

# IgE Levels in Intestinal Juice

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*This investigation was performed to determine the possible significance of the IgE levels in intestinal secretions for the recognition of type I food allergy (immediate hypersensitivity). Four groups of individuals were studied and compared with each other: (1) healthy controls and patients with gastrointestinal disorders not affecting the small intestine; (2) patients with small intestinal disease; (3) patients with various manifestations of allergy but without food intolerance; and (4) patients with atopic sensitivity to foods. Despite the presence of proteolysis in the intestinal fluid, a statistically highly significant increase of the intestinal IgE level was observed in patients of group 4 with type I food allergy and in patients of group 3 as compared with normals. This was associated with an increase in the serum IgE level. An increased synthesis of the IgE in the lamina propria of the small intestine is most probable, but not yet adequately established.*

Since the discovery of immunoglobulin E (IgE) almost simultaneously by Johansson (1) and by Ishizaka and Ishizaka (2), it has been demonstrated that the IgE globulin is associated with reaginic activity. Early studies on serum IgE revealed a definite correlation between elevated IgE levels and allergic disorders or parasitic infestations (3-5). Ishizaka and Ishizaka reported its presence in nasal secretions. In 1970, Tada and Ishizaka (6) showed that plasmacytes synthesizing IgE were distributed essentially in the tonsils, adenoid tissue, mucous membranes of the bronchi and intestine, and in the lymph nodes. This was confirmed by Yunginger and Gleich (7) and by Waldmann et al (8). IgE was found to be present also in other secretions and body fluids such as tears (9, 10) bronchial mucus (8), saliva (11), milk (12), cerebrospinal fluid (13), urine (11, 14, 15), pancreatic (16) and intestinal juices and serum (11, 17, 19).

Comparative studies of the ratios IgE/IgG, IgE/albumin, and IgE/total proteins in both serum and secretions have suggested a local synthesis of IgE in the latter. Thus, the presence of IgE in the secretions is probably not a simple transmural transfer of serum IgE. The local synthesis of IgE was found to be high in the mucous membranes of patients with allergic rhinitis or nasal polyps (7, 20, 23) and inflammation of the middle ear (24), in the bronchial mucous membranes of patients with asthma (25), and in the mucous membranes of the intestinal tract (26-29).

The question arises whether the local synthesis of IgE in the digestive mucous membranes is related to the inflammation of the mucosae, to the general allergic background or to food allergy.

Our purpose in this study is to evaluate in healthy individuals and patients with various digestive disorders: (1) the level of IgE in the intestinal juice of normals and patients with various manifestations of type I food allergy of the Gell and Combs (30) classification; (2) the relationship between the inflammation and atrophy of the intestinal mucous membranes and the intestinal IgE levels; and (3) the feasibility of the detection of the intestinal atopy by measurement of the intestinal IgE, using methods available for its assay.

