

Quantitative comparison of immunoglobulins in atopic (reaginic) and nonatopic (nonreaginic) individuals: Higher γ D levels in atopic sera

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A quantitative comparison of γ G, γ A, γ M, and γ D was made between 67 sera from persons with high titers of reaginic (skin-sensitizing) antibody and 51 sera from individuals lacking detectable reagin. A statistically significant elevation of γ D and slight increases of γ G and γ A were present in the reaginic sera when considered as a group. In addition, a correlation between age and γ A levels was present with increasing concentrations throughout adult life. The increase in γ D is probably not a consequence of the presence of reaginic antibody per se but rather represents an additional quantitative difference in the immunologic makeup of atopic persons. The basis for this quantitative alteration is uncertain at the present time since the functional role of γ D has not been determined. The distribution of immunoglobulins in the 118 subjects studied followed a log normal rather than arithmetic normal distribution, similar to the antibody titers of experimental animals immunized with standard amounts of antigens.

The diagnosis of atopic diseases depends on a combination of immunological and clinical criteria. By definition, the characteristic immunological criterion which distinguishes persons with atopic rhinitis and/or asthma from others with similar clinical symptoms is the uniform presence of reagin, the heat labile, passively transferable skin-sensitizing antibody in atopic sera.¹ Reagin can also be demonstrated in a significant percentage of persons who have never exhibited respiratory symptoms despite considerable exposure to airborne antigens capable of eliciting immediate wheal-and-flare reactions.^{2, 3}

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The present study was undertaken to determine the quantitative relationship of γ G, γ A, γ M, and γ D immunoglobulins in persons with atopic disorders as compared to nonatopic persons. The sole criterion used for classification of individuals as atopic or nonatopic was the degree of immediate wheal-and-flare reactivity to a large number of naturally occurring pollen antigens present in their environment. Only highly reactive or completely nonreactive individuals were selected in order to amplify any possible difference in immunoglobulin concentrations which could be correlated with the presence or absence of reaginic (skin-sensitizing) antibody.

MATERIALS AND METHODS

Sera from approximately 600 individuals who had undergone skin testing at this institution between Jan. 1, 1963, and July 1, 1965, were available for study. The sera were obtained prior to skin testing and hyposensitization treatment and were stored at -20° C. Each person was tested with a minimum of 70 different allergenic extracts derived from 45 or more pollens, 16 environmental substances such as animal hair and danders, dust, feathers, kapok, etc., 5 molds, and 6 common foods. Approximately 0.02 ml. of the antigen solution was injected intradermally and the skin response interpreted after 20 minutes. A 2+ reaction consisted of a wheal at least 10 mm. in largest diameter with accompanying erythema. From the above individuals, the following two serum classes were selected.

Reaginic class: sera from 67 persons 4 to 70 years old, median age 20 years, who gave multiple 2+ or greater wheal-and-flare reactions to 2×10^{-6} weight by volume dilutions of pollen antigens by direct intradermal testing or 2+ reactions to 2×10^{-4} dilutions with the passive transfer technique of Prausnitz and Kustner (P-K).

Nonreaginic class: sera from 51 persons 4 to 76 years old, median age 50 years, with absent wheal-and-flare reactivity to 2×10^{-4} dilutions of pollen antigens in addition to negative reactions to environmental, spore, and common food extracts at 2×10^{-2} dilutions by either direct or passive transfer (P-K) testing. Sera from 9 individuals employed as passive transfer (P-K) recipients were included in the nonreaginic class.

Immunoglobulin measurement

The quantitative single radial diffusion technique as described by Mancini, Vaerman, Carbonara, and Heremans⁴ was employed. γ G, γ A, and γ M concentrations were measured in agar plates incorporated with specific goat antisera. The test sera and immunoglobulin standards were placed in the antigen wells and the precipitin rings measured after diffusion for 24 hours at 4° C. With this immunochemical technique, the log concentration of the specific protein being measured is proportional to the diameter of the precipitin ring. The same lot of immunodiffusion plates* and standards was used throughout. The reference standards were dilutions of a concentrated pooled normal sera in which

*Immunoplates: Hyland Laboratories, Los Angeles, Calif.

the respective immunoglobulin concentrations had been determined by comparison with known concentrations of purified γ G, γ A, and γ M isolated from normal serum. The antibody specificity of the commercial immunodiffusion plates was established using purified immunoglobulins obtained from both normal and myeloma sera (γ G and γ A), a macroglobulinemic serum (γ M), and with kappa and lambda light chains isolated from myeloma urine. Approximately 15 sera were tested at a time. Two sera from reaginic individuals were tested in duplicate during each separate determination to establish the reproducibility of the method in our hands.

