probably explains reports which indicate that there is no immunoglobulin in acid gastric juice\textsuperscript{49}. Achlorhydric gastric juice also poses problems, because although there may be high concentrations of immunoglobulin, non-immunoglobulin precipitates may interfere with quantitative measurement\textsuperscript{41}. Small intestinal aspirates have been fairly extensively studied both quantitatively and qualitatively\textsuperscript{42,50–53}. Although secretory IgA is resistant to digestion by trypsin, this does not apply to IgG and IgM and in any report it should be demonstrated that the sampling and storage methods used provide adequate inhibition of proteolytic activity. A further problem is the formation of protein precipitates, unrelated to specific antiserum-immunoglobulin precipitates\textsuperscript{50,54}. These are mainly due to the reaction of trypsin or chymotrypsin with a non-immunoglobulin component of certain animal sera, and can be avoided either by choice of suitable antisera or preferably by using the globulin fraction of the antiserum rather than the whole serum\textsuperscript{55,56}

Measurement of IgA levels in stool requires an extensive preparation of the stool to obtain a clear globulin-containing fraction and the preparation losses may be considerable so that quantitative levels quoted can only be considered to be estimates.

**Problems with Standards**

A further difficulty in the assessment of IgA levels in the gut is that the proportion of monomer, dimer, and higher molecular weight forms is not known. The standards used in quantitating IgA in secretions should contain IgA in the same form as in secretions or results will be misleading (the use of a 7S serum IgA standard to quantitate 11S secretory IgA will lead to an underestimate of approximately threefold\textsuperscript{7}). Estimates have been made of the molecular weight distribution of IgA in gut secretions\textsuperscript{42,57,58} and these have
been taken into account when levels have been measured, but even with these refinements the results can only be considered as estimates.

PROBLEMS WITH ANTISERA
As Brandtzaeg has shown very fully, antiserum to IgA and secretory piece can be produced in a number of ways using different forms of immunogen, and thus producing antiserum with markedly different specificities. In addition to determinants specific for the IgA heavy chain, he has demonstrated a determinant associated with polymers of IgA but absent from monomers, a determinant unique to free secretory piece, and a determinant specific for bound secretory piece but concealed on free secretory piece. He has also shown that the use of different antiserum may produce significantly different results when quantitating the same sample. It is therefore important that the specificity and method of preparation of antiserum should be described when results are quoted.

PROBLEMS WHEN SAMPLES ARE CONCENTRATED
Because concentrations of IgA may be low, especially in saliva, concentration of samples may be necessary. Significant protein loss can occur with some of the procedures used and it is obviously desirable to use sensitive methods which can be used on unconcentrated samples. The method of radioactive single radial immunodiffusion should be particularly suitable for use on gut secretions.

Antibody Activity in the Gastrointestinal Tract

METHODS OF DEMONSTRATING ANTIBODY ACTIVITY
Early studies of antibody in stool produced inconsistent results and the reasons for this have been discussed extensively. The antibody activity in a specimen may show marked variations according to how it is assessed. The most reliable results are produced by methods which directly measure antigen-antibody combination such as the radioimmunoassay system described by Farr or its modifications. Freter has demonstrated that antibody to Vibrio cholera can be consistently demonstrated in the stool of volunteers given oral vaccine when the Farr test is used, but when tested by agglutination results were inconsistent and titres low. Among the reasons suggested for this discrepancy are partial degradation of antibody by proteolytic enzyme; the presence of agglutination inhibitors in stool extracts; and formation of non-specific precipitates in the extracts.

Other direct methods of detecting antigen-antibody combination include immunofluorescence and radioimmunoelectrophoresis or radioimmunoimmunodiffusion. Immunofluorescence has been used to detect antibody in saliva and in colonic washings with apparently satisfactory results, but other workers have found the method to be unsatisfactory with intestinal secretions, and our own observations confirm these problems. The technical difficulty of using this technique with unfractonated gut secretions demands extensive controls. Radioimmunodiffusion and radioimmunoimmunodiffusion present problems due to the binding of labelled antigen to non-specific precipitates, and therefore it is necessary to demonstrate that precipitates which appear to bind antigen do in fact contain immunoglobulin and that non-specific binding does not occur. Although tests depending on the effects
of antigen-antibody combination may pose technical problems, they should not necessarily be rejected, as such tests may provide information related to possible physiological function of antibody. A recent review by Minden and Farr emphasizes the difference in results which may be obtained using different methods to study the antibody activity in any one sample.

