

Total Serum IgE Concentrations in Adolescents and Adults Using the Phadebas IgE PRIST® Technique

JEAN BOUSQUET,¹ YVES COULOMB,² HANS ARRENDAL,³ MICHÈLE ROBINET-LEVY² and FRANÇOIS B. MICHEL¹

¹Clinique de Maladies Respiratoires, ²Centre de Transfusion Sanguine de l'Hérault, France, and ³Pharmacia Diagnostics AB, Uppsala, Sweden

Total serum IgE measured with the Phadebas PRIST® technique was titrated in 117 normal non-allergic subjects, 237 allergic adolescents or adults and 89 non-allergic patients who suffered from asthma, rhinitis, or conjunctivitis. All subjects were of Caucasian origin. In normal subjects, mean total serum IgE was 38 ± 43 kU/l. This value is exactly the same as that found in a study of Caucasian New Zealanders and very similar to the values found in most U.S. studies. This suggests that the mean total serum IgE concentration is remarkably constant in normal non-allergic Caucasians. The upper limit of the normal range is considered to be 150 kU/l. 38% of allergic patients have total IgE concentrations within the normal range. Some pollen or hymenoptera venom-sensitive patients have a total serum IgE concentration below 20 kU/l. The non-allergic patients had a mean IgE concentration of 94 ± 93 kU/l, and 25% of them had a total serum IgE above the normal range. Asthmatic patients had higher mean IgE levels than those who were suffering from either rhinitis or conjunctivitis.

Key words: asthma; atopy; conjunctivitis; IgE; normal values; rhinitis.

Accepted for publication 29 January 1982

CLINICAL ASPECTS

Total serum IgE was measured by means of the PRIST® technique, and examined according to the sensitivities of the patients and their allergic disorders. Patients who presented multiple sensitivities had higher total mean serum IgE concentrations than those suffering from animal dander or pollen allergy. Some allergic patients had normal or low serum IgE concentrations. Patients with mite or mould allergy had intermediate range IgE.

Patients with allergic asthma had higher mean IgE concentrations than those who had rhinitis and/or conjunctivitis. Patients with both atopic dermatitis and allergic asthma had very high IgE concentrations. Non-allergic asthmatics still had elevated total serum IgE concentrations.

Total serum IgE is measured using several techniques. The PRIST technique (4), available commercially, was demonstrated to be as sensitive as double-antibody radioimmunoassay (7) which has the disadvantage of requiring the addition of a second antiserum after the competitive binding assay (12). The PRIST technique was shown to be more sensitive than the RIST technique (9) especially for the determi-

nation of IgE concentrations below 100 kU/l (14, 17) and in cord serum (15).

A few investigations of a normal population sample have been done using the PRIST technique (3, 18, 20, 21, 22). The determination of serum IgE levels in atopic disease was conducted already in 1968 (8) in Scandinavian countries and several other reports have been published since (1, 2, 11, 18) but some of them

were done using the RIST technique. No complete data is available which compares normal values, concentrations in allergic patients, and concentrations according to the sensitization of the patients, with the PRIST technique. In the present study 117 Caucasian normal subjects were compared with 237 Caucasian allergic patients ranging in age from 13 to 76 years and 89 Caucasian patients who attended an allergy clinic but who were not classified as allergic.

MATERIAL AND METHODS

Subjects tested

All subjects were of Caucasian origin.

Two groups of subjects were defined. The first, a control group, comprised 117 volunteers free of atopic and allergic disorders (age range: 17–45 years; mean: 30.1 ± 7.1 years). They had no allergic symptoms and all had a negative family history.

The second group comprised patients who were admitted to the out-patient Allergy Clinic of the Montpellier hospital (age range: 13–76 years; mean: 31.3 ± 18.5 years).

Patients and control subjects who had a disease known to increase IgE concentrations were excluded from the study: Hodgkin's disease, parasitosis, Loeffler syndrome, collagenosis (10), acute infection (Bousquet et al., in preparation), and chronic obstructive lung disease (18).

