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# IMMUNOGLOBULIN E (IgE) AND IgE-CONTAINING CELLS IN HUMAN GASTROINTESTINAL FLUIDS AND TISSUES

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## SUMMARY

Human gastric, small intestinal, colonic and rectal mucosae were examined for IgE-containing cells by single- and double-antibody immunofluorescence techniques, and IgE in intestinal fluids was measured by a double-antibody radioimmunoassay. IgE-containing cells were identified in all tissue specimens and comprised about 2% of all immunoglobulin-containing cells. Although less numerous than cells containing IgA, IgM or IgG, they were remarkably numerous in relation to the concentration of IgE in serum (about 0·001% of total immunoglobulin). IgE immunocytes were significantly more numerous in stomach and proximal small bowel than in colon and rectum, and were very numerous at bases of gastric and duodenal peptic ulcers. Measurable IgE was found in seventy-eight of eighty-five (92%) intestinal fluids. Sucrose gradient ultracentrifugation analysis of four of the fluids revealed that the immunologically reactive IgE was largely in fractions corresponding to molecules of lower molecular weight than that of albumin, which suggests that IgE in gut contents is degraded by proteolytic enzymes. The presence of IgE-forming cells in gastrointestinal tissues, and IgE or a fragment of IgE in intestinal fluids, suggests that IgE antibodies are available for participation in local reaginic-type reactions in the gut.

## INTRODUCTION

Tada & Ishizaka (1970) found IgE-forming cells preferentially located along mucous membranes of the respiratory and gastrointestinal tracts, and suggested that IgE antibodies might be involved in reaginic reactions on mucosal surfaces. Since that report, however, little has been learned about IgE in the pathophysiology of the human gut. Several investigators (Hobbs *et al.*, 1969; Savilahti, 1972; Søltoft & Søeberg, 1972) found few or no IgE-forming cells in gut tissues. No systematic study of IgE cell populations in various

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regions of the digestive tract has been conducted. Also, attempts to find IgE in intestinal fluids have been generally unproductive. Hobbs *et al.* (1969), using rather insensitive methods, detected no IgE in upper bowel fluids of patients with coeliac disease. Waldman, Virchow & Rowe (1973) suggested that IgE fragments might be present in intestinal fluids because some fluids interfered with precipitin reactions between IgE and anti-IgE in radioimmunodiffusion experiments, but the immunoglobulin was not conclusively demonstrated. However, IgE is present in several other secretions, such as tears, respiratory mucus and urine (Barratt, Turner & Johansson, 1971; Brauninger & Centifanto, 1971; Deuschl & Johansson, 1974; Ishizaka & Newcomb, 1970; Newcomb & Ishizaka, 1970; Özkaragöz, Smith & Gökcen, 1972; Waldman *et al.*, 1973). These findings suggest that IgE may be demonstrable in gut fluids by the use of a sufficiently sensitive method that does not involve precipitin reactions.

Because IgE, either in gut mucosae or secretions, might be important in gastrointestinal immunological reactions, we set out to determine the distribution of IgE immunocytes along the bowel and the IgE content of intestinal fluids in healthy subjects and patients with various digestive diseases.

## MATERIALS AND METHODS

### *Subjects*

Fifty-three gastrointestinal mucosal specimens were taken from forty-seven adult subjects (Table 1). All gastric, duodenal and jejunal specimens and two-thirds of rectal and colonic specimens were from patients with some type of gastrointestinal disorder. In-

TABLE 1. Diagnoses in patients studied immunohistologically

| Tissue | Diagnosis | Number of cases |
|---|---|---|
| Stomach | Duodenal ulcer | 8 |
| | Gastric ulcer | 3 |
| | Zollinger–Ellison syndrome | 1 |
| | Other | 2 |
| Duodenum–jejunum | Duodenal ulcer | 4 |
| | Gastric ulcer | 1 |
| | Zollinger–Ellison syndrome | 1 |
| | Coeliac disease | 1 |
| | Pancreatic carcinoma | 1 |
| | Other | 4 |
| Colon–rectum | Normal | 9 |
| | Colonic carcinoma | 3 |
| | Villous adenoma | 2 |
| | Chronic ulcerative colitis | 1 |
| | Regional enteritis | 2 |
| | Other or nondiagnostic | 2 |
| | No disease | 8 |
| | | Total: 53 |

testinal fluids were collected from some of these individuals and additional subjects with Crohn's disease, intestinal parasites, coeliac disease, intestinal bacterial overgrowth, or no digestive symptoms.