**THE RANGE OF ANTIBODY ACTIVITY**

Tomasi, Tan, Solomon, and Prendergast demonstrated antibody activity against blood group substances (isohaemagglutinins) in human parotid saliva and colostrum, and showed that these were of IgA class. The development of serum isohaemagglutinins is thought to be due to exposure to antigenically cross-reacting bacteria and viruses and presumably the same is true for exocrine isohaemagglutinins. Certainly gastrointestinal secretions have antibody activity to a wide range of commensal organisms: these are frequently termed 'natural' antibodies to distinguish them from antibodies which follow infection or immunization, though this distinction is somewhat meaningless. Antibodies directed against bacteria can be found in saliva, achlorhydric gastric juice, and small intestinal secretions.

There is evidence from animal work which suggests that antibodies to parasites are present in intestinal secretions. In man there is little evidence for the existence of secretory antibody to intestinal parasites, although it has been observed that patients with panhypogammaglobulinaemia have a high incidence of infection with *Giardia lamblia.*

Antibody activity in intestinal secretions is also present to viruses as well as dietary constituents. The development of such natural antibodies is possibly related to the antigenic stimulus provided by the normal microbial flora and dietary proteins.

**THE DISSOCIATION OF GASTROINTESTINAL AND SERUM ANTIBODY RESPONSES**

The clinical studies referred to below show clearly that the secreted antibody response differs from the serum response, and studies of the immune response to ingested antigens in animals confirm this strikingly different pattern. When ferritin is fed to rats in drinking water, large numbers of immunocytes in the gut lamina propria can be shown to produce antibody to ferritin. These cells are all of the IgA type. In the mesenteric nodes and spleen, a few antiferritin-producing cells can be seen, and again these are mainly of IgA type. By contrast, when ferritin is given parenterally, the great majority of antiferritin cells are in the lymph nodes and spleen. These cells are of IgM type initially but after further immunization they are mainly of IgG type. Similar findings have been reported for the response to ingested red cells and albumin. These studies provide a clear indication that in the species studied the antibody response to ingested antigen consists of IgA antibody which is produced mainly by cells in the gut wall.

Burrows, Elliot, and Havens and Burrows and Havens showed that experimental cholera in the guinea pig is followed by a rise in antibody titre in both serum and faeces. Coproantibody appeared earlier, reached peak titres sooner, and declined before serum antibody. Resistance to infection appeared to correlate better with faecal than serum antibody.

Eddie, Schulkind, and Robbins studied the effect of various immunization schedules on rabbits using *Salmonella typhimurium* and studying antibody
responses in serum, intestinal fluid, and colostrum. Direct contact of the secretory tissue with living bacteria gave rise to IgG and IgA antibodies in external secretions. This did not occur when dead organisms were used. Parenteral inoculation of living or dead organisms gave rise to high serum antibody titres but no secretory antibody. This immune response by the intestine appears to be localized to the site exposed to antigen and the intestine does not behave as a single unit. Ogra and Karzon\textsuperscript{93} instilled polio vaccine into the distal limb of a double-barrelled colostomy. Secretory IgA antibody appeared in this segment, but only low titres were found in the proximal colon, and no antibody activity whatsoever in the nasopharynx. Following oral challenge, the virus was able to colonize the nasopharynx but not the colon.

When considering the gut humoral immune response it is important to distinguish between studies which demonstrate solely local effects of local stimulation, and work in which, because of the absorption of antigen, a systemic immune response may contribute to what is observed locally. Orally administered antigen can give rise to serum antibody as well as antibody in gut secretions\textsuperscript{68,92,94,95}. This could be due to absorption of antigen, or proliferation of organisms, allowing antigen to reach systemic antibody-producing sites. Alternatively, immunocompetent cells from the local site could migrate to sites of systemic antibody production.

Following parenteral administration of antigen antibody may be detected in gut secretions\textsuperscript{68,92,97}, although this is not confirmed in all studies\textsuperscript{92,95}; however, there is no doubt that parenteral immunization against infections localized to the gut does confer protection\textsuperscript{98,99}. The gut antibody response to parenteral immunization could be due to the effect of parenterally administered antigen reaching gastrointestinal sites of antibody production. This is supported by the observations of Koshland\textsuperscript{100}, suggesting that the use of adjuvants localized parenterally administered antigen so that it did not reach the gut in sufficient amounts to lead to the production of antibody.

It has also been shown by passive immunization experiments\textsuperscript{99} that serum antibody may reach the gut, probably by passive leakage of antibody.

In conclusion these various studies support the concept of local immunological responses in the gastrointestinal tract. However, in any one situation there may be reasons why serum and gut antibodies show some degree of correlation.

