

Isotopic and Enzymatic IgE Assays in Non-Allergic Subjects

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In order to establish normal values in an adult population for serum IgE concentration, sera were obtained from 446 ambulatory Canadian caucasian subjects with negative allergy histories. A standard isotopic procedure, the Phadebas paper radioimmunosorbent test (PRIST), was compared with the new enzymatic counterpart, the Phadezyme PRIST. By the isotopic method, serum IgE concentrations in women and men were comparable from one age group to another with no age-related trend in the seven age groups examined (15–20, 21–30, 31–40, 41–50, 51–60, 61–70, above 70). The median and 95th percentile units (U)/ml respectively were 17.5 and 145 for 224 women and 25.5 and 275 for 222 men. Mean values $\pm$ 1 SD for women were 43 ± 102 and for men, 58 ± 137 . Levels were significantly higher in men as a group. Sera with IgE concentrations above 100 U and a sampling of additional sera were tested for specific IgE antibodies to 13 common allergens by the radioallergosorbent test (RAST). After exclusion of RAST-positive sera, the mean U/ml values $\pm$ 1 SD were 22 ± 29 for 204 women and 37 ± 54 for 196 men. Geometric mean U/ml values for these sera were 14.6 for women and 22.3 for men and the median and 95th percentile U/ml respectively for the women were 15 and 66, for the men, 24 and 135. These 95th percentile values are considered the upper limits of normal in this population. The RAST identification of antibodies to allergens to which sensitization was demonstrated provided a potential explanation for serum IgE concentrations above 100 U/ml in less than 30% of the sera in this population with negative allergy histories. The isotopic method and the counterpart enzymatic method (Phadezyme PRIST) were highly comparable; the correlation coefficient (r) for all the sera was $+0.93$ ($P < 0.001$). IgE levels were significantly higher in male smokers than non-smokers by both methods but these differences were not significant in women.

Key words: enzymatic assay; isotopic assay; IgE determination; non-allergics.

Accepted for publication 28 February 1983

CLINICAL ASPECTS

In a non-allergic Canadian population, the median and 95th percentile U/ml respectively for serum IgE concentrations for women were 15 and 66 and for men 24 and 135. The 95th percentile values are considered the upper limits of normal. In this normal population there was no relationship to age, but among the men, levels were significantly higher for smokers than for non-smokers. The isotopic and enzymatic methods of IgE determination were highly comparable and either is appropriate for routine diagnostic use.

The identification of an unique immunoglobulin class, IgE (8, 10), in which human reaginic antibodies are found, and the generation of specific antisera to this immunoglobulin, provided the basis for the immunochemical assessment of allergic individuals. The Phadebas PRIST (Pharmacia Diagnostics) (12) has found

world-wide use for the measurement of serum IgE concentration. The procedure depends on anti-IgE in solid phase serially incubated with test serum and labelled IgE. The resulting solid phase counts are inversely proportional to the IgE concentration of the test serum.

Radioimmunoassay in the form of Phadebas

RAST (Pharmacia Diagnostics) (5, 17) provides a relative semiquantitative measurement of serum IgE antibodies to specific antigen. Results correlate well with the occurrence of allergic symptoms from natural exposure to the corresponding antigen. The test antigen in solid phase is serially incubated with the test serum and labelled anti-IgE. The resulting solid phase counts are directly proportional to the serum concentration of IgE antibodies to the test antigen.

The serum IgE concentration tends to be elevated in the patients with allergic conditions including asthma, atopic eczema and allergic rhinitis (9, 15). It is typically markedly elevated in parasitic infestations (9), IgE myeloma (20) and the so-called hyper IgE syndrome (6) and can also be elevated in a variety of other conditions including pemphigus (2) and Hodgkin's disease (1).

Accurate standardization of the immunoassay for quantitation of IgE depends upon a rigorously restricted specificity of the indicator reagent in the test, labelled anti-IgE, for the Fc portion of the epsilon chain of IgE. The clinical usefulness of the test depends in large part on the success in separating allergic from non-allergic patients. To this end, it was considered potentially useful to determine serum IgE concentrations in non-allergic adult Canadians in order to establish the limits of normal. At the same time, the standard isotopic method was compared with the enzymatic counterpart (Phadezyme PRIST) of this immunoassay.