*Collection of tissues and fluids*

Gastric fundic and certain colonic, rectal and duodenal tissue specimens were obtained surgically. Additional mucosal biopsies were obtained from the rectum at sigmoidoscopy and from the area of the duodenojejunal junction with the multipurpose biopsy tube (Quinton Instruments, Seattle, Washington).

Small intestinal fluids were collected from fasting subjects via a polyvinyl tube positioned at the duodenojejunal junction or, in some subjects, at multiple sites. Fluids were snap-frozen in a dry ice–acetone slurry and stored at −20°C until analysis. At the time of IgE measurement, fluids were thawed at 4°C, soybean or egg-white trypsin inhibitor was added to a concentration of 1 mg/ml, and insoluble debris was removed by centrifugation at 5800 rev/min (4°C).

*Antibody preparations*

Rabbit antiglobulins to human IgG, IgA, IgD, IgM and IgE, conjugated with fluorescein isothiocyanate (FITC), were purchased from Behring Diagnostics (Somerville, New Jersey). Antigenic specificity of the antibodies was tested by immunoelectrophoresis and immunodiffusion against normal human serum and by blocking their binding to tissue antigens in immunohistological preparations with unlabelled antisera. The anti-IgE globulin formed no precipitin line with normal human serum, but formed a line of identical specificity with IgE myeloma protein PS (obtained from Dr K. Ishizaka) and IgE-rich serum (WHO reference serum number 68/341).

Rabbit anti-IgA, conjugated with tetramethylrhodamine isothiocyanate (TRITC), was prepared in our laboratory. Rabbits were hyperimmunized with 11S IgA prepared from human colostrum (Brown, Newcomb & Ishizaka, 1970). The γ-globulin fraction of antiserum obtained by ammonium sulphate precipitation was absorbed with soluble human IgG and specifically purified by affinity chromatography with human serum IgA coupled to Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, New Jersey). Protein eluted with 3 M NaSCN (Dandliker & Saussure, 1968) was concentrated by vacuum dialysis and found to react monospecifically with normal human serum or whole human colostrum in immunoelectrophoresis or immunodiffusion analyses; it did not react with the secretory component of IgA free in human colostrum. Conjugation with TRITC (BBL Division Becton-Dickinson Company, Cockeysville, Maryland) was performed by the method of Cebra & Goldstein (1965) as in earlier work (Chen, 1971).

Unconjugated rabbit antiglobulin to human IgE myeloma protein PS was a gift from Dr K. Ishizaka (Ishizaka, Ishizaka & Lee, 1970), and rabbit antiglobulin to IgE myeloma protein ND was a gift from Dr S. G. O. Johansson (Johansson & Bennich, 1967). Rabbit anti-IgA and anti-IgM globulins were those used previously (Brown *et al.*, 1970).

*Immunohistological techniques*

Tissue specimens were fixed in 95% ethanol at 4°C for 12–24 hr and embedded in paraffin. Slices 3 μm thick were cut in a microtome, placed on nonfluorescent glass slides, and dried at 38°C for 30 min. After removal of paraffin by three changes of xylene within 15 min, the

slices were passed gently from pure ethyl alcohol to 45% ethyl alcohol. Sections were washed in cold phosphate-buffered saline (PBS), and excess fluid was removed with blotting paper. One of the FITC-conjugated anti-human immunoglobulin antibody preparations (diluted ten times in PBS) was applied, and the slides were incubated at room temperature for 4–12 hr in a moist chamber and washed several times with PBS (4°C, 30 min). In single-antibody experiments, sections then were mounted with buffered glycerine and examined by a fluorescence microscope (Leitz Dialux equipped with BG 38, $UV_1$, and K 510 filters and Orthomat camera). In double-antibody experiments, FITC conjugates were followed by application of TRITC-conjugated anti-IgA. After 4 hr at room temperature, sections were washed, mounted and examined as above. Fluorescent cells were counted directly and expressed as number of cells per twenty high-power fields (×400) of tissue, including epithelium and lamina propria.