**STUDIES ON HUMAN SECRETORY ANTIBODY, INCLUDING ORAL IMMUNIZATION**

**Antibody to bacteria**

An antibody response in intestinal secretions has been demonstrated during acute cholera infection\textsuperscript{101}, and more recent work\textsuperscript{98} using radioimmuno-diffusion claims to show that the antibody in jejunal juice in acute cholera exists in the IgG, A, and M classes. Intestinal antibody has also been reported to follow infection with other bacteria, eg, *E. coli*\textsuperscript{102,103} and Shigella\textsuperscript{57}, and in the latter study anti-shigella antibody also appeared to exist in all three major immunoglobulin classes. In view of these findings, the possibility of oral immunization against enteric bacterial infections is of great interest.

In human subjects, Freter\textsuperscript{63} showed that a killed oral vaccine provided a more reliable stimulus of coproantibody than parenteral vaccination. The
coproantibody response was of short duration but could be maintained by regular oral immunization: faecal and serum antibody appeared to be independent. Oral vaccination with attenuated strains of Shigellae has provided protection against natural infection in monkeys and in man. In typhoid fever killed vaccines given orally in man provide only slight protection against infection; living attenuated strains appear to be more successful—at least in animals. As has been mentioned, animal work suggests that high titres of bacterial antibody in the gut and serum follow oral administration of living, but not dead, organisms. It has been shown that live attenuated cholera vaccine produces better levels of serum antibody and coproantibody than are obtained using killed oral vaccine, and that the live vaccine was safe for human use. Further studies on the effectiveness of live oral vaccines in protecting against enteric infections are needed.

**Antibody activity to viruses**
The gut can mount a local response to poliovirus. Infection of the intestinal tract with living attenuated poliovirus in infants bears little relation to serum antibody titres. Previous intestinal infection with virus resulted in complete or partial resistance to re-infection, even when no demonstrable serum antibody followed the first infection. Antibody to poliovirus has been found in the stools of children with poliomyelitis or following oral Sabin vaccination. Ogra, Karzon, Righthand, and MacGillivray showed that oral immunization of infants with live attenuated poliovirus led to the appearance of antibody in secretions (duodenal fluid or nasal washings). Parenteral immunization with killed virus did not lead to production of antibody in the secretions. Immunization by either route led to the appearance of serum antibody, and, as discussed above, there are a number of reasons why an orally administered antigen may lead to antibody in serum. Other studies have confirmed that antibody against viruses in saliva, small intestinal fluid, and faeces is of IgA class.

**Antibody activity to dietary antigens**
As well as bacterial and viral antigens, the gut mucosa comes into contact with foreign proteins which are ingested as part of the normal diet. Thus, it is not surprising that antibody to dietary proteins has been detected in intestinal secretions. Early studies concentrated on disease states. In gastrointestinal milk sensitivity it was documented that affected infants had precipitins to milk in the stool. However, antibody to milk proteins in the absence of intestinal disease has been found in faeces and parotid saliva. In coeliac disease intestinal secretions may contain antibody to dietary protein, although here again a recent report suggests that the incidence is not significantly greater than in a control group. Two attempts have been made to define the class of antibody responsible for these reactions. Neither can be considered technically entirely satisfactory. In both, goat antisera to human immunoglobulin were used, and as outlined earlier enteric contents can give rise to non-immunological lines.

**The Protective Role of Colostrum**
In any review of gastrointestinal antibody it is essential to consider the role of colostrum and milk. In many animal species, eg, cows and pigs, there is no
transplacental passage of antibody and the animal absorbs antibody from colostrum in the first few hours of life. Failure to obtain colostrum renders the animals very susceptible to septicaemic illness\textsuperscript{118} due to infection with \textit{E. coli} and this can be prevented by feeding the IgM fraction of colostrum\textsuperscript{119}. In the human infant, transplacental transfer of IgG occurs, but IgA in serum is absent at birth\textsuperscript{120}. Neonatal saliva contains neither IgG nor IgA, the latter appearing by about the fourth week of life, and reaching adult levels by about six weeks\textsuperscript{120-122}. Normal meconium contains IgG, and in normal infants no IgA or IgM (although a recent report indicates that the meconium of infants with cystic fibrosis does contain IgA, which is at least partly of secretory type\textsuperscript{123}).

Human colostrum is rich in immunoglobulin, particularly IgA\textsuperscript{28}, and it contains antibody activity against bacteria\textsuperscript{28,66,124,125} and viruses\textsuperscript{114,126,127}. This antibody activity is usually of IgA type\textsuperscript{28,128-130}. These antibodies can survive passage through the neonate gastrointestinal tract\textsuperscript{124,125}. Secretory IgA antibodies from colostrum appear to be resistant to trypsin\textsuperscript{36,124}. Although IgA is sensitive to pepsin and HCl, these are absent from the stomach in the first weeks of life, or present in very low concentrations\textsuperscript{131}.