Definition of the symptoms

Asthma was confirmed by a history consistent with intermittent airways obstruction: wheezing and pulmonary function tests performed on all the patients showed a reversible airways obstruction. The diagnosis of conjunctivitis was based on clinical examination, and that of rhinitis on a history of rhinorrhoea and stuffy nose. In most rhinitis patients nasal provocation tests were performed with allergen. The patients with atopic dermatitis were selected according to several criteria: All patients had erythema with vesiculation or induration and characteristically localised lichenification. Some also had

Table 1
Skin test panel

	ID	Prick test	Normal subjects	Patients
House dust	10^{-4}		+	+
<i>Dermatophagoides pteronyssinus</i>	"		+	+
<i>Dermatophagoides farinae</i>	"			+
Feathers	"			+
<i>Alternaria</i>	"		+	+
<i>Aspergillus</i>	"			+
<i>Cladosporium</i>	"		+	+
<i>Mucor</i>	"			+
<i>Rhizopus</i>	"			+
<i>Botrytis</i>	"			+
<i>Neurospora</i>	"			+
<i>Pullularia</i>	"			+
<i>Stemphylium</i>	"			+
<i>Fusarium</i>	"			+
<i>Epicoecum</i>	"			+
<i>Helminthosporium</i>	"			+
<i>Candida albicans</i>	"			+
<i>Trichophyton</i>	"			+
<i>Epidermophyton</i>	"			+
M.R.V.	"			+
Mixed grasses		1/20	+	+
Parietaria	"		+	+
Plantain	"			+
Oak tree	"			+
Wormwood	"			+
Short ragweed	"			+
Giant ragweed	"			+
Mugwort	"			+
Alder	"			+
Birch	"			+
Cypress	"			+
Hornbeam	"			+
Chestnut	"			+
Fat hen	"			+
Plane tree	"		+	+
Ash	"			+
Olive	"		+	+
Juniper	"			+
Beech	"			+
Horse-chestnut	"			+
Paper mulberry	"			+
Japan mulberry	"		+	+
Hazel	"			+
Elm	"			+
Nettle	"			+
Sorrel	"			+
Poplar	"			+
Willow	"			+
Cat dander		"	+	+
Dog dander		"	+	+
Horse dander		"		+

ID: intra dermal test, 10^{-4} (w/v).

Table 2
Global results

Groups		A	B	C	D	E	F	G	H	I
Allergenic sensitivity		0	0	Animal dander	Pollens	Moulds	Mites	House dust + mites	Multiple sensitivity	Venoms
No. of subjects		117	89	9	58	6	6	36	107	15
Age (years)	mean	30.1	41.5	16.5	31.3	14.7	33.2	37.2	29.1	27.8
	SD	7.1	19.1	3.2	14.6	7.3	13.1	18.5	16.5	13
Asthma		0	46	7	15	5	5	33	86	
Asthma + atopic dermatitis		0	0	0	0	0	0	0	14	
Rhinitis		0	35	1	39	1	1	3	22	
Conjunctivitis		0	8	1	4	0	0	0	0	
Atopic dermatitis		0	0	0	0	0	0	1	3	
IgE (kU/l)	mean	38	94	113	185	312	272	398	957	322
	SD	43 (a)	93 (b)	68	265	193	178	358	1286	318
	limits	1-210	1-410	30-290	7-700	110-320	85-590	22-1800	47-5820	20-1000

P a/b < 0.01 (the statistical analysis was made using the Mann & Whitney *U* test).

xerosis, or ichthyosis, or hyperlinear palms. Most patients had a personal or family history of other atopic diseases.

Urticaria was excluded from the study since its origin is often difficult to determine.