*Radioimmunoassay of IgE*

The double-antibody radioimmunoassay for IgE was a modification of methods described recently (Gleich, Averbeck & Swedlund, 1971; Polmar, Waldmann & Terry, 1973). IgE myeloma protein PS was radioiodinated according to the method of Klinman & Taylor (1969). Two millicuries of $^{125}I$ (Amersham-Searle, Arlington Heights, Illinois) were used to label 80 μg of IgE in the presence of 40 μg of chloramine T (1 min; room temperature). Forty micrograms of sodium metabisulphite and then 2 ml of 1% human serum albumin were added. After dialysis to remove unbound radioisotope, 95–100% radioactivity was precipitable by trichloroacetic acid or an excess of anti-IgE ND.

In the assay of IgE, 0·4 ml of 1% bovine serum albumin (in 0·1 M PBS, pH 7·4) was added to plastic vials (Falcon Plastics, Oxnard, California). Duplicate or triplicate 0·1-ml or 0·2-ml samples of serum, intestinal fluid or standards were added. Next were added 0·1 ml of $^{125}I$-labelled IgE (about 0·15 ng) and 0·1 ml of a dilution of anti-IgE ND (in non-immune rabbit γ-globulin, 50 μg of N/ml) that precipitated 50% to 60% of the $^{125}I$-labelled IgE. The mixture was incubated for 24 hr at 4°C, and 0·1 ml of goat anti-rabbit γ-globulin, which had been absorbed with normal human serum conjugated to Sepharose 4B, was added. After incubation (24 hr, 4°C), the tubes were centrifuged at 2500 rev/min for 30 min (4°C), and radioactivity of the precipitates was counted in an automatic gamma counter. The amount of IgE in samples was estimated by reference to the inhibition curve produced by dilutions of a sample of IgE-rich serum that had been calibrated against the WHO reference standard 68/341. The slope of this curve was similar to that produced by either IgE myeloma protein PS or the reference serum added to intestinal fluid (from which endogenous IgE had been removed by an anti-IgE–Sepharose immunosorbent). One international unit (i.u.) of IgE was equivalent to about 2 ng of IgE. Sensitivity of the assay was about 2 i.u. of IgE per millilitre.

*Ultracentrifugation analysis of IgE in intestinal fluids*

The four intestinal fluid specimens containing the largest quantities of IgE were analysed by sucrose gradient ultracentrifugation. 0·2 ml of fluid, to which human IgG and serum albumin had been added as markers, was applied to a 5–20% sucrose gradient and centrifuged at 35,000 rev/min for about 17 hr (4°C) in the swinging bucket rotor of a preparative ultracentrifuge (Beckman Model L, Beckman Instruments, Palo Alto, California). 0·5-ml fractions were collected by piercing the bottom of tubes. IgE was measured in duplicate