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TABLE 1. IDENTIFICATION OF PATIENTS IN GROUP 4A\*

| Name | Sex | Age | Food allergens  | Cutaneous tests<br>(IDR or prick tests) | PK tests       |            | In the serum  |                    | Rabbit basophil<br>degranulation<br>tests<br>(Shelley) | Sublingual<br>and oral<br>challenge tests | Type of<br>allergy‡ |
|------|-----|-----|-----------------|-----------------------------------------|----------------|------------|---------------|--------------------|--------------------------------------------------------|-------------------------------------------|---------------------|
|      |     |     |                 |                                         | For<br>IgG-STS | For<br>IgE | RAST<br>score | IgE†<br>(units/ml) |                                                        |                                           |                     |
| IMH  | ♀   | 42  | Fish, shellfish | IDR                                     | ++<br>++++     |            |               | 264                | ++                                                     | Hives, Quincke edema                      | I                   |
| SCH  | ♀   | 26  | Wheat, fish     | IDR                                     | —<br>—         |            | 0             | 55                 | ++<br>++                                               | Migraine, postprandial<br>vomiting        | III                 |
| THI  | ♂   | 40  | Shellfish       | IDR                                     | ++++           |            | 2             | 11000              |                                                        | Hives                                     | I                   |
| KRA  | ♂   | 16  | Egg             | IDR                                     | ++++           |            | 1             | 176                | +                                                      | Anaphylactic shock                        | I                   |
| BOD  | ♂   | 23  | Fish            | IDR                                     | ++             |            | 0             | 330                | +                                                      | Negative                                  | I                   |
| VUL  | ♀   | 42  | Egg             | IDR                                     | ++             | —          | 0             | 330                | +                                                      | Hives                                     | I                   |
| LAN  | ♂   | 25  | Mustard         | IDR                                     | ++++           |            |               |                    |                                                        | Anaphylactic shock                        | I                   |
| VIA  | ♂   | 18  | Shellfish       |                                         |                |            | 2             | 550                |                                                        | Abdominal pain,<br>vomiting               | I                   |
| MAS  | ♀   | 34  | Wheat           | IDR                                     | +++            | +(1/16)    | 3             | 1980               | +                                                      | Asthma                                    | I                   |
| BRI  | ♀   | 72  | Shellfish       | IDR                                     | —              |            | 2             | 10                 |                                                        | Cystitis, hematuria                       | I                   |
| ZOU  | ♀   | 35  | Vegetables      | IDR                                     | ++             | —          | 2             | 140                | —                                                      | Quincke edema (face)                      | I                   |
| HEI  | ♂   | 50  | Amarant dye     |                                         |                |            |               | 198                |                                                        | Hives                                     | I                   |
| ROS  | ♀   | 40  | Swiss cheese    | IDR                                     | ++             |            |               | 22                 |                                                        | Hives                                     | I                   |
| DEW  | ♀   | 22  | Celery          | PRICK                                   | +              | +          |               | 550                |                                                        | Quincke edema                             | I                   |
| SIM  | ♂   | 40  | Shellfish       | PRICK                                   | ++             | +          |               | 308                | —                                                      | Hives, Quincke edema                      | I                   |
| JAL  | ♀   | 42  | Egg             | PRICK                                   | ++             | +(1/8)     | 4             | 121                | +                                                      |                                           | I                   |
| GIR  | ♂   | 18  | Celery          | PRICK                                   | ++             | +          |               | 286                |                                                        |                                           | I                   |
| GAS  | ♀   | 33  | Shellfish       | PRICK                                   | ++             |            |               |                    |                                                        | Anaphylactic shock                        | I                   |

\*Abbreviations used: IDR: intradermal reaction; PK: Prausnitz-Küstner reaction; STS: short-term sensitizing; LTT: lymphoblast transformation test; RAST: radioallergosorbent test; RIST: radioimmunosorbent test.

†Determined by the RIST.

‡Gell and Combs classification.

## MATERIALS AND METHODS

### Patients

One hundred seventy-four individuals were studied. These were divided into four groups:

**Group 1.** Controls, comprising 21 healthy individuals and 25 patients with gastrointestinal disorders not affecting the small intestine, such as diseases of the colon, gastritis, and alcoholic liver cirrhosis.

**Group 2.** Patients with small intestinal ailments, comprising 21 individuals with celiac disease, malabsorption syndrome, or Crohn's disease. Four patients with intestinal parasitosis were not included in this group and were studied separately.

**Group 3.** Atopic patients comprising 45 individuals with various allergic manifestations from non-gastrointestinal organs (rhinitis, asthma, atopic eczema, and urticaria) and with no food intolerance and gastrointestinal symptoms.

**Group 4.** Atopic patients comprising 62 individuals with chronic urticaria or immediate allergic reactions following ingestion of foods (urticaria, Quincke's edema, asthma, anaphylactic shock). These patients were subdivided into two subgroups:

**Subgroup 4A.** This group was comprised of 17 individuals with type I food allergies. This was confirmed by skin tests: intradermal or prick test (31, 32), Prausnitz-Küstner test for IgE (33), and serum IgE (34), RAST test (35), lymphoblast transformation test (LTT) (36) test of

degranulation of rabbits basophils [Shelley test (37)], and sublingual or oral challenge test (38, 39). (Table 1).

**Subgroup 4B.** This group was comprised of 45 individuals with chronic urticaria caused by an unbalanced diet, consumption of foods rich in histamine or having histamine-releasing properties, and presenting digestive clinical problems. These individuals were often affected by colonic fermentation and did not tolerate well the introduction by tube of histamine chloride, 2.75 mg/kg body weight, into the small intestine, which is normally well tolerated. In these patients, there was a correlation between the alimentary factors and histamine reaction, but, strictly speaking, the latter response does not pertain to the immediate type of allergy (40).

None of our patients in these four groups had watery diarrheas, which would suggest hypersecretion of intestinal juice or transsudation of the water from the plasma.