There is some debate as to whether colostral antibodies are derived from serum or synthesized in the breast. IgA antibodies may be present in colostrum, which are not demonstrable in serum\textsuperscript{130,132}. Tissue culture studies on human and monkey mammary gland would also seem to indicate that IgA antibody is synthesized locally\textsuperscript{133}. Leucocytes derived from colostrum and cultured \textit{in vitro} appear to be able to synthesize IgA only\textsuperscript{134}, whereas peripheral blood leucocytes can synthesize all three immunoglobulin classes\textsuperscript{135,136}.

In human subjects it is now established that breast-fed infants have a lower incidence of enteric infection with \textit{E. coli} \textsuperscript{137-139} and less septicaemic illness\textsuperscript{140} than bottle-fed infants. It has been suggested that the low pH of faeces from breast-fed infants may inhibit \textit{E. coli}\textsuperscript{141} and that this effect is due to the poor buffering capacity of breast milk\textsuperscript{142}.

It is tempting to try to relate the beneficial effects of milk to its content of specific antibody, but the evidence for this is limited. A recent report shows that milk has a bacteriostatic effect on growth of \textit{E. coli} related to a combined effect of specific antibody and lactoferrin\textsuperscript{143}.

The Function of Secretory IgA in the Gastrointestinal Tract

\textbf{COMPLEMENT FIXATION}

The mode of action of IgA remains obscure. It is certainly able to agglutinate bacteria\textsuperscript{36}, but it is very doubtful if it can be bactericidal—this requires complement for the lysis of bacteria, and IgA antibodies are unable to fix complement\textsuperscript{23,132,144}. One report suggests that IgA can accomplish bacteriolyis in the presence of complement and lysozyme\textsuperscript{130}; however, it is possible that the IgA fraction used in this study was not pure, and this work has not been confirmed\textsuperscript{92}.

\textbf{OPSONIZATION}

It has been suggested that IgA may act as an opsonin, rendering bacteria more available for phagocytosis\textsuperscript{145,146}, although the evidence is conflicting\textsuperscript{92}. It is difficult to envisage the value of such a system to the intestinal tract,
ANTITOXIN ACTIVITY

IgA could act as an antitoxin. Antitoxin has been detected in intestinal secretions of rabbits\(^{97,147}\) and monkeys\(^{148}\) after oral administration of cholera toxin. Diphtheria antitoxin of IgA class has been demonstrated in nasal fluid after local antigenic stimulation\(^{149}\). No intestinal antitoxin against *E. coli* enterotoxin has yet been found.

INTERFERENCE WITH BACTERIAL GROWTH

Brandtzæg\(^{65}\) has shown *in vivo* coating of streptococci with IgA from saliva. Coated organisms exhibited interference with growth—the ‘long chain phenomenon’ which occurs when bacteria are grown in media which contain antibody\(^{150,151}\).

AGGLUTINATION AND THE PREVENTION OF ADHERENCE

It may be that agglutination of bacteria in itself is beneficial. As already mentioned, secretory IgA may be more efficient in agglutinating bacteria than serum IgA because of its dimeric structure. Bacteria are thought to be removed from the bowel by peristalsis\(^{152}\), and it is possible that aggregation of bacteria hastens this process. Potentially pathogenic organisms appear to be more adherent to mucosal surfaces than avirulent strains\(^{153,154}\) and selective attachment of bacteria to mucosa may be an important factor in the control of local bacterial populations\(^{155}\). Freter has shown that one effect of antibody is to prevent adherence of pathogens (*V. cholerae*) to intestinal mucosa\(^{156}\) or to inhibit the growth of bacteria which adhere to the mucosa\(^{157}\), but these experiments were done using serum antibody.

ANTIVIRAL ACTION

Antiviral activity can be demonstrated by the prevention of viral replication in tissue culture\(^{114,126}\) and by the prevention of viral colonization of locally immunized segments of gut\(^{48}\). The exact mechanism of the antiviral action is not known.