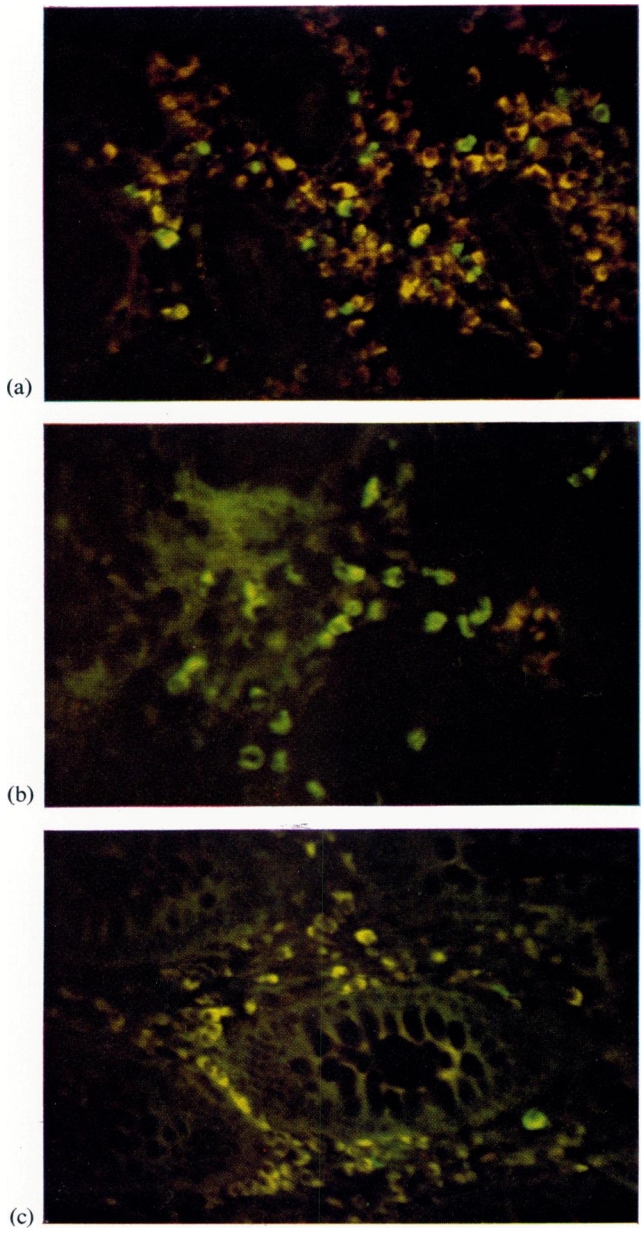


FIG. 1. Gastrointestinal mucosae reacted with TRITC-conjugated anti-IgA (yellow–orange) and FITC-conjugated anti-IgE (green). (a) Gastric mucosa. (Magnification ×175). (b) Base of an active duodenal ulcer. (Magnification ×280.) (c) Colon. (Magnification ×175.)

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0·2-ml samples of the fractions by the radioimmunoassay; IgG and albumin were measured by radial immunodiffusion.

## RESULTS

### *IgE-containing cells*

IgE-containing mucosal cells were clearly distinguished from cells of other Ig classes. FITC–anti-IgE staining was completely blocked by prior addition of unlabelled anti-IgE (either anti-IgE ND or anti-IgE PS), whereas unlabelled antibodies to IgG, IgM, IgA or IgD had no inhibitory effect. In addition, cells reacting with FITC–anti-IgE were contrasted with cells reacting with TRITC–anti-IgA (Fig. 1). The IgE immunocytes generally were in similar mucosal locations and had the same morphological features as immunocytes of other immunoglobulin classes. That is, they were within the lamina propria between secretory glands or beneath crypt epithelium and had typical plasma or lymphoid cell structure. In addition, IgE staining was occasionally seen at the tips of epithelial cells, perhaps in mucus attached to these cells.

IgE-containing cells were identified in all specimens examined, although they were less numerous than IgA, IgG or IgM immunocytes (Table 2). They comprised about 2% of the total immunofluorescent-positive cell population. Since negligible numbers of IgD-type cells were found, data concerning these cells are not included in Table 2. As is evident from the large standard deviations of cell counts, the numbers of IgE-containing cells in a given organ varied widely among individuals. IgE immunocytes also tended to be non-uniform in distribution within a tissue specimen and sometimes occurred in clusters. The total number of immunoglobulin-forming cells of all classes, the absolute number of IgE-containing cells, and the percentage of IgE-containing cells decreased along the bowel from stomach to rectum. Correspondingly, a slight but not statistically significant increase in the percentage of IgA-containing cells in the distal bowel was observed.

In gastric and duodenal specimens that contained peptic ulcers, IgE immunocytes were much more numerous (at least ten times) at bases of the ulcers than elsewhere in these tissues (Fig. 1b). (For estimates of IgE immunocytes given in Table 2, only data for tissues not adjacent to ulcers are included.) In rectal tissues, the numbers of IgE-type cells in two patients with Crohn's colitis and one with chronic ulcerative colitis were three to five times greater than in individuals with no or other colonic diseases.

### *IgE in small bowel fluids*

To confirm the specificity of the radioimmunoassay for IgE in intestinal fluids, portions of thirty fluids were reacted with a solid immunosorbent consisting of Sepharose 4B coupled to either rabbit anti-IgE PS or nonimmune rabbit γ-globulin. Residual inhibition of $^{125}$I-labelled IgE binding to anti-IgE in the assay by all anti-IgE-treated specimens (save for one very viscous, bile-rich specimen) corresponded to less than 3 i.u. of IgE/ml. Rabbit γ-globulin–Sepharose did not remove IgE activity. Specimens with less than 3 i.u. of IgE activity per millilitre were considered to contain no IgE.