### Intestinal Juice Aspiration

A duodenojejunal tube was introduced down below the Treitz angle in patients fasted for 12 hr. The intestinal juice, essentially representing baseline secretion, was withdrawn without hormonal stimulation at intervals of 15 min for 1 hr. The volume collected varied from 2 to 20 ml in various patients. The secretions were immediately mixed with phosphate buffer solution (0.1 M, pH 7.5) containing 0.2% w/w sodium azide and 10,000 units/ml of Zymofren, in a proportion of 3 ml buffer per 10 ml of secre-

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TABLE 2. COMPARISON OF THE INTESTINAL IGe LEVELS OBTAINED WITH PHARMACIA RIST AND PRIST METHODS\*

| Sample | IGe (RIST)<br>(units/ml) | IGe (PRIST)<br>(units/ml) |
|--------|--------------------------|---------------------------|
| 129    | 34                       | 3.2                       |
| 130    | 50                       | 2.8                       |
| 131    | 19                       | 2                         |
| 132    | 120                      | 4                         |
| 133    | 100                      | 2.6                       |
| 134    | 105                      | 8                         |
| 135    | 22                       | 2.4                       |
| 136    | 220                      | 3.2                       |
| 137    | 230                      | 2.6                       |
| 138    | 26                       | 3                         |
| 139    | 190                      | 2                         |
| 140    | 370                      | 3.8                       |
| 141    | 380                      | 24                        |
| 143    | 22                       | 2.4                       |
| 146    | 165                      | 2.4                       |
| 147    | 50                       | 2.4                       |
| 149    | 170                      | 2.5                       |
| 150    | 160                      | 2.4                       |
| 152    | 165                      | 2                         |
| 153    | 185                      | 2.2                       |
| 157    | 180                      | 6                         |
| 158    | 42                       | 2.4                       |
| 161    | 127                      | 2.8                       |

\*1 unit (IGe) = 2.42 ng (World Health Organization Reference Standard 68-341 for IGe, 1970).

tion. Zymofren is a basic biological polypeptide extracted from the ox lung, with 16 amino acids, of molecular weight 6500 daltons. It was used here as an inhibitor of proteases and suppressed the action of trypsin, chymotrypsin, and fibrinolysin, which may destroy IGe. The final dilution of each sample was 1.36. The samples were stored at  $-25^{\circ}\text{C}$ , and subsequently treated by the technique of Girard and de Kalbermatten (28). After thawing and centrifugation at 12,000 rpm for 30 min to remove cell fragments, the juices were dialyzed overnight at  $4^{\circ}\text{C}$  against distilled water, and recentrifuged again. No further concentration was necessary for the measurement of the IGe.

### Measurement of IGe

The IGe levels were determined using two radioimmunoassay methods: (1) the competitive method of radio immunosorbent test RIST (41), which we used in all the samples; (2) the sandwich method of paper-disk radio immunosorbent test PRIST (42), which we used in 23 of these samples for comparative purpose.

## RESULTS

**Comparison of Intestinal IGe Levels as Determined by the RIST and PRIST Methods.** The two methods were compared in 23 samples from the patients of all four groups taken at random. The results are shown in Table 2. A clear-cut discrepancy of the two methods is apparent. The variations in concentration of IGe found in intestinal secretions of vari-

ous individuals are similar to those found in milk (43) and to lesser extent in serum (43-45). The IGe levels as determined by the PRIST method were always much lower than those found by RIST and amounted to about 5% of RIST values on average. As will be discussed later, the PRIST method can identify complete IGe molecules only, which is not the case with the RIST technique. The low levels obtained by the PRIST method suggest an almost total proteolysis of the IGe molecules in the intestinal juice. The comparative study of intestinal IGe in the four groups of patients was therefore performed with RIST which detects both the digested and nondigested IGe molecules.

**Intestinal IGe Levels as Determined by RIST.** The mean levels of intestinal IGe in the four groups studied were evaluated statistically using the Student-Fischer test. They are listed in Table 3. The individual values of IGe in the group 4A are listed in Table 4.

Diseases of the small intestine (group 2) did not cause a significant change in RIST values, the mean being  $87 \pm 126$  units/ml. There was a nonsignificant elevation of intestinal IGe level in non-gastrointestinal atopy (group 3), with a mean of  $110 \pm 114$  units/ml and a  $P$  value of  $< 0.03$ , as compared with group 1. There was, however, a high increase of the RIST values in cases of type I food allergy (group 4A). Here, the mean value was  $261 \pm 494$  units/ml, and the difference from the mean normal value in controls of group 1 was statistically highly significant ( $P = 0.01$ ) despite the wide spread of values. The comparison of group 4A with group 3 shows also a statistically significant difference ( $P = 0.05$ ). Group 4B did not show a statistically significant difference when compared with normal group 1 or groups 3 or 4A.

Since the individual variations in IGe levels were considerable among patients presenting type I food allergy (group 4A), we have considered only levels significantly elevated as suggestive of this type of allergy.