Skin tests

Skin tests to the common aero-allergens of the South of France were performed on all patients and normal subjects using prick tests and intradermal tests as shown in Table 1. A negative control solution was used for both intradermal and prick tests and consisted of the diluant of extracts used for the two routes of administration, i.e. saline for intradermal test and 50% glycerol for prick test. The criteria of positivity of the skin tests were examined according to the method of Ménardo et al. (13). Briefly, they

used codein phosphate as a positive control in the prick tests (a 5% solution in 50% glycerol) and in the intradermal tests (10^{-4} w/v solution in saline). The wheal induced by allergens is classified as positive when it is equal or more than 80% of the wheal induced by codein phosphate. This criterion of positivity was shown to give better results than simply measuring the size of the wheal induced by allergens. Only a limited number of allergens were tested in volunteers who claimed to be symptom-free.

Only patients who had gastrointestinal symptoms or symptoms suggesting a food allergy or intolerance were tested for food allergy. Tests included skin prick tests using a 5% or 10% (according to allergens) w/v glycerinated extract.

All extracts were obtained from the Laboratoire des Stallergènes, Fresnes, France.

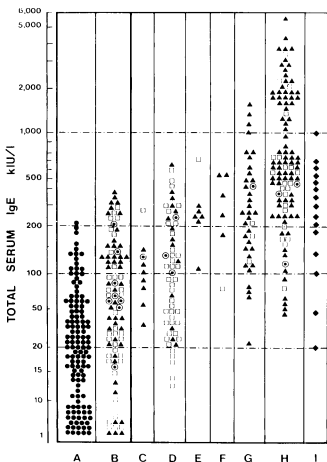


Fig. 1.: Global results.

- A: normal subjects
- B: non-allergic subjects
- C: animal dander sensitivity
- D: pollen sensitivity
- E: mould sensitivity
- F: mite sensitivity
- G: house dust (and mite) sensitivity
- H: multiple sensitizations
- I: hymenoptera venom sensitivity
- : normal subjects
- ▲: asthma (and rhinitis)
- : rhinitis alone
- : conjunctivitis
- ◆: venom sensitivity

IgE determination

Total serum IgE was titrated using the PRIST technique (4) (Phadebas PRIST®, Pharmacia Diagnostic AB, Uppsala, Sweden). Specific serum IgE antibodies were assayed using the RAST test (Phadebas RAST®, Pharmacia Diagnostic AB, Uppsala, Sweden).

For each patient and control subject the following allergens were examined: house dust, mites (*Dermatophagoides pteronyssinus*) and grass pollens (*Dactylis glomerata*, *Phleum pratense*). Furthermore, the patients (group B-I) (Table 2) were RAST screened according to the results of the skin test. Food allergens were not included in the screening but were sought when there was a history of suspected food allergy.

Criteria defining allergy

The criteria defining an allergic patient were the association of a clinical history, suggesting allergy, positive skin tests measured by comparison with codein phosphate (13) and the presence of specific IgE antibodies when RAST disks were commercially available. When they were not commercialized, and/or a weak skin test reactivity and/or a RAST class 1 was observed, nasal and/or bronchial provocation tests were performed. If a discrepancy was shown between *in vivo* and/or *in vitro* tests, the patients were excluded since only undoubtedly allergic patients should be considered. This occurred in a limited number of cases and the patients were not included in the present study.

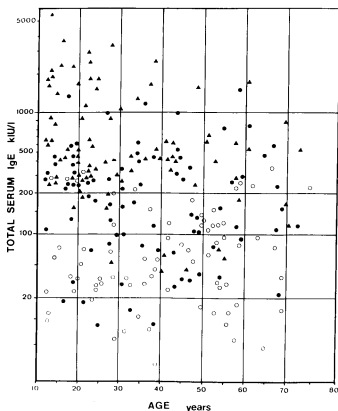


Fig. 2. Total serum IgE according to the age of the patients.

- : non-allergic patients
 ●: monosensitization
 ▲: polysensitization

RESULTS

Populations studied

The 117 volunteers in the non-allergic group (A) had neither positive skin test nor serum specific IgE antibodies.

