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HUMAN FAECAL IMMUNOGLOBULINS IN HEALTHY INFANTS AND CHILDREN, AND IN SOME WITH DISEASES AFFECTING THE INTESTINAL TRACT OR THE IMMUNE SYSTEM

B. HANEBERG AND D. AARSKOG

Department of Pediatrics and Broegelmann Research Laboratory for Microbiology, University of Bergen, Norway

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SUMMARY

IgA, IgG and IgM in faeces were quantified by single radial immunodiffusion using extracts of freeze-dried faeces. IgA in small specimens of faeces seemed to mirror the total amount of IgA secreted into the gut at the time of sampling. Presumptive normal values for faecal IgA concentrations in infants and children were established. Agglutinins to rabbit erythrocytes served as markers for the antibody activity. Infants and children just recovered from enteritis of probably infectious origin had higher concentrations of both IgA and agglutinins in faeces. Faeces from three out of five patients with ulcerative colitis in remission, contained IgG in markedly increased concentrations. Two patients with IgA deficiency had no detectable IgA in faeces, but had increased levels of faecal IgM which also agglutinated rabbit erythrocytes. Immunoglobulins were not demonstrated in faeces from three patients with agammaglobulinaemia. The findings indicate that faeces can be used for assay of immunoglobulins of the intestinal tract.

INTRODUCTION

Immunoglobulins are normally found in extracts of freeze-dried faeces from healthy infants and children (Haneberg, 1974b; Haneberg & Tønder, 1973). IgA is the dominant class, and agglutinins to rabbit erythrocytes can be used as markers for immunological activity (Haneberg, 1974a). The methods developed using faecal material seemed practical for the investigation of intestinal immunoglobulins which have been the subject of much interest, as can be seen from the review by Shearman, Parkin & McClelland (1972).

In this paper we describe the results of experiments designed to clarify the limitations and the usefulness of faeces as a material for the investigation of intestinal immunoglobulins. Normal levels of immunoglobulins in faeces from infants and children of various age groups have been established. They were compared with the findings in faeces from patients just recovered from enteritis of probably infectious origin, or with verified septicemia and/or

Correspondence: Dr Bjørn Haneberg, Barneklinikken, Haukeland sykehus, N-5016 Bergen, Norway.

clinical signs of osteomyelitis. Children with ulcerative colitis in remission, as well as infants and children with some immunoglobulin deficiency states, were also investigated.

MATERIALS AND METHODS

Individuals. The group of healthy infants and children comprised 161 individuals, aged from 1 day to 12 years, randomly selected at well-baby clinics and among children of hospital employees. Some were also patients at the Children's Hospital, Bergen, admitted for undescended testicles, nocturnal enuresis, or congenital heart disease without signs of failure. None of them had signs of infection or gastrointestinal disease. The infants did not receive human milk for 2 weeks or more prior to the sampling of faeces, and were fed solids from the age of 2–3 months.

Seventeen individuals (5 months–10 years of age) were studied 3–7 days after a bout of diarrhoea lasting for 2–3 days, sometimes accompanied by vomiting. Since the enteritis affected previously healthy individuals in minor epidemics, an infectious aetiology was assumed. At the time faeces were collected, they had all recovered and had regular, formed stools.

Fifteen infants and children (6 months to 9 years of age) with septic infections, were studied. In seven of these, repeated blood cultures were positive for either *Staphylococcus aureus*, β-haemolytic *Streptococcus*, *Hemophilus influenzae* or *Pneumococcus*. In the remaining cases the diagnosis of osteomyelitis was based on radiological and clinical signs. None of the patients had signs of gastrointestinal dysfunction. Samples of faeces were collected 1–3 weeks after initiation of fever.

Five patients with ulcerative colitis were studied when in clinical remission, i.e. when their stools were regular and formed, and without blood. The diagnosis was based on history of bloody diarrhoea and rectoscopic as well as X-ray findings.