MATERIAL AND METHODS

Subjects

Volunteers were recruited from friends, relatives and patients at various out-patient clinics including Surgery, Ophthalmology, Orthopaedics, Allergy and Neurology, in addition to staff members of McMaster Health Sciences Centre. The project was approved by the Research Advisory Group of the McMaster division of the Chedoke-McMaster Hospital.

Each prospective volunteer was questioned and a doubtful or positive response to enquiries

about the following allergy-related conditions resulted in exclusion from the study: hay fever (allergic rhinitis); bronchial asthma; recurrent ear infections; bronchitis or pneumonia; drug or food allergy (rash, hives, diarrhoea, vomiting) within the past year; eczema, hives or dermatitis; parasitic infestations; allergic reactions to insect stings (bee, yellow jacket, hornets, wasp). In addition a smoking history was obtained from the final 268 subjects entered into the study. Sera were collected from 446 volunteers in the 6-month period of January-June, 1980. More than 95% of the subjects were caucasian. Signed informed consent was obtained from each subject.

Specimens and materials

Blood was collected into Vacutainer tubes without anticoagulant and stored overnight at 4°C. The next morning the clot was removed and the specimens were centrifuged at 2000 r.p.m. for 10 min. The separated serum samples were then stored at -20°C until assayed.

Serum IgE concentrations were measured in duplicate by the Phadebas PRIST and the counterpart enzyme-linked assay, Phadezyme PRIST, with test kits provided by Pharmacia (Canada) Inc. according to the method provided. For the Phadebas PRIST, 100 µl test serum, diluted 1/10 in IgE-free (horse) serum, was added to paper discs coated with anti-IgE and incubated 3 h at room temperature. The discs were then washed three times with 2.5 ml washing solution (0.9% NaCl plus Tween), allowing it to stand each time for 10 min after which the fluid was aspirated. After this washing, ¹²⁵I-labelled anti-IgE (100 µl) was added to the discs and incubated overnight at room temperature. Following this incubation, the discs were washed three times as described above and then counted in an automatic gamma counter. With each test a set of standards was included which consisted of lyophilised serum supplied with the kit and reconstituted for inclusion in the test. A standard curve was plotted against which the test samples were read. Allowance was made for the dilution factor in calculation of the final results. The

Phadezyme PRIST method was identical up to the end of the first wash. At that stage, anti-IgE, conjugated to β -galactosidase, was added to the discs and followed by overnight incubation. Washing as before was followed by the addition of substrate for incubation at 37°C for 60 min. The reaction was stopped with an inhibiting solution and the optical density was read on a spectrophotometer at 420 nm. The serum IgE concentration is directly proportional to binding of the label. One international unit equals about 2 ng.

Levels of IgE antibodies to 13 common allergens were measured with Phadebas RAST kits provided by Pharmacia (Canada) Inc. These assays were performed on i) all sera in which the IgE concentration was found to be 100 U/ml or higher and ii) an approximately equal number of sera with IgE levels below 100 U/ml randomly selected from each of the seven age groups of the men and women studied. The test depends on an insoluble disc coated with antigen. Test serum is added, followed by labelled anti-IgE. For a particular antigen, the concentration of serum IgE antibody to the antigen is directly proportional to the binding of the label. Sera with 0.35% or greater binding of labelled anti-IgE are considered RAST-positive.

RAST-inhibition studies were performed with six sera positive in the house dust mite RAST and, for comparison, with sera from 10 patients clinically allergic to ragweed. In these tests, 50 μ l of serum was incubated with 10 μ l house dust mite (*D. farinae*) antigen or 10 μ l buffer and the corresponding sera were then incubated with discs sensitized with house dust mite antigen and ragweed antigen, respectively. The percent inhibition was determined by calculating the counts with serum plus buffer minus counts with serum plus inhibitor divided by counts with serum plus buffer with the result multiplied by 100.