Patients who were admitted to the out-patient Allergy Clinic of the Montpellier Hospital were classified into seven groups according to their allergenic sensitization (Table 2). Group B comprised 89 patients, 46 of whom had asthma, 35 rhinitis, and eight had other symptoms but no allergenic sensitivity as determined by skin tests and RAST. Group C comprised nine patients with a sensitivity to animal dander; seven of these patients had symptoms of asthma. Group

D comprised 58 pollen-sensitive patients, 39 of whom had manifestations of hay fever, 15 asthma often associated with hay fever, and four had conjunctivitis associated with their hay fever. In group E only six patients were sensitized to moulds; five of these patients had asthma and one had hay fever. Group F comprised six mite-sensitive patients, five of whom had asthma and one, hay fever. Group G comprised 36 patients with house dust and mite sensitivity; most of these patients had asthma, three had perennial rhinitis alone and one patient had atopic dermatitis. Group H comprised 107 patients with a multiple allergenic sensitization of whom 86 had asthma alone or associated with atopic dermatitis (14 cases) and perennial rhin-

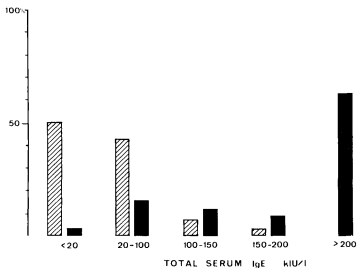


Fig. 3: Percentage of subjects according to the total serum IgE titration. Allergic patients ■; normal subjects ▨.

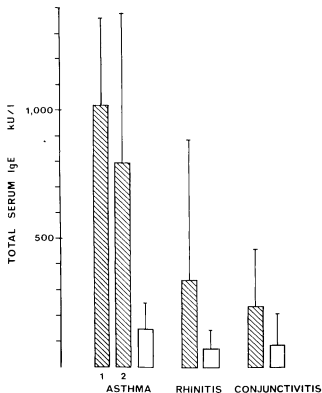


Fig. 4. Total serum IgE according to the disease (1: asthma and atopic dermatitis, 2: asthma alone). Allergic patients ▨; non-allergic patients □.

itis (8 cases) 22 had rhinitis alone and three atopic dermatitis alone. Finally, group I comprised 15 subjects with a hymenoptera venom allergy.

Serum IgE concentrations according to the age and allergens

Fig. 1 shows the distribution of total IgE concentration according to the different groups of patients (groups B to I). Non-allergic subjects placed in group A were not included. Roughly, the younger the patient group, the higher the mean IgE concentration. Moreover, in adolescents and young adults (12–30 years) (Fig. 2) the percentage of polysensitized patients is higher than in older patients. Those patients who showed no sensitivity to common aero-allergens ranged from 22% to 54% of the age groups. Over the age of 60, 75% of the patients who claimed to have allergic symptoms presented an allergic sensitization.

Sex differences between normal subjects

Although more females (81 cases) than males (36 cases) were tested, the population is large enough to allow a comparison. The group of males had a slightly higher geometric mean level (42.1 ± 36.6 kU/l) than females (32.6 ± 37.3 kU/l) but the difference is not significant.

Serum IgE concentrations according to the allergenic sensitivities

The global results are given in Table 2 and Fig. 1. In normal subjects the mean IgE levels were 38 ± 43 kU/l whereas the so-called non-allergic patients (group B) had mean serum total IgE concentrations significantly ($P < 0.01$) higher (94 ± 93 kU/l) than the normal subjects.

In patients sensitized to either pollens or animal danders the total serum IgE was always below 700 kU/l and their mean titers were respectively 185 ± 265 kU/l and 113 ± 68 kU/l. Moreover, five patients with hay fever had total serum IgE below 20 kU/l.

Patients with allergic symptoms due to moulds, mites and/or house dust had serum

total IgE levels in the intermediate range. Mean titers ranged from 272 to 398 kU/l.

Hymenoptera venom-sensitized patients had a wide range of total serum IgE concentration (20–1000 kU/l).