MASKING OF ANTIGENIC SITES

IgA may mask antigenic sites. Bacteria coated with secretory IgA appear to be non-antigenic in rabbits. Furthermore, if bacteria are exposed to secretory IgA antibody, the bactericidal effect of IgG and IgM antibody is blocked\(^{92}\). One hypothesis suggests that IgA is directed only against commensal bacteria, leaving potential pathogens uncoated so that these can then stimulate the production of bactericidal IgG and IgM antibodies\(^{158}\). It has been suggested also that IgA may play a similar role in relation to dietary antigen\(^{159}\), preventing the formation of IgG and IgM class antibodies. It has been mentioned that antibody to dietary antigens is present in intestinal secretions even in normal subjects\(^{56,118}\). In patients with congenital deficiency of IgA, there is a greatly increased incidence of serum antibody to milk proteins\(^{160-162}\) and ruminant IgM\(^{161-163}\), this antibody being of IgG class. An increased incidence of serum precipitins to dietary antigens has also been found in coeliac disease\(^{66,164,165}\), and inborn absence of IgA seems to be associated with the development of some cases of coeliac disease\(^{66,167}\). Coeliac disease could
represent a functional failure of the masking action of IgA antibodies against dietary proteins\textsuperscript{169}.

This attractive hypothesis is far from proven. Neonates rapidly develop serum IgG and IgM antibody to ingested bovine serum albumin\textsuperscript{84}. There may be increased absorption of dietary protein in this period\textsuperscript{168,169}. It is possible that in the absence of local IgA in the gut in the first few weeks of life\textsuperscript{122} these ingested proteins may be able to produce a systemic antibody response. However, this does not explain the high incidence of anti-BSA antibodies reported in older subjects\textsuperscript{170,171}. There is little evidence for quantitative IgA deficiency in intestinal secretions in the majority of coeliacs, and indeed the levels may be raised\textsuperscript{52,63}. Furthermore, antibodies to commensal bacteria are not exclusively IgA, and IgA antibody can appear in response to pathogenic bacteria\textsuperscript{57,68}. Additional work is clearly necessary, and in studies on intestinal antibody the class of immunoglobulin involved should be determined.

**Gut Immunoglobulin and Antibodies in Pathological States**

Lehner\textsuperscript{172} has shown that the salivary IgA levels of individuals prone to caries are significantly lower than those of caries-free individuals, despite the fact that the group prone to caries had higher serum IgA levels. By contrast, patients with recurrent oral ulceration showed no difference in salivary IgA levels compared with controls.

Brandtzaeg has shown high levels of IgA in saliva of patients with macroglobulinaemia and presents evidence that these are probably due to selective transfer of IgM from the serum. In contrast, elevated saliva IgG levels in patients with IgG myeloma were probably dependent on the raised serum levels with no evidence of selective transport\textsuperscript{57}.

Aspirates from the normal acid-secreting stomach have been reported to contain no immunoglobulin\textsuperscript{49} but appreciable amounts can be detected using suitable sampling methods\textsuperscript{41}. In achlorhydric patients, the gastric juice contains high levels of immunoglobulin\textsuperscript{41}. These levels cannot at present be related to studies on the immunoglobulin-containing cells in the gastric mucosa, which show that in the normal gastric mucosa there is a majority of IgA-producing plasma cells\textsuperscript{49,173}. In atrophic gastritis, the proportion of IgA-staining cells has been reported to be much less than normal\textsuperscript{59} and in another study\textsuperscript{178} the proportion of IgA-staining cells was shown to be much increased.

Autoantibodies to intrinsic factor in gastric juice have been shown to occur in both IgG and IgA classes\textsuperscript{174–176}, and in the case of intrinsic factor type II antibodies, IgA antibodies predominated. Parietal cell antibodies in gastric juice were seen to be predominantly IgA, or IgG and IgA combined. There appears to be no correlation between the immunoglobulin class of the autoantibodies in gastric juice and in serum\textsuperscript{175}.

In adult coeliac disease there are marked alterations in the immunoglobulin-containing cells in the lamina propria and small intestine, the proportion of IgM-containing cells being increased\textsuperscript{52,177}, and these changes persist after treatment with a gluten-free diet. In one study\textsuperscript{177} the number of IgG-containing cells was also elevated.

The jejunal juice in untreated coeliac disease has been reported to contain increased amounts of IgM\textsuperscript{55} and increased amounts of IgG, A, and M\textsuperscript{58}. 
In one study, IgM levels returned to normal on a gluten-free diet whereas in the other report there was no change following treatment.

In ulcerative colitis, the density of IgA-containing cells in rectal mucosa is lower than in normal tissue, although still greater than that of IgG- and IgM-containing cells. More extracellular IgA was seen. There is also a report of the presence of large numbers of IgD-containing cells in rectal mucosa in ulcerative colitis. A recent report suggests the presence of faecal antibodies in colitic patients which react with the patient's own anaerobic flora.

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References

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