One patient with low (> 2 s.d. for age) but easily measurable serum IgA concentration and two others with IgA deficiency (serum levels < 4 mg/100 ml) were also included in the study. One of them was detected among the patients with osteomyelitis and another was found among the children with ulcerative colitis. The third was mentally retarded and had neurological and clinical signs of ataxia telangiectasia. In addition three other individuals with agammaglobulinaemia, probably sex-linked, were included. One of these (H.Ø.) had frequent loose stools during infancy, all had seemingly normal bowel function at the time of study.

Extracts of faeces. Small batches (1–2 g) of faeces were collected in plastic containers and frozen within 2 hr after defaecation. The samples obtained at 1–3 days of age consisted of meconium. As outlined before (Haneberg & Tønder, 1973), extracts were prepared from the faeces after freeze-drying, using 10 ml of phosphate-buffered saline (PBS), pH 7·2, per gram of dry matter. From fourteen individuals the total amount of faeces was also collected over 3 days. All the faecal samples studied were negative for blood by the benzidine test (Haneberg & Tønder, 1973).

Sera. From fifty-two of the healthy individuals and from all patients serum was obtained at the time of faecal sampling. Pooled serum from ten healthy blood donors served as normal human serum (NHS), the IgA concentration being 198 mg/100 ml. Standard human serum (Batch number 173, Behringwerke AG, Marburg-Lahn, Germany) which, according to the manufacturer's manual, contained 230 mg of IgA/100 ml and were related to a WHO reference sample, were used as control for immunoglobulin measurements.

Normal rabbit serum and rabbit antisera to whole human serum, human faecal extracts, human salivary IgA, serum IgG and IgM were obtained as outlined before (Haneberg & Tønder, 1973). Commercial rabbit antisera to human serum IgA, IgG (Fab), albumin, α_1-antitrypsin, α_2-macroglobulin and to free secretory component (Behringwerke AG) were also used.

All sera were stored at −20°C and those used for agglutination techniques were heated to 56°C for 30 min to destroy complement activity.

Agglutination of rabbit erythrocytes. Titres of agglutinins to rabbit erythrocytes were determined as described previously (Haneberg, 1974a) with faecal extracts, fractions of these and sera. Inhibition of agglutination and antiglobulin test were carried out with antisera to IgA, IgM and IgG, also as described.

Gel filtration. Extracts of faeces, either dialysed or concentrated by negative pressure dialysis against Tris–NaCl buffer (0·05 M Tris–NaCl + 0·14 M NaCl), pH8, were separated through a Sephadex G-200 column measuring 1·5 × 45 cm, as in a former study (Haneberg & Tønder, 1973).

Purification of faecal IgA. Faecal IgA was isolated as outlined for milk IgA (Brandtzaeg, Fjellanger &

Gjeruldsen, 1970). Extracts of faeces were filtered through Sephadex G-200 column, and the fractions containing IgA were pooled and dialysed against 0·01 M phosphate buffer, pH 7·5, before being applied to a DEAE–cellulose column measuring 1·2 × 27 cm. Elution was stepwise, the same buffer being used with 0·04, 0·1, 0·2, 0·3, 0·4 and 1 M NaCl. The flow rate was kept relatively constant at 11 ml/cm²/hr, and fractions about 1 ml each were collected during continuous registration of u.v.-light transmittance at 280 nm (Uvicord Absorptiometer, LKB-Produkter AB, Stockholm, Sweden).

Other methods. Single radial immunodiffusion, double diffusion and immunoelectrophoresis in agar were carried out as described previously (Haneberg & Tønder, 1973).

Protein concentrations in fractions of faecal extracts were determined according to the method of Folin–Ciocalteu (Kabat & Mayer, 1964), using Beckman DU-2 spectrophotometer (Beckman Instruments Incorporated, Palo Alto, U.S.A.). As standards were used dilutions in PBS of Gammaglobulin 16·5% (Kabi AB. Stockholm, Sweden).

The pH of faeces was measured as described earlier (Haneberg, 1974b) with Lyphan indicator paper (Dr Gerhard Kloz, West Berlin, Germany).

Conventional statistical methods were applied, including Student's *t*-test.

RESULTS

Quantitative determination of IgA

Since diffusion of IgA in agar is dependent on its molecular size, it was necessary to purify IgA from faecal extracts to obtain a correction factor for faecal IgA values in single radial immunodiffusion based on serum IgA as standard.