The concentrations of γ D were determined on serum samples identified by number only in Dr. John Fahey's laboratory, National Cancer Institute, Bethesda, Maryland. The same quantitative technique⁵ was employed with the use of rabbit antisera specific for γ D (R133A, Sept. 27, 1965). The lowest γ D concentration which can be detected with this technique at present is 0.3 mg. per 100 ml. Concentrations in the lower detectable range from 0.3 to 1.4 mg. per 100 ml. cannot be measured precisely and, therefore, all values in this range are expressed as (0.7) milligrams per 100 ml.⁶

Statistical analyses

Concentrations of the four immunoglobulins in the population studied were not distributed in a gaussian manner. In order to normalize the distribution for statistical analysis, the respective immunoglobulin levels in each subject were transformed logarithmically to base 10.^{7, 8} The Student t test was employed for statistical analysis. The mean and standard deviations for the non-transformed immunoglobulin concentrations were also determined.

RESULTS

The reproducibility of the γ G, γ A, and γ M quantitation by the single radial diffusion technique was determined by twelve duplicate measurements on two sera. The respective coefficients of variation for γ G were 8.1 and 10.2 per cent, γ A 7.0 and 12.5 per cent, and γ M 6.6 and 9.0 per cent. γ D quantitation has a 19.5 per cent coefficient of variation.⁶

Tables I and II compare the concentrations of the four immunoglobulins in the 118 sera studied, with the clinical diagnoses, age, sex, and method(s) of skin testing employed to classify an individual as either reaginic (Table I) or nonreaginic (Table II). In order to delineate the relationship of age to immunoglobulin levels, the two sera classes were subdivided into four age groups: (1) 4 to 10 years, (2) 11 to 30 years, (3) 31 to 60 years, and (4) $\geq$ 61 years. The mean and median concentrations of immunoglobulins and standard deviations of the mean in the various age groups are presented in Table III.

γ G

The mean γ G concentration in the reaginic class was 1,637 mg. per 100 ml. (median 1,600) compared to 1,547 (median 1,500) in the nonreaginic class. Higher mean levels were present in reaginic individuals at every age except the 4 to 10 year group (Table III). Neither the over-all nor the age group differences

Table I. *Reaginic class: age, sex, method of skin testing, clinical diagnosis, and immunoglobulin levels in 67 individuals with high levels of reagin**