**Intestinal Parasitosis.** Five patients with active intestinal parasitosis (*Necator americanus*, ankylostoma duodenale, and bilharzia) were studied. The levels of intestinal IGe were normal in three patients (25-62 units/ml) and highly elevated in two (1162-1700 units/ml) (Tables 5 and 6).

In the case of bilharziosis (*Schistosoma mansoni*), the IGe levels correlated well with the effectiveness of the therapy. After an initial unsuccessful treatment with Niridazole, the IGe rose

TABLE 3. COMPARATIVE VALUES OF INTESTINAL IgE IN THE FOUR GROUPS OF PATIENTS

| Group | Number of cases | Mean IgE level (RIST)<br>with standard deviation (unit/ml) | Student's t test |                 |
|-------|-----------------|------------------------------------------------------------|------------------|-----------------|
|       |                 |                                                            | Reference group  | P values        |
| 1     | 46              | 68 ± 69                                                    |                  |                 |
| 2     | 21              | 87 ± 126                                                   | 1                | Not significant |
| 3     | 45              | 110 ± 114                                                  | 1                | <0.03           |
|       |                 |                                                            | 4A               | =0.05           |
|       |                 |                                                            | 4B               | Not significant |
| 4A    | 17              | 261 ± 494                                                  | 1                | <0.01           |
| 4B    | 45              | 110 ± 145                                                  | 1                | Not significant |
|       |                 |                                                            | 4A               | <0.06           |

above 1100 units/ml, and five precipitin lines were observed on double-immunodiffusion test of the serum against the *Schistosoma* antigens. Following successful treatment with Niridazole and Pyrantel, the IgE level decreased to 100 units/ml and only one precipitin line was detectable in the serum at that time (Table 6). It increased again one year later despite clinical recovery. Serum IgE levels were consistently high but lower than in the ankylostoma case.

**Comparison of IgE Levels in Serum and Intestinal Juice.** The comparison of the serum and intestinal IgE levels in Table 4 of the same group of patients with food allergy indicates that there is no significant correlation between them. However, in 11 of these cases, serum levels were higher than intestinal ones, sometimes by an order of almost 100- to 200-fold (cases MAS and KRA). Only in two cases the levels were about the same and in two they were lower.

## DISCUSSION

The considerable difference existing between the IgE levels measured by the PRIST and RIST methods poses the problem of the interpretation of results yielded by each of these techniques. The results appear to depend on the degree of fragmentation of the IgE molecule by proteolytic agents in the intestinal lumen (46).

As has been shown by Bennich and Von Bahr-Lindstrom (47) (Figure 1), the extreme  $F_c$  of the IgE molecule carries two binding sites for anti-IgE that correspond to similar regions of each of the two heavy chains. This region has been named  $F''_c \in 1$ . The PRIST technique requires that these two sites be retained on the molecule. One permits the binding of IgE to anti-IgE fixed on the paper disk, the other permits its binding to [ $^{125}$ I] anti-IgE, added

subsequently to the reaction medium. This method necessitates, and therefore can detect only, intact IgE molecules.

In contrast, the RIST technique requires only one fixation site to operate, when combined with anti-IgE fixed on Sephadex. Thus, the isolated fragments  $1/2 F''_c \in 1$ , resulting from the degradation of the IgE can be detected and counted by this method. In other words, RIST permits determination of a mixture of intact and fragmented IgE without discriminating among them. The values obtained by RIST are always greater than those obtained with PRIST, because if  $\alpha$  designates the fraction of IgE digested by proteolysis ( $\alpha < 1$ ), PRIST will detect only  $(1 - \alpha)$  IgE molecules while the RIST will recognize  $(1 - \alpha) + 2\alpha$ , ie,  $(1 + \alpha)$  IgE molecules.

From Table 2 it is apparent that about 95% of IgE is destroyed on the average by enzymatic proteolysis in intestinal juice *in vivo*. The existence of such a proteolysis is likely because IgE does not possess a protective secretory component as does the secretory IgA (48). IgE molecules become fragmented into portions  $F_{ab}$  and  $F_c$  within  $F''_c \in 1$ . This

TABLE 4. LEVELS OF INTESTINAL AND SERUM IgE IN 17 CASES OF TYPE I FOOD ALLERGY

| Patient | IgE (units/ml)* |       | Patient | IgE (units/ml) |       |
|---------|-----------------|-------|---------|----------------|-------|
|         | Intestine       | Serum |         | Intestine      | Serum |
| IMH     | 40              | 264   | BRI     | 22             | 10    |
| SCH     | 175             | 55    | ZOU     | 219            | 140   |
| THI     | 1756            |       | HEI     | 11             | 198   |
| KRA     | 60              | 11000 | ROS     | 40             | 22    |
| BOD     | 1320            | 176   | DEW     | 40             | 550   |
| VUL     | 85              | 330   | SIM     | 65             | 308   |
| LAN     | 198             |       | JAL     | 43             | 121   |
| VIA     | 300             | 550   | GIR     | 36             | 286   |
| MAS     | 22              | 1980  |         |                |       |

\*Determined by RIST.