Statistical methods

Log transformation of IgE concentrations was performed to calculate the geometric mean values for men and women.

Linear correlation coefficients were determined for a number of comparisons: i) Phadebas PRIST with Phadezyme PRIST results in men and women; ii) age relationship of Phadebas PRIST and Phadezyme PRIST results in men and women; iii) Phadebas PRIST and Phadezyme PRIST results with Phadebas RAST determinations in men and women.

Unpaired t tests were performed to compare i) men with women for the serum IgE concentrations; ii) house dust mite RAST with ragweed RAST in inhibition by house dust mite antigen. The Mann-Whitney U test with 2-tailed analysis was used to compare smokers with non-smokers.

RESULTS

Serum IgE concentrations in international U/ml in women and men in the seven age groups studied are recorded in Fig. 1. There was no age-related change in IgE concentration. Levels were higher in men, as a group, than women ($t = 3.21$, $P < 0.001$). The mean U/ml $\pm$ SD were 43 ± 102 for 224 women and 58 ± 137 for 222 men. The median and 95th percentile values respectively were 17.5 and 145 for the women and 25.5 and 275 for the men.

RAST determinations with 13 antigens were performed on 142 sera. Positive results are illustrated in Fig. 2. The three antigens which yielded the largest number of positive tests in these subjects with negative histories were yellow jacket venom, rye grass pollen and house dust mite, respectively. The specificity of these determinations was examined with house dust mite antigen inhibition of the house dust mite RAST with six house dust mite RAST-positive sera and the ragweed RAST with the sera of 10 ragweed-allergic patients (Fig. 3). There was a significant difference between the two groups of tests ($t = 2.77$, $P < 0.02$) with inhibition by house dust mite antigen of the corresponding RAST.

IgE concentrations were analysed after exclusion of all sera with one or more positive RAST determinations. The mean U/ml $\pm$ 1SD for this population was 22 ± 29 for 204 women and 37 ± 54 for 196 men. The geometric mean value for the women was 14.6 and for the men 22.3. The median and 95th percentile values

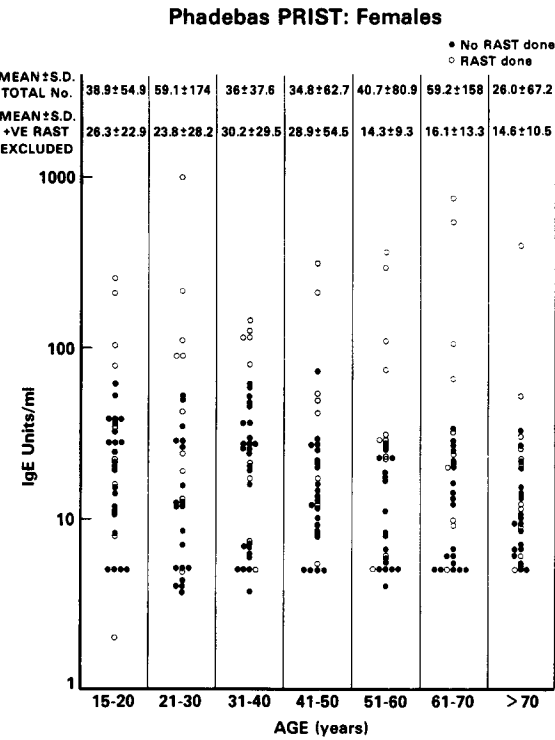
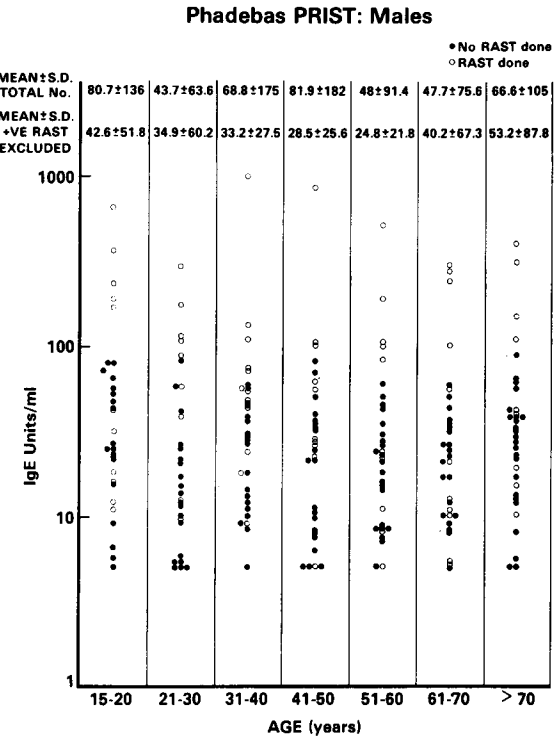