Although we have expressed IgE content of fluids in i.u./ml, this expression should be considered meaningful only in relative terms because of the possibility that varied proportions of IgE fragments and intact IgE in the fluids had variable affinity for anti-IgE in the radioimmunoassay.

TABLE 2. Immunoglobulin-containing cells in gastrointestinal mucosae

| Organ | Number of cases | Total immunoglobulin-forming cells/ twenty high power fields (×400) | IgE-forming cells per twenty high power fields (×400) | Percentage of total immunoglobulin-containing cells | | | |
|---|---|---|---|---|---|---|---|
| | | | | IgE | IgA | IgM | IgG |
| Stomach | 14 | 860 (±251) | 29* (±30) | 3·0* (±2·9) | 77·8 (±6·7) | 13·1 (±4·6) | 6·1 (±2·3) |
| Duodenum–jejunum | 12 | 649 (±199) | 17† (±14) | 2·8† (±2·5) | 79·8 (±4·0) | 13·0 (±3·3) | 4·4 (±1·5) |
| Rectum–colon | 27 | 508 (±192) | 6 (±9) | 1·1 (±1·5) | 82·1 (±9·3) | 11·7 (±8·4) | 5·1 (±2·4) |

* Stomach *vs* rectum–colon, $P < 0{\cdot}005$.
† Duodenum–jejunum *vs* rectum–colon, $P < 0{\cdot}01$.

A measurable amount of IgE-reacting material was found in seventy-eight of eighty-five (92%) intestinal fluids (Fig. 2). In healthy individuals, the amount corresponded to 0–68 i.u./ml. There were no consistent differences between IgE content at various levels of the gut in individuals from whom multiple specimens were obtained. IgE concentrations were slightly but not significantly below control values in patients with Crohn's disease, intestinal parasites, bacterial overgrowth or adult coeliac disease. Serum IgE levels in these patients also did not differ significantly from normal (Brown, Lansford & Hornbrook, 1973). Mean serum IgE concentration in parasitized patients was 355 i.u./ml (normal = 106), and the maximum concentration was 1150 (normal = 1000).

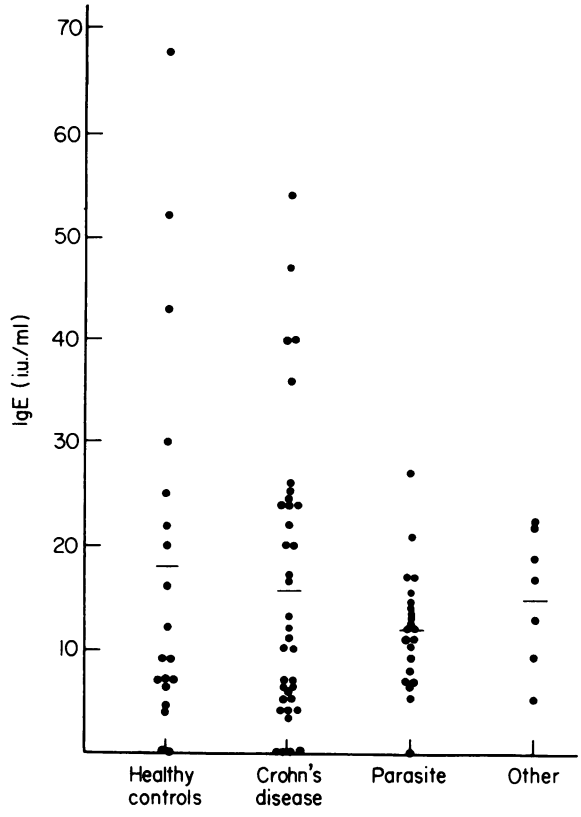


FIG. 2. IgE in small intestinal fluids from healthy control subjects and patients with Crohn's disease, intestinal parasites (*Entamoeba histolytica, Giardia lamblia, Trichuris trichiura*) or 'other' conditions (four patients with adult coeliac disease and three with intestinal bacterial overgrowth syndromes).