Identi- fication No.	Age	Sex	Skin test	Clinical diagnosis	Immunoglobulin (mg. per 100 ml.)			
					G	A	M	D
150	4	M	P-K	Sinusitis, asthma	1,075	220	28	1.8
90	5	F	P-K	AR, asthma	1,800	52	92	2.8
165	5	F	P-K	AR, asthma, eczema	1,150	90	56	2.5
162	6	M	P-K	AR, asthma, eczema	950	31	56	1.5
145	8	M	P-K	AR, asthma	1,450	250	49	10.0
127	8	F	P-K	AR	1,300	93	74	4.4
114	8	M	P-K	AR, asthma	1,500	190	62	4.0
101	8	M	P-K	AR	1,100	37	42	2.9
53	9	M	P-K	AR	1,650	94	43	4.0
142	10	M	P-K	AR, asthma	1,300	160	83	2.5
117	10	M	P-K	AR, asthma	1,600	84	81	10.0
126	10	M	P-K	AR	1,075	115	38	(0.7)
130	10	M	P-K	AR, asthma, eczema	820	30	67	6.0
143	10	F	P-K	AR, asthma, eczema	1,550	17	53	2.5
55	11	M	D	AR	2,300	270	57	2.0
159	11	M	P-K	AR, asthma, chronic bronchitis, sinusitis	1,075	145	54	3.7
13	12	M	D	AR	1,850	230	118	8.0
168	12	M	P-K	AR, asthma	1,600	250	40	5.6
176	12	M	D	AR, asthma	1,200	140	65	3.7
179	12	M	D	AR, asthma	2,200	300	38	2.2
63	13	F	D	AR, asthma	1,850	220	130	9.1
111	13	M	P-K	AR, acute sinusitis	1,750	73	55	2.8
158	13	F	P-K	Chronic sinusitis, asthma	1,750	145	60	7.0
69	14	F	D	AR, asthma, sinusitis, recurrent pneumonitis	1,850	420	130	12.0
96	16	M	D	AR, asthma	1,825	245	78	1.5
178	16	M	D	Asthma	1,300	235	50	5.0
36	17	M	P-K	AR	2,025	190	47	5.1
137	17	F	D	AR, asthma, eczema	1,200	130	130	7.7
153	17	M	D, P-K	AR, asthma	1,225	100	49	< 0.3
109	18	F	P-K	AR, asthma, eczema	2,300	250	110	2.6
131	18	M	P-K	AR, asthma, chronic sinusitis	1,500	340	110	8.5
177	18	M	D	AR, asthma	1,500	220	60	5.0
133	19	M	D, P-K	AR, chronic tonsillitis	1,300	175	58	5.5
128	23	M	D	AR	1,300	235	90	2.0
71	24	M	D	AR, asthma	2,450	700	68	30.0
105	25	M	D	AR, asthma	1,850	180	160	2.0
22	26	F	D	AR	1,750	395	66	(0.7)
8	30	M	D	AR, diabetes	1,680	300	50	5.3
174	30	F	D	AR, asthma	1,600	96	92	1.8
24	31	F	D	AR, asthma	1,500	280	98	(0.7)
59	32	M	D, P-K	AR, asthma	1,650	180	62	4.0
65	32	F	D	AR, asthma, urticaria	1,850	330	90	(0.7)
141	32	M	D	AR, asthma, sinusitis	1,300	145	69	5.6
99	35	F	D, P-K	AR, asthma, sinusitis	1,050	120	100	1.5
11	36	M	D	AR, asthma, sinusitis	1,600	255	55	5.3
28	36	M	D	AR, asthma	980	150	105	10.0
51	36	M	D	AR, sinusitis	2,800	300	74	3.8
32	37	M	D	AR, asthma	2,025	150	95	2.6
19	39	F	D	AR	1,100	215	55	6.2
166	40	F	D, P-K	AR, asthma	1,100	165	40	5.6
67	42	F	D	Asthma, possible rheumatoid arthritis	2,000	300	82	5.6
40	45	M	D	AR, asthma	2,000	290	140	24.0
75	46	M	D	AR, asthma	2,450	280	62	5.6
12	47	M	D	Pharyngitis, unknown etiology	1,400	360	73	56.0
44	47	M	D, P-K	Asthma, chronic bronchitis	2,600	230	52	5.6

*Abbreviations: D, direct; P-K, passive transfer; AR, atopic rhinitis.

Table I. Continued

Identification No.	Age	Sex	Skin test	Clinical diagnosis	Immunoglobulin (mg. per 100 ml.)			
					G	A	M	D
146	47	M	D	AR, asthma, sinusitis	2,000	290	65	(0.7)
34	50	M	D, P-K	Asthma, chronic bronchitis	1,950	380	61	12.0
57	51	F	D	AR, asthma	1,650	290	62	1.5
73	52	M	D	AR	1,525	460	68	(0.7)
77	53	M	D	AR, asthma	1,950	380	120	2.4
7	55	F	D, P-K	AR, asthma, chronic bronchitis, sinusitis	2,000	280	85	2.6
157	59	F	D	AR, asthma	2,150	200	115	< 0.3
155	60	F	D	AR	1,850	380	87	1.8
138	62	M	D	AR, asthma, chronic bronchitis	1,050	380	83	2.8
79	65	M	D, P-K	AR, bronchitis	2,200	190	51	< 0.3
31	66	F	D, P-K	Asthma, emphysema	1,350	270	61	6.2
149	70	F	D	AR, sinusitis	2,150	345	65	2.5

were statistically significant. No age-related variation in γ G was observed (Table III, Fig. 1).