By gel filtration and ion exchange chromatography, IgA was isolated from: pooled extracts of faeces from three healthy children; an extract of faeces from one child just recovered from 'infectious' enteritis; and an extract of faeces from one child with ulcerative colitis. Reasonably pure preparations were obtained with eluting buffer containing 0·2 M NaCl; only IgA could be demonstrated in the unconcentrated fractions. However, after 100-fold concentration, traces of α_1-antitrypsin were demonstrated while IgG and IgM amounted to less than 0·03 and 1·3% of total protein concentrations, respectively. No other serum or faecal components were demonstrated using antisera to whole human serum, faecal extract and to IgG (Fab) and α_2-macroglobulin.

Results of gel filtration of the IgA preparation from the healthy individuals gave evidence that IgA in the purified preparation had roughly the same distribution of molecular sizes as the IgA of the initial material. In this experiment IgA was demonstrated in all the fractions ranging from those representing the molecular size of IgM to those of IgG while the highest concentrations were found in the fractions between these.

A linear relationship was evident between total protein concentrations in dilutions of these IgA-preparations and the corresponding concentrations of IgA, measured as the percentage of NHS in single radial immunodiffusion. Therefore, correction factors were established for concentrations of IgA in extracts of faeces from different individuals (Table 1). The results were dependent on the antiserum used; with our antiserum to secretory IgA not reacting with free secretory component the values were 63–52% higher than with an antiserum to serum IgA (Behringwerke). When compared to concentrations (w/v) of IgA in NHS, the correction factors were 5·3 and 3·3, respectively. In the following, the corrections of IgA concentrations were thus based on values obtained with the same type and the same batch of antiserum throughout the study, i.e. our antiserum to secretory IgA.

Small-molecular impurities in the IgA preparations were not responsible for a substantial part of protein concentrations; less than 5% difference was found for correction factors after prolonged dialysis.

TABLE 1. Correction factors for concentrations of IgA in extracts of faeces based on the percentage of NHS values in single radial immunodiffusion with two different anti-IgA sera

	Antiserum to:	
Extracts of faeces from	Secretory IgA	Serum IgA
Healthy individuals (pool from three)	10·6	6·5
Patient recovered from infectious enteritis	11·4	7·5
Patient with ulcerative colitis	4·3	Not done

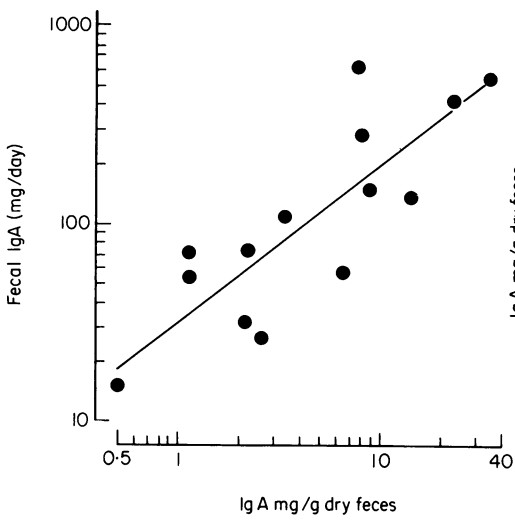

FIG. 1

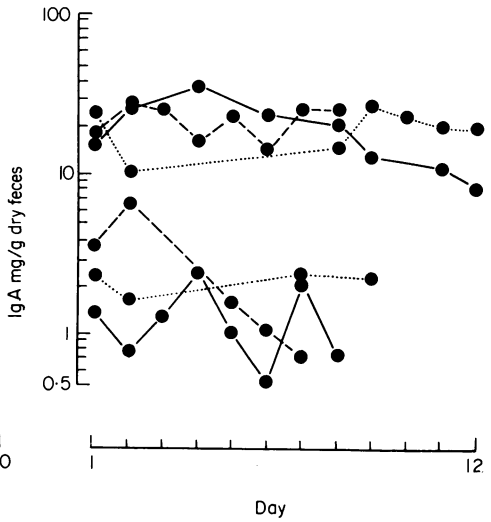

FIG. 2

FIG. 1. The total amounts of IgA in extracts of the daily bulk of faeces, related to the corrected concentrations of IgA in small samples of faeces from fourteen individuals. The regression line was calculated after linear transformation of the scales. The linear correlation coefficient was 0·84, which differed from zero to a highly significant degree ($P < 0·001$).