Fig. 1. Serum IgE concentration measurements by the Phadebas PRIST procedure expressed in U/ml. Results for women: upper graph; men: lower graph. Vertical axis shows serum IgE concentration and horizontal axis the seven age ranges studied. Open circles: sera also tested for specific IgE antibodies (RAST); closed circles: sera not tested. Mean $\pm$ 1 SD for the total in each age group is at the top of each column and the corresponding value after exclusion of RAST-positive sera is immediately below. There was no significant age-related trend. Results in men, as a group, were higher than in women.



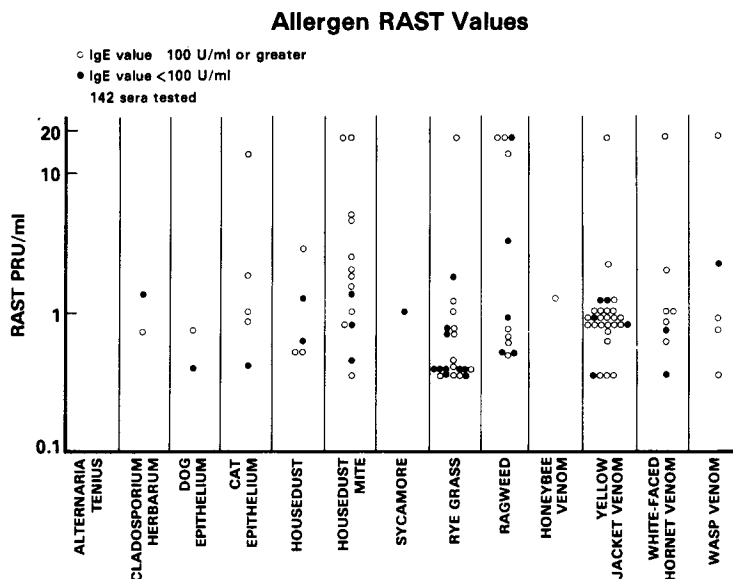


Fig. 2. RAST determinations on 142 sera to detect specific IgE antibodies to 13 common allergens. Vertical axis: RAST units representing all positive tests; horizontal axis: the 13 allergens. Closed circles: total IgE levels below 100 U/ml; open circles: total IgE levels at 100 U/ml or higher. In these subjects with negative allergy histories, the largest number of positive RAST determinations was with yellow jacket venom, followed by rye grass pollen and house dust mite.

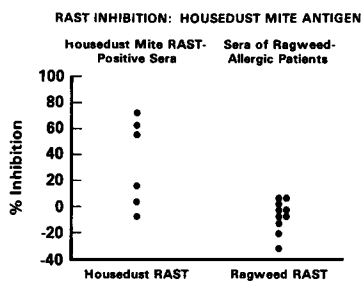


Fig. 3. RAST inhibition with house dust mite antigen: inhibition by house dust mite antigen of the house dust RAST with house dust mite positive sera was compared with inhibition of the ragweed RAST with the sera of ragweed-allergic patients. House dust mite antigen inhibited the house dust mite RAST significantly more than the ragweed RAST. Results demonstrate a specificity of house dust mite RAST and confirm the IgE-dependent sensitization of subjects.

respectively were 15 and 66 for the women and 24 and 135 for the men.