Analysis by sucrose gradient ultracentrifugation (Fig. 3) of four of the intestinal fluids revealed that nearly all IgE-reacting material was in fractions that corresponded to molecules of lower molecular weight than that of human serum albumin (69,000). In control specimens, IgE myeloma protein PS that was added to pooled intestinal fluids (treated with anti-IgE–Sepharose to remove endogenous IgE) was recovered in fractions corresponding to IgG.

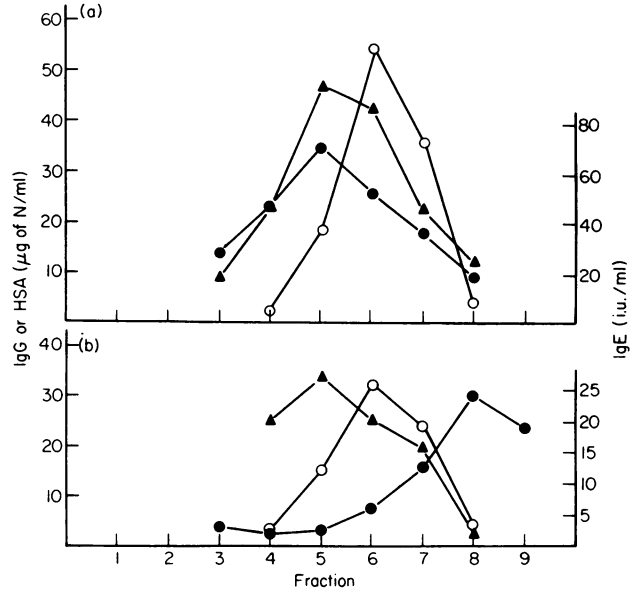


FIG. 3. Sucrose gradient ultracentrifugation of IgE (●) in intestinal fluid. (a) Control sample of pooled intestinal fluid to which IgE myeloma protein PS was added. (b) Intestinal fluid from a healthy individual, representative of four such fluids analysed. Purified human IgG (▲) and serum albumin (○) were added to all specimens as markers of known molecular weight.

## DISCUSSION

Results of this study convincingly demonstrate that human gastrointestinal tissues contain IgE-forming cells, and that IgE or a fragment of IgE is present in a high percentage of intestinal fluids.

Immunohistological aspects of this study are in fundamental agreement with those of Tada & Ishizaka (1970), who reported that IgE-containing cells in man and monkey are much more numerous in respiratory mucosa, gastrointestinal mucosa and regional lymph nodes than in spleen or subcutaneous lymph nodes. Those authors actually found IgE-type cells to be more numerous than IgG-type cells in human gastric and rectal mucosae. Although we found that IgG immunocytes slightly outnumbered IgE immunocytes, this minor difference in results might be explained by the larger number of specimens examined in our work, for the number of IgE mucosal immunocytes varies considerably among individuals.

Our findings contrast with those of three other studies. Savilahti (1972) found no IgE cells in about one-half of rectal and small bowel biopsies from children, and considerably fewer IgE cells than IgG cells in the remainder; Søltoft & Søeberg (1972) found IgE cells in only one-half of small intestinal biopsies from patients with hepatitis or minor digestive symptoms; Hobbs *et al.* (1969) found no IgE-containing cells in patients with coeliac disease or in control subjects. We have difficulty explaining the discrepant results of these studies and ours. Differences cannot be attributed to staining techniques, tissue fixation or antibody preparations employed. Direct antibody techniques were used in our study and by

Savilahti (1972) and Søltoft and Søeberg (1972). Those authors used frozen sections, whereas we used alcohol-fixed sections, but we have found IgE cells to be at least as numerous in frozen sections. We used rabbit antibodies prepared against IgE myeloma protein PS, whereas others (Savilahti, 1972; Søltoft & Søeberg, 1972) used sheep antibodies, presumably prepared against IgE myeloma protein ND, but both types of antibodies have been used successfully in measurement of IgE in serum (Gleich *et al.*, 1971; Polmar *et al.*, 1973) and thus evidently react well with the Fc component of normal IgE. Age of subjects could be a factor. Most of our subjects were adults, but those studied by Savilahti (1972) and probably many studied by Hobbs *et al.* (1969) were children. Conceivably, IgE intestinal immunocytes become more numerous after early childhood, as is reportedly true for IgA immunocytes (Savilahti, 1972).