FIG. 2. The corrected concentrations of IgA in extracts of faeces from six individuals, aged from 1 month to 12 years. Small samples of faeces were collected on various days, each sample marked represented one defaecation.

The concentrations of IgA related to the dry weight of faeces were not dependent on the water content of fresh faeces. This seemed valid even though the wet-weight/dry-weight ratio in faeces from the healthy individuals varied between 1·8 and 7·0, with mean value 3·8 and standard deviation ±1·9. For meconium obtained at 1–3 days of age, this mean ratio was 5·3, otherwise there was no difference in ratio with age.

The IgA concentrations in extracts of small samples of faeces correlated well with the total daily amounts of IgA in dry faeces excreted over a 3-day period (Fig. 1). Thus, the concentration of IgA in a single small sample of faeces can for practical purposes be used as a measure of the excretion of IgA with faeces at that time. The finding that the faecal IgA concentrations, in the same individuals, varied little from day to day (Fig. 2), supports this.

Normal levels of immunoglobulins

From the above it seemed justified to establish normal levels of IgA concentrations in extracts of small samples of faeces at various ages (Fig. 3). IgA was found in such extracts from 134 of the 140 normal individuals aged 1 month or older. The range of concentrations was quite large for all age groups. The highest levels were found in 1–2-month-old infants, being significantly higher than at 7–12 months ($P<0·001$) or at 9–12 years of age ($P<0·01$).

As many as 129 of the 140 extracts of faeces from individuals aged 1 month or older agglutinated rabbit erythrocytes to titre 1 or higher (Fig. 3). The titres reached a relatively stable niveau from 1 to 2 months of age, no statistical difference being found between the levels at 7–12 months and those at 1–2 months of age, or at a later age. The agglutination titres in faecal extracts did not correlate with the corresponding titres in sera. However,

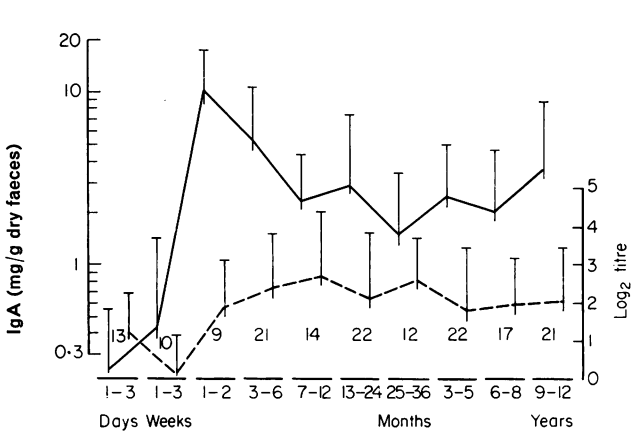

FIG. 3

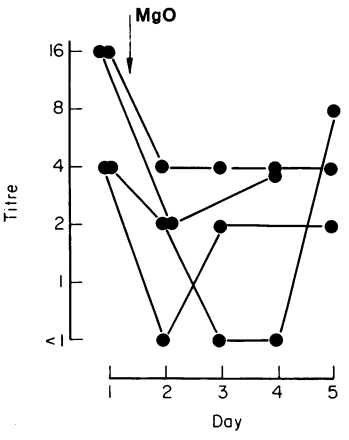

FIG. 4

FIG. 3. The mean corrected concentrations of IgA (continuous line), and agglutination titres to rabbit erythrocytes (dashed line), in extracts of single small samples of faeces from infants and children of various age groups. The material collected at 1–3 days of age was meconium. The upper standard deviations are indicated by vertical lines. The number of individuals in each age group is also given.

FIG. 4. The titres of agglutinins to rabbit erythrocytes in extracts of faeces from three children in four trials with MgO by mouth as a laxative.