γ A

An age-related variation in γ A concentrations was found with progressively higher concentrations in each succeeding age group in both reaginic and non-reaginic sera. This age-related increase was statistically significant, $p < 0.01$, between the 4 to 10 year and 11 to 30 year age groups.

In all age groups the mean γ A concentration was higher in the reaginic class (Table III, Fig. 1). The mean increase ranged from 17 mg. per 100 ml. in the 4 to 10 year group to 42 mg. in the ≥ 61 year group. These differences were not statistically significant.

If the age-related variation in γ A is not considered, the concentrations in the two classes are essentially the same, mean 225 mg. per 100 ml. (median 220) in the 67 reaginic sera and 221 mg. per 100 ml. (median 200) in the 52 nonreaginic sera. The smaller over-all intraclass difference is the result of a greater proportion of young individuals in the reaginic class, median age 20 years, compared to the nonreaginic, median age 50 years.

γ M

There was no consistent relationship between γ M concentrations and reaginic status or age. The levels in the reaginic and nonreaginic classes and in each age group were very similar (Table III, Fig. 1).

γ D

The over-all reaginic class mean γ D level was 5.5 mg. per 100 ml. (median 3.7) compared to the nonreaginic mean of 3.2 (median 1.5). This difference is highly significant, $p < 0.01$ (Fig. 2). In all age groups the reaginic median γ D level was higher. However, in the ≥ 61 year group a higher mean γ D was present in the nonreaginic sera (Table III, Fig. 1).

Table II. *Nonreaginic class: age, sex, method of skin testing, clinical diagnosis, and immunoglobulin levels in 51 individuals without detectable reagin**