The values of the positive RAST determinations with individual sera were summed and these aggregate values were examined in relation to total serum IgE concentrations as illustrated in Fig. 4. There was no demonstrated correlation between IgE concentrations and ag-

gregate RAST values for women ($r = +0.13$, $P > 0.05$); however there was a significant direct correlation for men ($r = +0.44$, $P < 0.001$).

Total serum IgE measurements by the Phadebas PRIST compared closely with values obtained by the corresponding enzymatic method, the Phadezyme PRIST (Fig. 5). The correlation coefficient (r) for all the sera was $+0.93$ ($P < 0.001$). Statistical analyses of Phadezyme PRIST values in relation to age and RAST values yielded results comparable to those obtained with the Phadebas PRIST.

Subjects questioned about smoking were categorized as smokers, non-smokers and past smokers. RAST-positive sera were excluded and IgE levels were then analysed for 236 subjects (Table 1). The number of people is relatively small. Median values for IgE were chosen for analysis to minimize the effects of outlying values. The median values for IgE determined by the isotopic and enzymatic methods were significantly higher in smokers than non-smokers in the male population ($P < 0.05$); the differences were not significant in the female population.

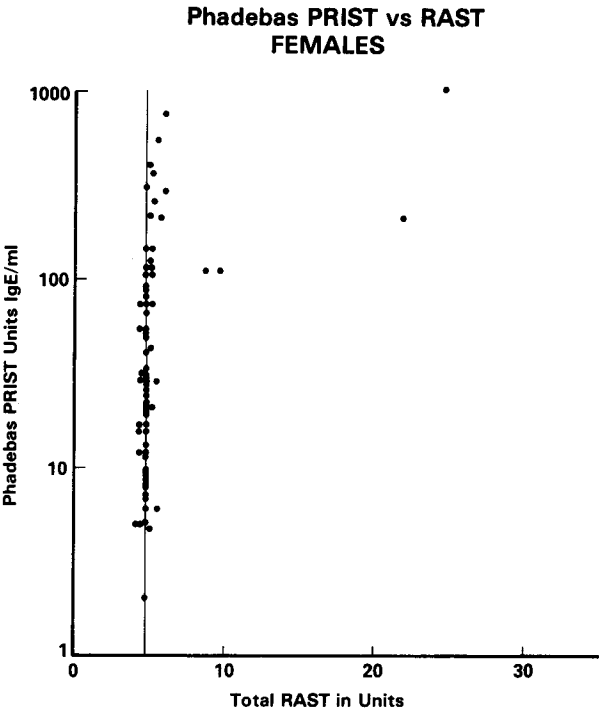
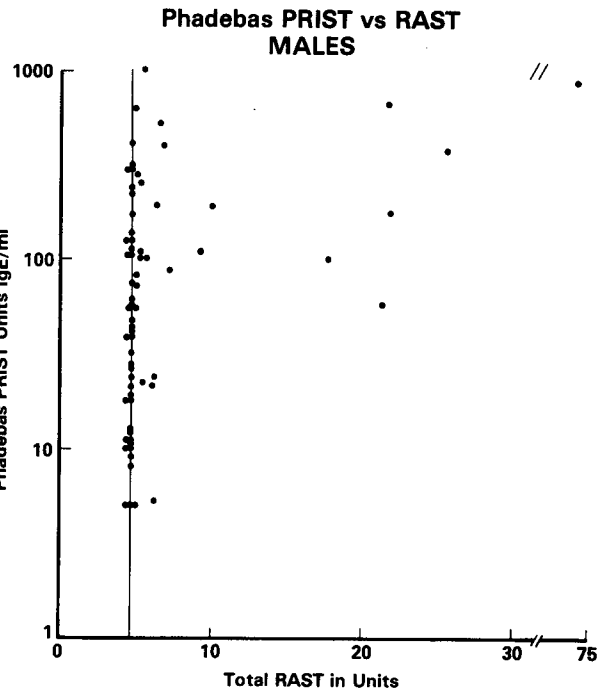


Fig. 4. Serum IgE concentrations in relation to RAST results. Upper graph: values for women; lower graph: values for men. Vertical axis: IgE concentration in U/ml; horizontal axis: total RAST units, a value derived from summation with each individual serum of the numerical value assigned to each positive RAST result. Vertical line at the left is considered to represent the limit of background RAST activity. The RAST determination provided a potential explanation for IgE concentrations above 100 U/ml in the sera of no more than 4 women and 12 men.