TABLE 2. IgG and IgM in extracts of faeces from healthy infants and children, as well as from some just recovered from 'infectious' enteritis. The numbers of individuals with measurable concentration i.e. ⩾0·07 and ⩾0·08 mg/g dry faeces respectively, are given

			IgG	IgM
Individuals	Age	Number	Number (range)	Number (range)
Healthy	1–3 days (meconium)	13	2 (0·2–0·5)	0
	1–3 weeks	10	6 (0·1–0·4)	4 (0·2–2·8)
	1–2 months	9	0	4 (0·8–3·1)
	3–6 months	21	1 (0·1)	6 (0·2–1·0)
	7–12 months	14	0	4 (0·2–1·5)
	13–24 months	22	1 (0·1)	2 (0·2–0·2)
	25–36 months	12	0	0
	3–5 years	22	0	3 (1·0–1·8)
	6–8 years	17	1 (0·4)	3 (0·3–0·4)
	9–12 years	21	1 (0·3)	2 (0·5–3·4)
Recovered from enteritis	5 months–10 years	17	5 (0·2–0·8)	7 (0·4–1·5)

The ranges of the measured concentration (mg/g dry faeces) are shown in parentheses.

at 1–6 months of age, when serum titres were on the increase, an apparently linear relationship was found; the correlation coefficient was 0·45 but did not differ significantly from zero ($0·1 < P < 0·2$).

IgG and IgM were present only in some of the faecal extracts (Table 2). IgG was more often found in the 1–3-week-old infants compared with older infants and children. The occurrence of IgM in the extracts did not show any convincing variation with age.

The effect of laxatives

Three children (author's own), aged 2½–7 years, were given one to two teaspoonfuls of magnesium oxide (MgO) powder by mouth in the evening of day 1. With one child this was repeated some months later. Liquid stools were passed the following day; the wet-weight/dry-weight ratio increased from 2·8–3·8 to 6·0–10·1 and the pH of faeces concomitantly changed from 5·5–6·5 to 8–8·5. The stools were again regular and formed from day 3 or 4 and the wet/dry weight ratio and pH returned to the previous levels by day 5. In three of the trials the IgA concentrations in relation to dry weight of faeces were largely unchanged. In the trial leading to the most prolonged diarrhoea IgA disappeared from the faecal extracts. IgG or IgM were not demonstrated in any of these extracts. The agglutination titres, however, fell two to three titre steps in all trials (Fig. 4) and returned to the former levels within 2 weeks. No rebound increase in agglutination titres of more than 1 titre step was observed.

'Infectious' enteritis

Faeces from seventeen infants and children just recovered from a bout of diarrhoea contained significantly higher levels of IgA than faeces from the 1–2-month-old controls

TABLE 3. Corrected concentrations of IgA and rabbit erythrocyte agglutinins in extracts of faeces from infants and children 3–7 days after a period of 'infectious' enteritis. As controls were used the two age groups among healthy individuals having the highest levels of faecal IgA or agglutinins, respectively

			IgA (mg/g dry faeces)				$\log_2$ titre			
Individuals	Number	Age	Mean	(Range)	s.d.	Significance	Mean	(Range)	s.d.	Significance
Healthy	9	1–2 months	10·5	(1·6–21·2)	7·5	$P<0·001$				
Recovered from enteritis	17	5 months–10 years	17·0	(1·6–33·4)	9·8		4·7	(3–7)	1·2	$P<0·001$
Healthy	14	7–12 months					2·7	(0–5)	1·7	

having the highest levels of faecal IgA among the healthy individuals (Table 3). The IgA concentrations were corrected with the same factor as that established for normal controls. The correction factor made out for one patient with enteritis was not lower than this control factor (Table 1), indicating that the higher levels of IgA in faeces from these patients were not based on increased amounts of low molecular weight IgA. Also, the agglutination titres were higher than in another control group, 7–12 months of age, having the greatest normal agglutinin activity.

IgG was found in faeces from nearly one-third of the patients, whereas in the controls aged 1 month or older IgG was measurable in only four out of 138 (Table 2). The occurrence of IgM in faeces did not differ greatly between these groups of individuals.