Finally, patients with multiple sensitivities often had IgE above 1000 kU/l and their mean titers were as high as 957 ± 1286 kU/l.

Overall (Fig. 3), 50% of normal subjects had serum total IgE below 20 kU/l whereas 70% of allergic patients had above 150 kU/l.

Serum IgE concentrations according to the disease

The results are presented in Fig. 4. Patients who had allergic asthma had a significantly higher mean level (812 ± 949 kU/l) of total serum IgE than those suffering from either allergic rhinitis (326 ± 500 kU/l, $P < 0.001$) or allergic conjunctivitis (216 ± 504 kU/l, $P < 0.01$). Patients suffering from both atopic dermatitis and asthma had higher median IgE concentrations (1023 ± 357 kU/l) than those having asthma alone (787 ± 867 kU/l). There was also a big difference between those with allergic asthma and those with non-allergic asthma (116 ± 108 kU/l, $P < 0.0001$). Patients with nasal or ocular symptoms had a higher mean level when they were allergic whilst patients with non-allergic asthma had a significantly higher level ($P < 0.025$) than those with either non-allergic rhinitis (73 ± 83 kU/l) or non-allergic conjunctivitis.

DISCUSSION

Elevated serum IgE concentrations are often found in allergic conditions such as asthma, hay fever and atopic dermatitis (for review see (10)).

However, normal serum IgE levels do occur in allergic patients (16). Most published studies conducted in adults have titrated IgE using the RIST technique or double-antibody radioimmunoassays. The RIST assay tends to overestimate low IgE concentrations (9) and the latter assay is not commercially available. Total serum IgE measured by the Phadebas IgE PRIST technique represents one of the best assays. Although several studies with this tech-

nique have involved children (1, 11) fewer have been conducted in adults (2).

In the present study only normal subjects and patients over 13 years of age were examined.

Total serum IgE concentrations of normal subjects were determined in 117 subjects having no personal history of allergy, no positive skin tests and no specific IgE antibodies as determined by screening. A limited number of allergens were tested in these volunteers claiming to be symptom-free. However, with this screening method more than 85% of the allergic patients of the Montpellier area were detected. Screening was found to be reliable since all volunteers were normal. The ages of these subjects ranged from 17 to 45 years since within these limits immunological changes are limited to minor differences. Over 45 years it is not certain whether modifications of IgE concentrations can be found. Sears et al. (20) used the same age limit in their study. Only 20 of them had IgE levels above 100 kU/l and four above 150 kU/l. This value can be considered as the highest normal level. Mean IgE concentrations were not significantly different in men and women and did not accord with the study of Pauwells et al. (18). Mean concentrations were similar to those published in former studies (20, 21, 22) but lower than those presented by Brown et al. (3).

The mean IgE value of our normal group with an age range of 17 to 45 years was exactly the same as that of the New Zealand group (range 17–45 years) with no personal or family history of allergy (20) and slightly higher than that of a Swedish group (23). These results suggest that IgE levels are remarkably constant in Caucasians whatever their place of birth and wherever they live. Moreover Sears et al. (20) found only one subject who had a total serum IgE level above 150 kU/l and suggested that this level could be the upper limit of normal range. The present study draws the same conclusions. Finally, the median values of IgE measured by the PRIST technique are similar to those measured by double-antibody radioimmunoassay (6).

No allergenic sensitization could be found in 89 patients (group B). Among them 45 suffered from asthma classified as the so-called

intrinsic asthma. Mean serum IgE concentrations were significantly higher ($P < 0.01$) than in normal control subjects and 25% of the patients had serum IgE above 150 kU/l. No disease known to increase IgE concentrations was theoretically present and the patients had not recently had any acute infection, nor did they have chronic obstructive lung disease. Their increased serum total IgE level is therefore suggestive of an allergic etiology and should encourage further etiologic investigation by clinical history, skin-testing, assays of circulating and cell bound IgE, or provocation test. Moreover, food allergy should be reassessed even although some patients had negative skin tests and RAST when a history compatible with such etiology was suspected. Bacterial allergy could also be examined. Skin tests to bacteria were routinely performed and no patient had an immediate positive reaction but the titration of specific IgE antibodies to bacteria may be more accurate (19). 35% of the patients in this group had a family history of allergy, and according to the recent paper of Sears et al. (20) this may partly account for a higher median IgE value, although it does not explain the whole discrepancy.