Identi- fication No.	Age	Sex	Skin test	Clinical diagnosis	Immunoglobulin (mg. per 100 ml.)			
					G	A	M	D
70	4	F	P-K	Recurrent bronchiolitis	1,150	120	110	(0.7)
164	5	M	P-K	Asthma—infectious	1,250	27	67	1.6
147	5	M	P-K	AR, asthma	1,400	38	105	2.2
160	6	M	P-K	AR	1,500	165	67	4.3
172	13	F	D	Upper respiratory infection	1,400	155	64	1.6
161	18	F	P-K	Asthma, chronic sinusitis	720	230	67	1.5
167	20	F	P-K	Vasomotor rhinitis, chronic sinusitis, urticaria	1,400	210	46	(0.7)
180	23	M	D	Chemical rhinitis	1,400	250	65	3.7
104	24	F	D	P-K recipient	1,500	180	68	5.3
156	25	F	D	Vasomotor rhinitis	1,500	170	115	1.5
113	30	F	D	Chemical rhinitis	1,400	210	75	(0.7)
54	33	M	D	Chronic sinusitis	1,650	250	165	<0.3
115	36	M	D	P-K recipient	1,500	210	55	4.0
170	37	F	P-K	Asthma, chronic bronchitis, sinusitis	1,600	230	67	4.1
56	39	F	D	Chronic sinusitis	2,150	400	83	7.7
94	40	F	D	P-K recipient	1,950	315	85	4.6
25	41	F	D	Asthma, chronic bronchitis	1,800	420	87	24.0
48	42	M	D	Vasomotor rhinitis, sinusitis	1,800	210	85	2.8
100	43	F	D	Asthma, chronic bronchitis	1,725	155	90	(0.7)
66	44	F	D	Urticaria	1,550	170	130	(0.7)
45	46	F	D	Neurodermatitis	1,200	105	100	2.0
95	48	F	D	P-K recipient, chronic bronchitis	2,250	340	78	7.4
106	48	M	D	P-K recipient	1,400	300	62	8.0
118	49	M	D	P-K recipient	1,600	55	120	(0.7)
58	50	F	D	Deviated nasal septum	1,850	340	38	6.0
72	50	F	D	Chronic bronchitis, sinusitis	1,650	390	100	3.4
134	51	F	D	Chronic bronchitis, emphysema	1,200	90	63	2.8
169	51	M	D	Asthma, chronic bronchitis	1,550	90	67	1.5
181	53	F	D	No organic disease	780	115	29	<0.3
154	54	F	D	Chronic bronchitis, sinusitis	1,850	220	40	<0.3
107	55	F	D	P-K recipient	1,100	180	62	(0.7)
14	56	F	P-K	Neurodermatitis	1,750	510	34	(0.7)
97	56	F	P-K	Urticaria	2,100	290	92	3.6
29	58	M	P-K	Chemical rhinitis	1,300	200	30	1.5
125	58	M	D	Chronic bronchitis	1,150	84	46	(0.7)
132	58	F	D	Conjunctivitis, diabetes mellitus	1,400	190	98	(0.7)
42	59	M	P-K	Chronic bronchitis	1,750	360	100	<0.3
27	60	F	D	Chronic bronchitis, sinusitis	1,300	220	105	14.0
116	60	F	D	P-K recipient, chronic sinusitis	1,700	84	74	<0.3
148	60	F	P-K	Vasomotor rhinitis	1,500	205	105	(0.7)
135	64	F	D	Chronic bronchitis, sinusitis	1,000	310	83	0.7
38	65	M	D	Chronic bronchitis, sinusitis	1,350	230	67	3.2
68	65	F	D	Dermatitis caused by hairspray	2,150	390	47	(0.7)
110	65	F	D	P-K recipient	2,900	180	90	<0.3
140	65	F	D	Emphysema	1,200	82	110	<0.3
139	65	M	D	Vasomotor rhinitis, asthma	1,550	190	110	2.8
18	67	F	D	Chronic bronchitis	1,400	105	89	1.5
151	68	M	D	Vasomotor rhinitis, chronic lymphatic leukemia	1,150	100	87	<0.3
129	70	F	D	Chronic pyelonephritis	1,500	190	26	(0.7)
108	73	M	P-K	Chronic bronchitis, emphysema	2,000	695	82	21.0
74	76	M	D	Chronic sinusitis	2,000	330	68	4.8

*Abbreviations: D, direct; P-K, passive transfer; AR, atopic rhinitis.

Table III. Over-all and age group immunoglobulin concentrations in reaginic and nonreaginic sera*

Age group (years)	Class	No.	Immunoglobulin (mg. per 100 ml.)							
			G		A		M		D	
			Mean $\pm$ S.D.	Me-dian	Mean $\pm$ S.D.	Me-dian	Mean $\pm$ S.D.	Me-dian	Mean $\pm$ S.D.	Me-dian
4-10	R	14	1,309 $\pm$ 291	1,300	105 $\pm$ 74	93	59 $\pm$ 19	56	4.0 $\pm$ 2.9	2.8
	N-R	4	1,325 $\pm$ 155	1,325	88 $\pm$ 66	76	87 $\pm$ 23	86	2.2 $\pm$ 1.5	1.9
11-30	R	25	1,689 $\pm$ 379	1,750	239 $\pm$ 130	230	79 $\pm$ 34	65	5.6 $\pm$ 5.9	5.0
	N-R	7	1,331 $\pm$ 274	1,400	201 $\pm$ 34	210	71 $\pm$ 21	67	2.1 $\pm$ 1.7	1.5
31-60	R	24	1,770 $\pm$ 480	1,850	267 $\pm$ 89	280	80 $\pm$ 24	73	6.9 $\pm$ 11.6	3.9
	N-R	29	1,590 $\pm$ 333	1,600	232 $\pm$ 117	210	79 $\pm$ 32	83	3.6 $\pm$ 5.1	1.5
61	R	4	1,688 $\pm$ 576	1,750	296 $\pm$ 84	308	65 $\pm$ 13	63	2.9 $\pm$ 2.5	2.9
	N-R	11	1,655 $\pm$ 558	1,500	255 $\pm$ 177	190	78 $\pm$ 25	82	3.3 $\pm$ 6.0	0.7
Over-all	R	67	1,637 $\pm$ 445	1,600	225 $\pm$ 120	220	74 $\pm$ 28	68	5.5 $\pm$ 8.0	3.7
	Range		820-2,800		17-700		29-160		< 0.3-56.0	
	N-R	51	1,547 $\pm$ 386	1,500	221 $\pm$ 127	200	78 $\pm$ 28	78	3.2 $\pm$ 4.7	1.5
	Range		720-2,900		27-695		26-165		< 0.3-24.0	