The serum levels of IgA and the agglutination titres in the patient group did not differ from the controls at the time of collection of faeces.

Septic infections

In fifteen patients with septicaemia or obvious osteomyelitis, but no signs of gastro-intestinal dysfunction, the faecal or serum IgA concentrations, and agglutination titres were found to be substantially the same as in twenty-one controls of the same age. One patient (T.L.), however, had low serum IgA concentration and was subsequently included in the group with immunoglobulin deficiencies.

Ulcerative colitis

In four out of the five patients normal levels of faecal IgA were measured (Table 4). The correction factor established for IgA in faeces from one of these was low (Table 1), suggesting an abundance of low molecular weight IgA compared to the controls. One patient (V.H.) had no detectable IgA in faeces and very low serum IgA concentration, and is therefore included in the immunodeficiency group.

Unusually high levels of IgG were found in faeces from three of these patients (O.E., Å.I. and V.H.) (Table 4). Their faecal agglutination titres were in the normal range. Anti-IgG did not increase the titres in the antiglobulin test, indicating that IgG in faeces was not active against rabbit erythrocytes. Albumin was found in faecal extracts from two of them (O.O. and Å.I.), in faeces from the others no albumin was detected. The high levels of IgG in faeces from two patients (O.E. and V.H.) with no detectable faecal albumin, cannot therefore be explained by a mere leakage of serum through an inflamed colonic mucosa.

Immunoglobulin deficiencies

One patient (T.L.) with low serum IgA concentration, i.e. below 2 s.d. of the mean, but not in the extreme range, had normal level of IgA in faeces (Table 4). In faeces from two other patients with extremely low serum IgA levels, IgA could not be detected. Both of these had unusually high levels of faecal IgM, which is also evident from the results of gel filtration (Fig. 5). An impressive first peak on the u.v. light transmittance curve fell close to the void volume of the column, and coincided with the appearance of IgM as well as the presence of agglutinins to rabbit erythrocytes. Except for the presence of IgG in a patient (V.H.) with ulcerative colitis the results of gel filtrations were strikingly similar in the two patients with IgA deficiency. The low-titred agglutinin activities were inhibited by antiserum to IgM, a further indication that IgM was responsible for this activity in faeces from these patients.

TABLE 4. Immunoglobulin concentrations and rabbit erythrocyte agglutination titres in sera and extracts of faeces from patients with ulcerative colitis and various immunoglobulin deficiencies. The limits of detection were 0·5, 0·7 and 0·8 mg/100 ml of serum-IgA, IgG and IgM, respectively. One gram dry faecal matter represents 10 ml faecal extract

Disease	Patient	Age (years)	Sex	Serum				Faeces			
				IgA	IgG	IgM	Titre	IgA	IgG	IgM	Titre
				(mg/100 ml)				(mg/g dry matter)			
Ulcerative colitis	O.O.	6	M	210	1230	90	512	1·3	0·2	n.d.	4
Ulcerative colitis	O.E.	10	M	120	1200	110	512	1·9	1·7	n.d.	8
Ulcerative colitis	Å.I.	11	M	350	2300	220	128	7·7	3·6	2·2	16
Ulcerative colitis	J.N.	13	M	180	1500	70	256	6·5	n.d.	0·2	8
Ulcerative colitis	V.H.	12	M	0·6	1290	40	64	n.d.	7·7	13·8	4
Ataxia telangiectasia	T.R.	7	F	1·4	1640	240	256	n.d.	n.d.	7·4	4
Osteomyelitis	T.L.	7	F	16	1040	90	128	0·8	n.d.	0·4	2
Agamma-globulinaemia	K.F.*	7	M	<4	210	<8	8	n.d.	n.d.	n.d.	<1
Agamma-globulinaemia	H.Ø.*	13	M	<4	240	<8	<2	n.d.	n.d.	n.d.	<1
Agamma-globulinaemia meningitis	K.L.†	3 months	M	4	180	<8	4	n.d.	n.d.	n.d.	<1

n.d. = Not detectable.