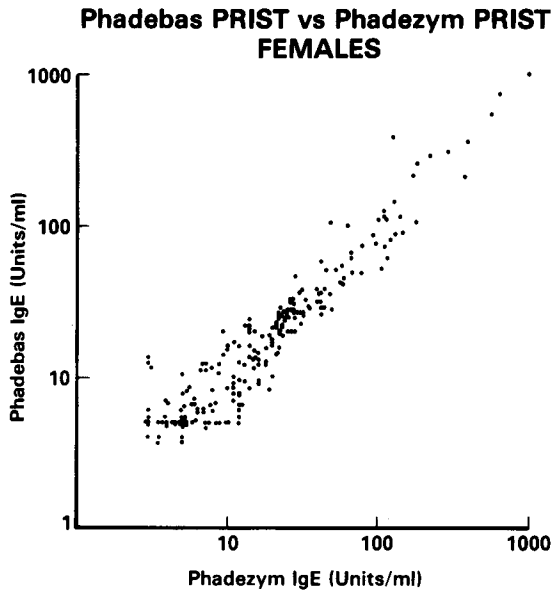
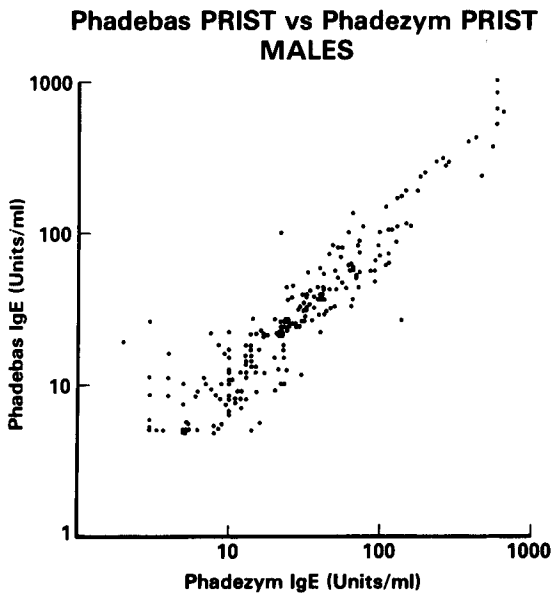


Fig. 5. Comparison of the isotopic (Phadebas PRIST) and enzymatic (Phadezym PRIST) methods of serum IgE quantitation. Upper graph: data for women; lower graph: data for men. Vertical axis: serum IgE concentration in U/ml by the isotopic method; horizontal axis: by the enzymatic method. The close relationship between results derived from the two methods is evident.



DISCUSSION

The relatively high percentage of allergic individuals in the general population complicates efforts to assign a normal range to serum IgE concentrations. The validity of a specified range can be increased by the exclusion of clinically

allergic individuals. This was accomplished by two procedures in the present study. The first was to exclude individuals with a positive history of allergy. The second was to attempt to identify additional allergic individuals through the use of the RAST with 13 common allergens. This approach is validated by previous studies where-

Table 1
Analysis of IgE levels by the isotopic and enzymatic methods

Females	Smoker n = 37	Non-smoker n = 64	Past smoker n = 16
Phadebas PRIST			
mean	20	30	22
SD	15	65	19
median	16	12	22
95th percentile	47	73	37
Phadezyme PRIST			
mean	20	26	20
SD	17	55	18
median	16	11	21
95th percentile	44	110	30
Males	Smoker n = 42	Non-smoker n = 45	Past smoker n = 32
Phadebas PRIST			
mean	33	31	22
SD	21	45	14
median	30	18	22
95th percentile	70	110	47
Phadezyme PRIST			
mean	35	29	23
SD	26	39	23
median	30	18	17
95th percentile	74	88	68

in a close relationship was demonstrated between a positive RAST and a susceptibility to allergic symptoms from exposure to the corresponding antigen (5, 9, 15). Sera with high IgE levels would be expected to more likely exhibit positive results in the RAST; accordingly, all sera with IgE levels of 100 U/ml or higher were chosen in addition to a more-or-less equal number of sera with lower IgE concentrations. The data with sera from men were sufficient to confirm the expected relationship of a significant positive correlation between serum IgE concentration and aggregate RAST value.