*Abbreviations: R, reaginic; N-R, nonreaginic.

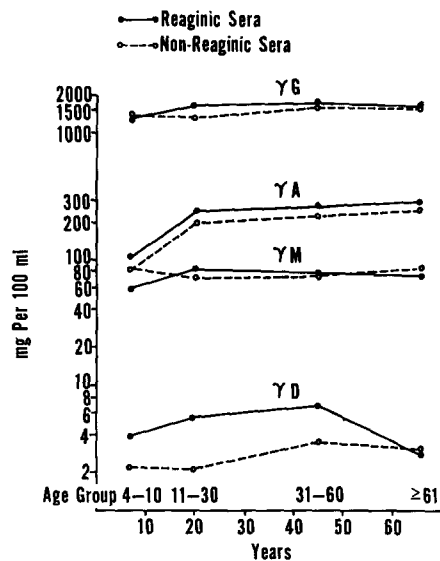
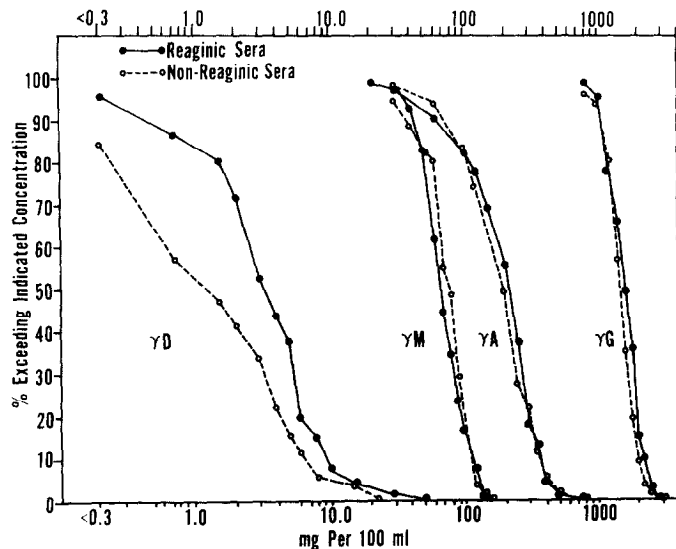


Fig. 1

Relationship of age and immunoglobulin concentrations. Mean γ A concentrations increase with age in both reaginic and nonreaginic sera. The difference between γ A concentrations in the 4 to 10 and 11 to 30 year groups is significant, $p < 0.01$.

DISCUSSION

The present study was undertaken to define and compare the concentrations of the four currently recognized immunoglobulin classes in sera from persons with high reaginic activity and those lacking detectable reagin. Significantly higher γ D levels were present in reaginic sera. Concentrations of γ G and γ A were also higher; however, these increases were not statistically significant.

**Fig. 2**

Cumulative percentage distribution of immunoglobulin concentrations. Log normal distributions are present. The difference between the γ D concentrations in reaginic and nonreaginic sera is significant, $p < 0.01$.

The finding that the concentrations of γ A continued to increase throughout early adulthood confirms and extends the recent observations of Stiehm and Fudenburg.⁹ Several previous studies have suggested that normal "adult" levels of γ A are established by the time the individual is 5 years of age.^{10, 11} The meaning of the continuing increase of γ A with age is unknown.