* Treated with Gammaglobulin 16·5% (Kabi).

† Treated with Gammaglobulin 16·5% (Kabi) and whole blood transfusions.

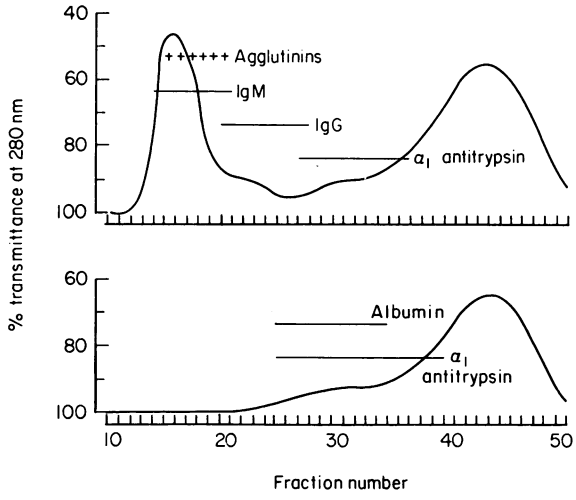

FIG. 5.

FIG. 5. Gel filtration through a Sephadex G-200 column, 1·5 × 45 cm of unconcentrated extract of faeces from a patient with ulcerative colitis and IgA deficiency (upper graph), and of a 10× concentrated extract of faeces from a patient with agammaglobulinaemia (lower graph). The flow rate was 11 ml/cm²/hr and fractions of about 2 ml each were collected. The protein concentrations in the fractions are indicated by u.v.-light transmittance. The presence of serum proteins, detected by double diffusion in agar, and of rabbit erythrocyte agglutinins in the fractions, are also marked.

In extracts of faeces from three patients with agammaglobulinaemia, no immunoglobulins or agglutinin activity were demonstrated (Table 4). Results of gel filtration of a concentrated faecal extract from one of these (K.F.), did not reveal high molecular weight proteins (Fig. 5). Secretory component was not demonstrated in any of the fractions, but was found by double diffusion of extracts from two other patients in this group. The failure to demonstrate secretory component in faeces from the third patient were not unsuspected since it was found in the extracts of faeces from only two-thirds of the healthy individuals.

DISCUSSION

In the present study with purified IgA from human faeces, a linear relationship was found between total protein concentrations and the measurements of IgA in single radial immunodiffusion based on serum IgA as standard. A correction factor could therefore be established as has earlier been done with IgA from human saliva and milk (Brandtzaeg *et al.*, 1970; Haneberg, 1974c). The influence on quantitation of IgA in single radial immunodiffusion because of size heterogeneity of IgA in secretions (Tomasi & Grey, 1972), is thereby reduced and either 7S serum IgA or 11S IgA can be employed as standards. The results varied with the antiserum used, as earlier observed by others (Samson, McClelland & Shearman, 1973), and it was therefore felt necessary to use the same batch of antiserum for quantitative measurements with faecal extracts as was used for the establishment of the correction factors.

Differences in quantitative relationship between high and low molecular weight IgA will influence the correction factor; with more low molecular weight IgA, the factor will be smaller. Since the factor for faecal IgA from one individual just recovered from 'infectious' enteritis was nearly the same as for the controls, the higher IgA values in the enteritis group most likely reflect increased intestinal IgA production. On the other hand, the low correction factor established for one patient with ulcerative colitis might suggest a passive leakage of low molecular IgA through the mucosal epithelium. Evidence for this is given by histological studies using immunofluorescence technique (Gelzayd *et al.*, 1968).

The molecular weight distribution of IgA in faeces may vary even between healthy individuals. For accurate estimation of IgA it would therefore be essential to establish a correction factor with a purified preparation from each individual sample. The excretion of dry faecal matter may also differ from time to time and between individuals. Therefore, the determination of IgA, related to dry weight of faeces, can be semi-quantitative at best. However, it is likely that the IgA in small samples of faeces reflects the secretion of IgA into the gut at the time when faeces were collected.