A number of reasons can be postulated to explain the presence of sensitivity, demonstrated by the RAST, in a population with a negative history. Individuals sensitized by a yellow jacket sting may present a negative history due to a lack of repeat challenge after

the occurrence of sensitization; alternatively, subjects may deny allergy to insect stings through failure to recognize large local reactions as a manifestation of sensitization (11). Persistent or recurrent symptoms such as nasal obstruction or cough might erroneously be attributed to processes other than reactions to an inhaled antigen such as house dust mite. However, most of the sera, including those with IgE concentrations at 100 U/ml or higher, had negative RAST results; the RAST with 13 antigens provided a potential explanation for IgE concentrations at 100 U/ml or higher in less than 30% of cases. It is possible that further searches for antibodies to common food and drug allergens, as well as other inhalant allergens, might provide a potential explanation for the high IgE concentrations in additional sera. Alternatively, these levels could reflect the presence of IgE antibodies with other types of specificities such as microbial antigens (16, 18) or autoantigens.

In allergic individuals, exposure to the offending antigen can lead to an increase in serum IgE concentration. In the present study, the number of individuals with unrecognized seasonal allergies is likely to be low. Nevertheless, there is probably some merit in avoiding seasons which could potentially lead to a rise in serum IgE levels. The present study was performed during the first half of the year with most sera collected prior to May. This would tend to avoid an elevation in serum IgE concentration due to grass pollen. Moreover, the sera were collected prior to the ragweed pollen season of the same year and many months after the season of the preceding year.

The main determinant of elevated serum IgE concentrations in Western countries is heredity (13). In some other parts of the world, an additional major influence is parasitic infestation (9). The observed higher serum IgE concentrations in men than women identifies another relevant factor; the differences, while highly significant and observed elsewhere (3, 7, 14), are not great and have not invariably been observed (19). These differences necessitate a separate handling of the data for men and women. There is a direct age-related increase

in serum IgE concentration in normal children up to the age of 15 (11). In the general population, a progressive increase in the frequency of allergy and in levels of serum IgE is evident until early adult life; following this, there is a gradual diminution in the occurrence of allergy (4) and in levels of serum IgE (17). However, in the present study, as has been observed elsewhere in selected non-allergic adults (3, 14), there was no significant age-related trends in men or women.

The development of enzyme-linked immunoassays has been motivated by a number of inherent advantages. Enzyme-labelled reagents are stable. The colorimetric measurements of enzyme assays can be performed without expensive instrumentation. A disadvantage of the enzymatic method is an additional step in the procedure. The present comparison between the isotopic and enzyme methods demonstrates that the procedures are, for practical purposes, interchangeable.

Data for serum IgE concentrations in populations are characterized by outlying numbers at the upper end of the scale. Geometric mean or median values permit the avoidance of an excessive influence of these numbers. These two types of analyses in the present study yielded values which were very close; the geometric mean and median values respectively for women were 14.6 and 15.0 U/ml and for men were 22.3 and 24 U/ml. It was elected to select the 95th percentile as the upper limit of normal. The proposed upper limit of normal for women is 66 U/ml and for men is 135 U/ml.

ACKNOWLEDGEMENTS

The financial support provided by Pharmacia (Canada) Inc. for this study is gratefully acknowledged. The authors wish to thank Sheldon Brookman, Ph.D. for his assistance and Mrs. Judy Walters, Dorothy Agnew and Bettyann Gibson for assistance in preparation of the manuscript.

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