The observation of more immunoglobulin-containing cells, including IgE cells, in proximal as compared to distal bowel may be evidence for more varied or intense antigenic exposure in the upper gut. Savilahti (1972) similarly observed in children that IgA, IgG and IgM immunocytes are more numerous in upper small bowel than in rectum.

Some of our observations concerning IgE cell populations in certain diseases deserve further attention, even though we have not studied enough patients within any one diagnostic category to provide conclusive evidence of disease-related alterations. The infiltration of tissue at bases of peptic ulcers with numerous IgE immunocytes suggests that a factor in ulcerogenesis could be IgE antibody-mediated release of histamine from mucosal mast cells. A similar immunohistological observation and increased IgE serum levels in peptic ulcer patients were reported in a recent abstract (Baird *et al.*, 1974). Likewise, increased numbers of IgE cells in rectal tissues from a few patients with Crohn's disease or chronic ulcerative colitis suggest a pathogenetic role for reaginic antibodies in these diseases, despite the existence of contradictory evidence (Watson & Bolt, 1968). That our observations reflect a non-specific involvement of IgE immunocytes in inflammation is, of course, possible.

Identification of IgE in intestinal fluids predominantly as a fragment of the intact molecule suggests that IgE in the upper gut is degraded by proteolytic enzymes, and corroborates previously reported suggestive evidence for degraded IgE in gut fluids (Waldman *et al.*, 1973). E myeloma proteins are cleaved readily by papain or pepsin (Bennich & Johansson, 1967; Ishizaka & Newcomb, 1970). It seems reasonable, therefore, that the ε-containing degradation product in gut fluids contains a fragment(s) of the Fc portion of IgE (Bennich & Johansson, 1967; Ishizaka & Newcomb, 1970).

It is not known whether the IgE in intestinal fluids is transudated serum IgE or is locally produced by mucosal immunocytes. Comparisons between concentration ratios of IgE:IgG or IgE:albumin in serum and similar ratios in nasal washings, sputum, saliva and tracheobronchial fluids indicate that IgE in these secretions is produced locally (Deuschl & Johansson, 1974; Newcomb & Ishizaka, 1970; Waldman *et al.*, 1973). Similar evidence in support of intestinal fluid IgE as a secretory immunoglobulin is impossible to obtain because all three of the above proteins are degraded by proteolytic enzymes.

Failure to find increased amounts of IgE in intestinal fluids of patients infected with *Giardia lamblia*, *Entamoeba histolytica* or *Trichuris trichiura* provides no evidence for involvement of IgE antibodies in these parasitic diseases. This is not an unexpected finding, however, because in contrast to certain helminthic infections (Johansson, Mellbin & Vahlquist, 1968; Rosenberg *et al.*, 1970; Rosenberg, Polmar & Whalen, 1971), serum IgE levels in these diseases usually are not elevated (Brown *et al.*, 1973). Intestinal fluid

IgE evidently has not been measured in parasitized patients with very high serum IgE concentrations.

The biological role of IgE in external body fluids is not well understood; but the suggestion has been made, from study of roundworm eradication in rats, that reaginic antibodies promote transfer of protective antibodies into intestinal fluids by release of vasodilator substances from mucosal mast cells (Murray, 1972). Existence of such a mechanism is unconfirmed (Ogilvie & Jones, 1971) but could be a means through which IgE antibodies serve beneficial purposes. IgE antibodies in bowel fluids or tissues could also participate in allergic-type reactions with certain food antigens (Heiner & Rose, 1970). Perhaps, however, fragmented IgE in intestinal fluids is biologically inactive, because Fc′ fragments of E myeloma proteins cannot block passive cutaneous sensitization by reaginic antibodies (Ishizaka & Newcomb, 1970; Stanworth *et al.*, 1968). In any event, the presence of IgE-forming cells in human gastrointestinal tissues, and IgE or a fragment of IgE in intestinal fluids suggests that IgE antibodies could be important in physiological or pathological reactions in the bowel. Study of this possibility is indicated.

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