The IgA concentrations in small samples of faeces from healthy infants and children indicate that the intestinal mucosa is immunologically mature at least from the age of 1 month. Moreover, the higher level at this early age, compared to that found at a later age, may reflect a vivid response to the introduction into the gut of dietary as well as microbial antigens (Shearman *et al.*, 1972).

Testing for agglutinins to rabbit erythrocytes has proved useful for the assay of IgA activity in faeces (Haneberg, 1974a); rabbits immunized with their own erythrocytes, agglutinated by human faecal extracts, produced precipitating antibodies to human IgA. In the healthy individuals the agglutination titres, like IgA concentrations in faeces, reached a relatively stable level from 1 month of age. The relatively high titres with extracts of meconium may be explained by swallowed amniotic fluid (Rule *et al.*, 1971), or by transudation of antibodies from the blood (Roulet & von Muralt, 1961). It was difficult to exclude contamination of blood in meconium by the benzidine test. The agglutinins in meconium, therefore, were not thought of as being of solely intestinal origin.

Diarrhoea induced by laxatives seemed to 'wash-out' the agglutinins recovered in faeces, and when diarrhoea was prolonged IgA also disappeared. In cases with ongoing severe diarrhoea, as in cholera and shigellosis, the specific faecal antibody activity (Freter *et al.*, 1965; Reed & Williams, 1971) would therefore be highly significant.

The high levels of faecal IgA in the patients with enteritis indicate a local stimulus by a supposedly infectious agent. The increased jejunal IgA synthesis demonstrated by others following experimental infection with the Norwalk agent (Agus *et al.*, 1974) is in line with this. Increased serum IgA levels in premature infants with gastroenteritis (Panayotou *et al.*, 1971) is further in support of increased intestinal production of IgA since tissues along the intestinal tract are main sources of IgA (Tomasi & Grey, 1972). The concomitant increased agglutinin activity against rabbit erythrocytes as demonstrated in our study is suggestive of an antigenic relationship between the surface of the rabbit erythrocytes and the presumptive infectious agent. Samples of faeces were not taken until after recovery from the disease, in order to obtain samples comparable to the controls. This also allowed time for the production of local antibody response.

The presence and the high levels of IgG in faeces from patients with ulcerative colitis, are analogous to the findings in saliva from individuals with inflamed gingival mucosa

(Brandtzaeg *et al.*, 1970). Signs of increased local IgG synthesis seem to be a general finding in chronic inflammatory conditions (Brandtzaeg, 1972; Brandtzaeg *et al.*, 1974), although some results of immunofluorescence studies obtained with rectal mucosa have been conflicting (Gelzayd *et al.*, 1968). The high levels of IgG in faeces, even in formed stools, free from blood, may therefore indicate a continuous inflammation of the colon.

The apparent lack of IgA in faeces from two patients with isolated serum IgA deficiency parallels the findings in saliva, parotid secretions and intestinal juice (Brandtzaeg, Fjellanger & Gjeruldsen, 1968; Savilahti, 1973; South *et al.*, 1968). The increased faecal IgM concentrations in our patients clearly indicate that IgM in intestinal secretions, as in salivary gland secretions (Brandtzaeg *et al.*, 1968; Savilahti, 1973) can compensate for IgA. This is substantiated by the demonstrations of low numbers of IgA- and increased numbers of IgM-containing plasma cells in the intestinal mucosa of patients with IgA deficiency (Crabbé & Heremans, 1966; Savilahti, 1973). Also faecal IgM in our patients seemed immunologically active against rabbit erythrocytes. We have not earlier been able to demonstrate such activity of faecal immunoglobulins other than for IgA. Possibly part of the large amounts of IgM may escape inactivation by proteolytic enzymes.

The absence of detectable immunoglobulins in faeces from three patients with agammaglobulinaemia was interesting. At least as far as IgA is concerned, this parallels the findings in saliva (South *et al.*, 1968). The importance of local immunoglobulins for the normal gross functioning of the intestines might therefore be questioned since none of the patients with agammaglobulinaemia had signs of gastrointestinal dysfunction.

In any event, the results so far give evidence that faecal material us useful for the evaluation of immunoglobulins of at least the low intestinal tract.

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