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TABLE 5. IgE IN CASES OF INTESTINAL PARASITES

| Patient | Parasites                       | Intestinal IgE<br>(units/ml)* | Serum IgE<br>(units/ml)* |
|---------|---------------------------------|-------------------------------|--------------------------|
| ORB     | <i>Necator americanus</i>       | 64                            | 154                      |
| ETO     | <i>Necator americanus</i>       | 52                            | 88                       |
| KAR     | Ankylostomiasis, anguilluliasis | 1700                          | 39                       |
| SYL     | Ankylostomiasis, leprosy        | 25                            | 4180                     |

\*Determined by RIST.

proteolysis in the intestinal juice had already been suspected by Brown et al (46) and by Waldmann et al (8). It has been noted during the assay of intestinal IgE by the radio immunodiffusion technique, when the ring precipitation of the standard IgE solutions was disturbed when the neighboring well contained intestinal juice. This phenomenon could be explained by the more rapid diffusion of the free fragments  $F''_c$  than that of the intact IgE. This would saturate the anti-IgE contained in the gel adjacent to the wells containing the standards. More recently, Brown and Lee (17) confirmed this hypothesis by discovering in the intestinal fluid nu-

merous fragments having a molecular weight of 40,000 daltons and carrying antigenic determinant E, while intact IgE has a molecular weight of 190,000. This explained the initial failures in detecting IgE in intestinal secretions (8). Nevertheless, in 1975, Brown et al (49), using the double-antibody radioimmunoassay (50), demonstrated IgE in the intestinal secretions. These levels are much lower, however, in the majority of our cases, than in the serum, even when determined by RIST (Tables 1 and 4).

There are doubts among some immunologists and allergists whether RIST is truly measuring antigenic

TABLE 6. IgE IN A PATIENT WITH BILHARZIOSIS (*Schistosoma mansoni*)

| Date<br>and<br>treatment       | Presence<br>of<br>fertilized<br>eggs<br>in feces | IgE (units/ml)* |       | Eosinophils<br>in<br>peripheral<br>blood | Precipitin<br>lines in<br>the serum† | Clinical<br>state                                                              |
|--------------------------------|--------------------------------------------------|-----------------|-------|------------------------------------------|--------------------------------------|--------------------------------------------------------------------------------|
|                                |                                                  | Intestine       | Serum |                                          |                                      |                                                                                |
| May 1975<br>after Niridazole   | +++                                              |                 |       |                                          | 6                                    | Intestinal<br>bilharziosis<br>with hepatic<br>involvement<br>by laparoscopy    |
| Sept. 1975<br>after Niridazole | 0                                                | 1162            |       | 0%                                       | 5                                    | Normal liver<br>by laparoscopy,<br>abdominal pain,<br>intermittent<br>diarrhea |
| Jan. 1976<br>after Pyrantel    | 0                                                |                 | 1980  | 12%                                      | 4                                    | Normal liver<br>by laparoscopy,<br>abdominal pain,<br>intermittent<br>diarrhea |
| April 1976<br>after Pyrantel   | 0                                                | 100             |       | 33%                                      | 1                                    | Normal liver<br>by laparoscopy,<br>abdominal pain,<br>intermittent<br>diarrhea |
| Sept. 1976                     | 0                                                |                 | 2680  | 16%                                      | 2                                    | Clinical<br>recovery                                                           |
| April 1977<br>after Pyrantel   | 0                                                | 4682            | 1760  | 5%                                       | 2                                    | Clinical<br>recovery                                                           |
| Jan. 1978                      | 0                                                | 580             | 2640  | 2%                                       | 1                                    | Clinical<br>recovery                                                           |

\*Determined by RIST.

†In Ouchterlony immunodiffusion against antigen.