Total serum IgE level was higher in allergic patients than in non-allergic control subjects. However, total serum IgE levels were within the normal range in 38% of the allergic patients (Fig. 3).

Moreover, total serum IgE concentration was below 20 kU/l in five pollen-sensitive and was 20 kU/l in one hymenoptera venom-sensitive patient. The mean total serum IgE level in the group of patients allergic to a single allergen (pollen, animal dander, venom) was significantly lower than that in polysensitized patients, although some of them had normal serum IgE.

Only six patients had mould allergy alone and a few others had this type of sensitization associated with other inhalant allergens. In the South of France, mould allergy is not a very important problem since the climate is dry. Patients included in group H had multiple sensitization and their mean IgE levels were the highest found (957 ± 1286 kU/l). In more than 30% of the patients serum total IgE was higher

than 1000 kU/l suggesting polyclonal synthesis of IgE. In some cases, specific IgE antibody concentration associated with a total serum IgE titer below 200 kU/l. Half of the patients included in group H were under 30 years of age.

Patients with extrinsic asthma had a higher total IgE median value than allergic patients who suffered from rhinitis or conjunctivitis. Patients with non-allergic asthma had a significantly lower ($P < 0.0001$) median IgE value. 23% of patients with extrinsic asthma had a normal serum IgE concentration. Thus a normal or even low serum IgE value does not exclude extrinsic allergy from the list of diagnostic possibilities. This is more evident with the PRIST technique than with the RIST since low serum IgE values were not accurately determined when using the latter method. Finally, both asthma groups (intrinsic and extrinsic) had significantly ($P < 0.001$ and $P < 0.01$) higher median IgE values than both the rhinitis and conjunctivitis groups (extrinsic and intrinsic). The reasons for these differences are not clear but might be partly explained by a relationship between prolonged antigenic exposure and IgE production.

Immediate hypersensitivity reactions appear to occur less frequently in older patients. Delespesse et al. (5) have shown a significant reduction of the serum IgE concentrations and specific IgE antibody response in ageing. Moreover, they found a lower skin response in the Prastnitz-Küstner reaction in the elderly. In the present data 21 patients were over 60 years of age, six of them had polysensitization and eight were sensitive to a single allergen. Furthermore, four patients had negative skin tests and a clinical history suggesting the presence of specific IgE antibodies in their serum. It therefore appears that allergy is still present in patients over the age of 60 but demonstration is more difficult.