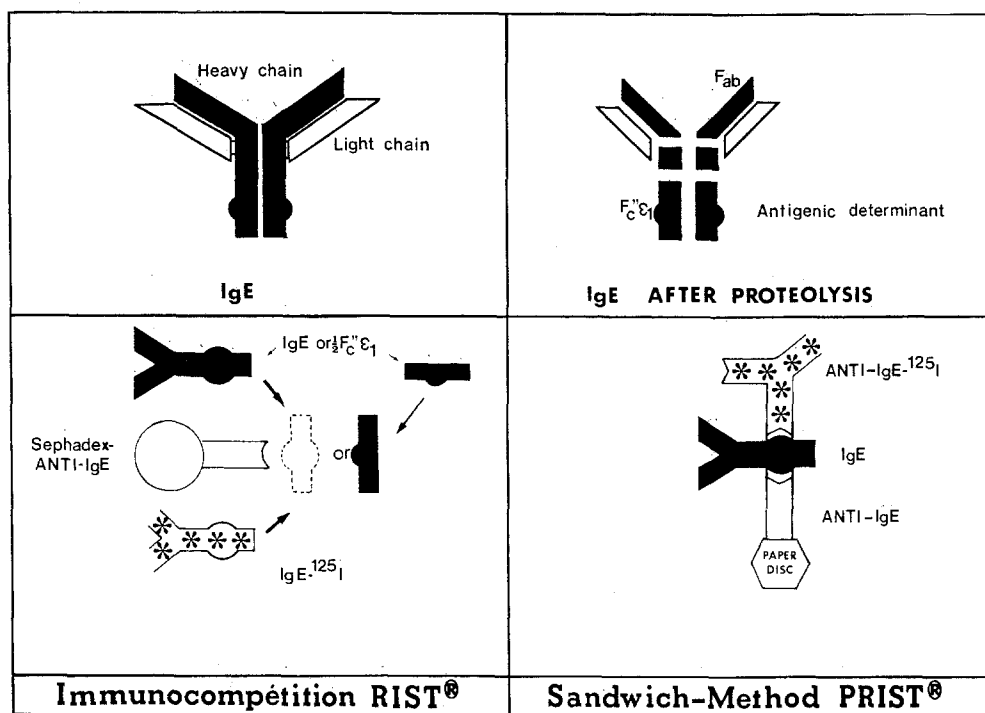


Fig 1. IgE in digestive secretions.

determinants of IgE. Underdown et al (51) have noted that RIST may give spuriously elevated levels of IgE in certain secretions. It would be therefore advisable to demonstrate that what we measure by the RIST procedure are really antigenic determinants of IgE. Two types of experiments were performed to this aim:

Since the IgE present in the sera of dog, cat, or guinea pigs is not cross-reactive with human IgE, it was of interest to determine whether intestinal secretions of one of these species would produce inhibition in RIST. To this aim we have used human intestinal secretion (HIS) containing 35 units/ml of IgE, as determined by the RIST method, and cat intestinal secretion (CIS), known not to cross-react with IgE. In the first set of experiments, listed in Table 7, we added increasing volumes of CIS to constant volumes of HIS, keeping the total volume of the mixture constant by addition of saline buffer solution. This progressive dilution of HIS by non-reactive CIS did not change the results of the RIST assay in any significant way. (The mean IgE concentration, which was 35 units/ml before addition of CIS, was 37.7 units/ml on the average after the addition of CIS, at 10 different concentrations.)

In the second experiment (Table 8) we have

shown that the treatment of HIS with Sephadex solid-phase anti-IgE causes removal of the reactivity of the IgE in the RIST assay of HIS. This is shown by the reduction of the IgE level in the RIST assay from 35 units/ml to less than 5 units/ml, on the average.

These two sets of data indicate that we are able to measure human IgE antigenic determinants by the RIST procedure.

The measurement of the IgE in the intestinal juice raises the issues of its adequacy. This is a part of the general problem of how to reliably measure antibody levels in secretions. This general problem has been extensively discussed by Shearman et al (52) in regard to the issue of its appropriateness in view of its important implications in disease.

The first prerequisite is a uniform way of dealing with the antibody assay. This has been attended to in all our samples by election of the RIST method as the proper choice for dealing with antibody assay in intestinal secretions. Its advantages over the PRIST method have been discussed, as well as its specificity for the measurement of the antigenic determinants of the human IgE. It should be also noted that the potential problem of the formation of non-specific protein precipitates unrelated to specific

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TABLE 7. NO INHIBITION OF RIST ASSAY BY ADDITIONAL DOSES OF CAT INTESTINAL SECRETION

|                                      | 50  | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50  | Mean |
|--------------------------------------|-----|----|----|----|----|----|----|----|----|----|-----|------|
| Volume of HIS ( $\mu$ l)             | 0   | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 50   |
| Volume of CIS ( $\mu$ l)             | 100 | 90 | 80 | 70 | 60 | 50 | 40 | 30 | 20 | 10 | 0   | 55   |
| Volume of buffered saline ( $\mu$ l) | 150 |    |    |    |    |    |    |    |    |    |     | 45   |
| Total volume ( $\mu$ l)              | 35  | 36 | 44 | 45 | 36 | 42 | 30 | 30 | 38 | 40 | 36  | 150  |
| IgE determined by RIST* (units/ml)   |     |    |    |    |    |    |    |    |    |    |     | 37.7 |

\*Each assay was performed in duplicate. HIS: human intestinal secretion; CIS: cat intestinal secretion.

antiserum does not exist in regard to the RIST assay, since it is not an immunoprecipitating technique.

The other problem most difficult to answer is the choice of the point of reference for the Ige value in the intestinal secretion, ie, whether this should be calculated per unit of protein, per unit of volume of the intestinal juice, or per total protein content or total volume of intestinal secretion. Because of the continuous degradation of the proteins in the small intestine, which is one of the main catabolic organs of the body, this determination cannot be done per unit of protein but only per unit of nitrogen (Kjeldahl) or tyrosine (Lowry) in the intestinal juice. Alternatively, this could be done simply in reference to the units of volume of the intestinal juice, a point of reference which we have elected and preferred to the questionable significance of the partly degraded protein content of the intestine. The other alternative is to refer the Ige content to the total volume or to the total nitrogen or tyrosine secretion of a segment of intestine under investigation. This can be done only with the use of perfusion methods which require special instrumentation and methodological training. The choice of the point of reference must therefore depend on the equipment and training of the investigator and on the aims of the study. The final solution of this problem must await development of an adequate technique to prevent the proteolytic degradation of the proteins of the small intestinal content.

Which method, then, should one choose for Ige detection in the intestinal juice? Although it is true that only the PRIST method represents a rigorous assay of intact Ige, its low content in the intestinal juice prevents its being considered as a significant parameter for the recognition of the food allergy. In contrast, the RIST technique, by also detecting the fragments  $F''_c$  of the Ige, informs us about the total amount of Ige present, including that which has been digested. As long as one could not completely suppress the Ige proteolysis, the use of RIST is justified. Some of the other techniques,

such as continuous perfusion of the mucosa with buffer and aspiration of the perfusate (53) hint of the possibility of reducing the proteolysis in this way by about 70%. This is still inadequate, however.

The mean level of intestinal Ige in our control group 1 was  $68 \pm 69$  units/ml by the RIST which is higher than the 17 units/ml (range 4-68 units/ml) obtained by Brown et al (49) with the double-antibody radioimmunoassay method. The same is true for the mean value of 27 units/ml (range 3-51 units/ml) obtained by Jones et al (18).

From the current investigation it appears that nonspecific inflammations of the small intestinal mucosa (celiac disease, Crohn's disease) do not change intestinal Ige levels. In contrast, parasites in the intestine may at times cause high Ige values in the intestinal juice. The relation between parasites and elevated intestinal Ige levels is evident in the case of bilharziosis here reported, in which the recovery was accompanied by a significant drop of the Ige level in intestinal juice. It is possible that the sequential assay of Ige in intestinal secretions may correlate with the effectiveness of the treatment of the intestinal parasites. This therapeutic effect may be suspected because of an increasing eosinophilia probably related to the passage of antigenic material into the circulation from lysis of parasites.

It is of interest that the atopy may be associated with a significant increase of intestinal Ige level. This has been noted by others in the serum, although there was no correlation between the intestinal and serum Ige in the same individual. The increase of Ige levels in the nasal and bronchial secretions of atopic individuals has been recognized

TABLE 8. REMOVAL OF REACTIVITY IN RIST BY TREATMENT OF HIS WITH SEPHADEX ANTI-IGE SOLID PHASE

|                 | Before treatment | After treatment |
|-----------------|------------------|-----------------|
| IgE (units/ml)* | 35               | <5              |

\*Mean of triplicate assays.