REFERENCES

1. Aas, K.: Diagnostics of immediate type respiratory allergy. *Pediatr. Clin. North Am.* 22, 33-42, 1975.
2. Björkstén, F. & Johansson, S. G. O.: In vitro diagnosis of atopic allergy. The occurrence of positive RAST results as function of age and total IgE concentrations. *Clin. Allergy* 5, 363-373, 1975.
3. Brown, W. G., Halonen, M. J., Kaltenborn, W. T. & Barbee, R. A.: The relationship of respiratory allergy, skin test reactivity, and serum IgE in a community population sample. *J. Allergy Clin. Immunol.* 63, 328-335, 1979.
4. Ceska, M. & Lundkvist, U.: A new simple radioimmunoassay method for the determination of IgE. *Immunochemistry* 9, 1021-1030, 1972.
5. Delespesse, G., De Maubeuge, J., Kemnes, B., Nicaise, R. & Govaerts, A.: IgE mediated hypersensitivity in ageing. *Clin. Allergy* 7, 155-160, 1977.
6. Gleich, G. J., Averbeck, A. K. & Swedlung, H. A.: Measurement of IgE in normal and allergic serum by radioimmunoassay. *J. Lab. Clin. Med.* 77, 690-701, 1971.
7. Gleich, G. J. & Dunette, S. L.: Comparison of procedures for measurement of IgE proteins in serum and secretions. *J. Allergy Clin. Immunol.* 59, 377-382, 1977.
8. Johansson, S. G. O.: Serum IgND levels in healthy children and adults. *Int. Arch. Allergy* 34, 1-12, 1968.
9. Johansson, S. G. O., Berglund, A. & Kjellman, N.-I. M.: Comparison of IgE values as determined by solid phase radioimmunoassay methods. *Clin. Allergy* 6, 91-99, 1976.
10. Johansson, S. G. O. & Foucard, T.: IgE in immunity and disease. In Middleton, E., Jr., Reed, C. E. & Ellis, E. F. (eds.): *Allergy, principles and practice*, pp. 551-562. Saint Louis, 1978.
11. Kerrebijn, K. F., Degenhart, H. J. & Hammers, A.: Relation between skin tests, inhalation tests and histamine-release from leukocytes and IgE in house-dust allergy. *Arch. Dis. Child.* 51, 252-258, 1976.
12. Lichtenstein, I. M. & Hamburger, R. N.: IgE and atopic disease. In Samter, M. et al. (eds.): *Immunologic diseases*, pp. 804-818. 3rd ed. Little Brown and Co., Boston, 1978.
13. Ménardo, J. L., Bousquet, J. & Michel, F. B.: Standardization of skin tests. In Molina, C. (ed.) *Proceedings of the European Meeting of Allergology and Clinical Immunology*. Clermont Ferrand, September 1981. (In press).
14. Merrett, F. G. & Merrett, J.: Methods of quantifying circulating IgE. *Clin. Allergy* 8, 543-557, 1978.
15. Michel, F. B., Bousquet, J., Greillier, P., Coulomb, Y. & Robinet-Lévy, M.: Comparison of cord blood immunoglobulin E concentration and maternal allergy for the prediction of atopic diseases in infancy. *J. Allergy Clin. Immunol.* 65, 422-430, 1980.
16. Nagaya, H.: Relationship between antigen-specific IgE antibody (RAST) and total serum IgE levels. *Ann. Allergy* 43, 267-270, 1979.
17. Nye, L., Merrett, F. G., Landon, J. & White, R. J.: A detailed investigation of circulating IgE levels in a normal population. *Clin. Allergy* 3, 13-21, 1978.
18. Pauwels, R. & Van Der Straeten, M.: Total serum IgE levels in normals and patients with chronic non-specific lung diseases. *Allergy* 33, 254-260, 1978.
19. Pauwels, R., Verschraegen, G. & Van Der Straeten, M.: IgE antibodies to bacteria in patients with bronchial asthma. *Allergy* 35, 665-669, 1980.
20. Sears, M. R., Chow, C. M. & Morseth, D. J.: Serum total

- IgE in normal subjects and the influence of a family history of allergy. *Clin. Allergy* 10, 423-431, 1980.
21. Wittig, H. J., Belloit, J., De Filippi, I. & Royal, G.: Age-related serum immunoglobulin E levels in healthy subjects and in patients with allergic disease. *J. Allergy Clin. Immunol.* 66, 305-313, 1980.
22. Wuthrich, B. & Wyss, S.: Ergebnisse eines Ring-Versuches des Schweiz. Schweiz. Med. Wochenschr. 109, 315-321, 1979.
23. Zetterstrom, O. & Johansson, S. G. O.: IgE concentrations measured by PRIST[®] in serum of healthy adults and in patients with respiratory allergy. *Allergy* 36, 537-547, 1981.

Address:

Jean Bousquet
Hopital l'Aiguelongue
Avenue du Major Flandre
34059 Montpellier
France

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.