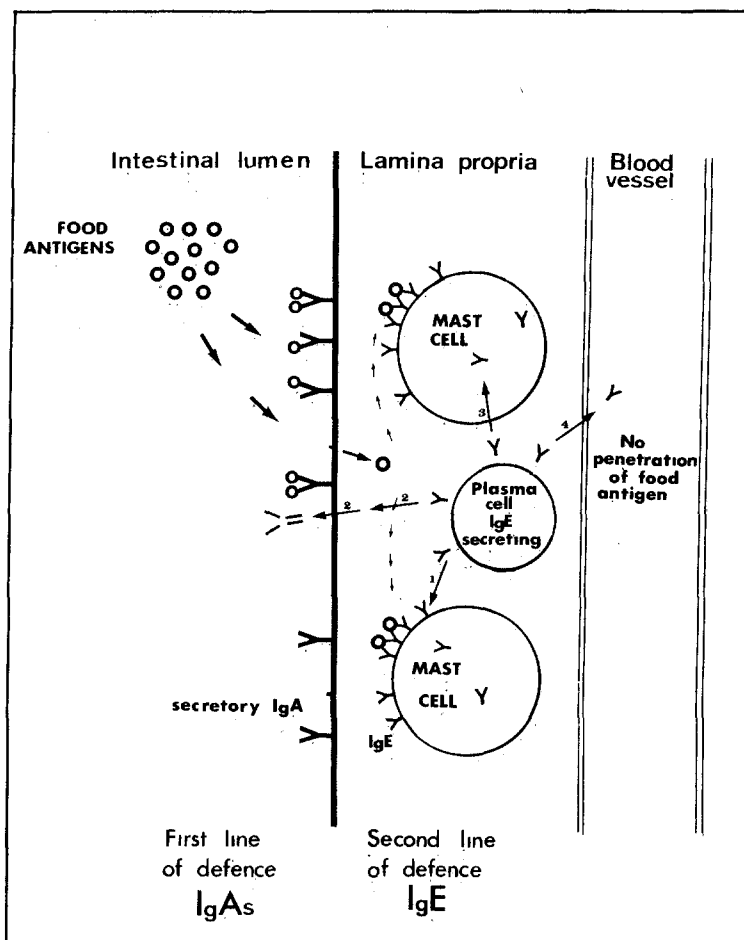


**Fig 2.** Hypothesis on the role of intestinal immunoglobulins IgE and IgAs: (1) Fixation on the surface of the local mast cells; (2) penetration in the intestinal lumen, proteolysis; (3) phagocytosis by local mast cells; and (4) penetration in the circulation.

by several authors (7, 11, 20, 54). Our group of 17 subjects with type I food allergy, which was well-documented by challenge tests, RAST, degranulation of basophils, and positive LTT, has demonstrated a statistically significant increase of the intestinal IgE levels as compared with values found in nonatopic subjects. These were also significantly increased, but to a lesser extent, as compared with atopic patients without food allergy and those presenting with histaminic disturbances following abnormal crossing of histamine through the intestinal mucosa.

This makes one think that a moderate increase of intestinal IgE could relate to a hyperpermeability of the small intestinal mucosa, resulting in the transudation of IgE. This is not necessarily due to a local allergic antigen-antibody reaction. We believe, however, that frankly elevated IgE levels in the in-

testinal juice in type I food allergy should be considered a reflection of the antigenic stimulation and a local allergic response. This could be confirmed by the demonstration of an antifeed antibody at the site of the IgE level elevation. This would require, however, a sampling with optimally reduced proteolysis of the intestinal IgE.

Since the physiological proteolysis degrades the IgE *in vivo* as soon as it passes from the lamina propria into the intestinal juice (the IgE is unprotected, as is the case with secretory IgA), this makes the active role of IgE in the intestinal lumen rather improbable. Since, however, the increase of IgE concentration in intestinal fluid may be the reflection of IgE synthesis in the lamina propria, one may consider that it is at this level that IgE plays a role against the atopic antigens. The results of the study of the jejunal mucosa of two nursed infants having

milk allergy and examined before and after provocation of the allergy symptoms is in line with this hypothesis (55).

The role of IgE in the mucosa of the digestive tract of patients with type I food allergy is under discussion. Various studies have clearly shown the development of antialimentary secretory IgA during experimental immunization with alimentary products. These antibodies may play a protective role in the intestinal lumen by noticeably decreasing the passage of native antigenic molecules across the mucosa (56-62). One may hypothesize that local IgE forms a "second line" of defense (Figure 2) by fixing itself on local mast cells and thus keeping back the food antigens at the level of the lamina propria. This would have no pathological consequence since it has been shown that the mast cells of the intestinal mucosa release histamine only with much difficulty (63). The locally synthesized IgE would have four possible destinies: (1) fixation on the surface of local mast cells permitting them to play their protective role by "trapping of antigen" by recognized reaginic antibodies in type I food allergy; (2) elimination by passage into the intestinal lumen where they undergo proteolysis; (3) destruction by local mast cells by phagocytosis (64); and (4) passage into the circulation which could lead to their fixation on the blood basophils and on the mast cells of other tissues (mainly in the skin and bronchi).

Of these four alternatives, only the first one would be of significance for the development of allergic reaction at the site of the intestine.

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