Since reaginic (skin-sensitizing) antibody is considered to be a prerequisite for the clinical manifestation of atopy, this immunological characteristic was utilized to select sera from individuals who differed primarily in this respect. Although clinical criteria were not considered in the classification of individuals, diagnoses of atopic rhinitis and/or asthma had been recorded for 66 of the 67 persons in the reaginic class. Upper or lower respiratory tract symptoms were present in all individuals classified as reaginic and 73 per cent of the nonreaginic class. The incidence of recurrent respiratory tract infection, such as, chronic bronchitis and/or sinusitis, was substantially higher (48 per cent) among the nonreaginic patients than among the reaginic (22 per cent).

In contrast to the present study, Huntley and Lyster^{11, 12} used clinical rather than immunological criteria to classify children as allergic or nonallergic. They also found no significant differences in γ G¹² or γ A and γ M¹¹ concentrations in their subjects. At the time of their studies, γ D had not been identified as an immunoglobulin class.

γ D was initially isolated from a myeloma serum and characterized as a separate immunoglobulin by Rowe and Fahey.¹³ They subsequently demonstrated its presence in nonmyeloma sera where the distinct γ D H-polypeptide (δ) chain is associated with a normal complement of kappa (type I) and lambda (type II)

L chains.¹⁴ Metabolic studies have revealed a median biological half-life of 2.8 days, which is distinctly shorter than that of other immunoglobulins.¹⁵ In addition, the in vivo distribution of γ D is predominantly intravascular (73 per cent), a feature distinguishing it from γ G and γ A.¹⁵ γ D concentrations of healthy adults range from undetectable levels (< 0.3) to 30 mg. per 100 ml. with a median level of 3.0 mg. per 100 ml.⁶ Unfortunately, no information is available concerning either the presence of previous atopic symptoms or the incidence of positive wheal-and-flare reactions in these subjects.⁶ On the basis of previous studies,^{2, 3} it is probable that a significant proportion would show evidence of reagin if skin tested. In the 118 patients included in this report, the median γ D concentration was 2.7 mg. per 100 ml., range < 0.3 to 56 mg. per 100 ml., indicating that the respiratory illness present in the majority of these subjects was not associated with an increase in γ D. The median γ D was the same (1.5 mg. per 100 ml.) in nonatopic patients with and those without significant respiratory illness (Table II), indicating that increased reagin titer was the only factor which correlated well with higher γ D concentrations (atopic median 3.7 mg. per 100 ml.).

Antibody activity associated with γ D has not, as yet,⁶ been demonstrated and its functional role remains unknown. Therefore, any interpretation relative to the higher γ D concentrations found in the sera from individuals with high titers of reaginic antibody must be made with caution. While it is tempting to speculate that reaginic antibody might be associated exclusively with the γ D immunoglobulin class, the following observations indicate that it is not. (a) In the present study, 3 out of 67 individuals (4.5 per cent) with high levels of reaginic antibody had undetectable γ D, that is, < 0.3 mg. per 100 ml. (Fig. 2). (b) Four individuals with γ D levels between 1.5 and 8.8 mg. per 100 ml. had reaginic antibody to bovine serum albumin (BSA) as demonstrated by passive transfer. These four sera were studied utilizing an indirect* radio-Ouchterlony technique for I^{131} BSA binding by γ D with negative results. (c) Ishizaka, Ishizaka, and Lee¹⁶ have reported that reaginic activity to ragweed pollen was not removed from 4 atopic sera following precipitation of γ D by specific antiserum.

The above observations by no means preclude the possibility that γ D reaginic antibody may occur since the physicochemical and immunochemical nature of reagin remains controversial. Thus far, skin-sensitizing activity has been reported to be associated with γ A,¹⁷⁻¹⁹ γ G,^{20, 21} and a new class of immunoglobulins designated γ E by Ishizaka, Ishizaka, and Hornbrook.²² The possibility exists that the unique physicochemical properties of skin-sensitizing antibody as manifested by its inactivation by heat and capacity to passively sensitize skin, could be determined by a common structural moiety which may occur in several immunoglobulin classes or subclasses.²⁰

Atopic persons as a group differ immunologically from nonatopic persons in terms of reaginic antibody and, as demonstrated in this study, γ D immunoglobulin concentrations. These alterations appear to be independent phenomena.

*The I^{131} BSA was added after the precipitin line, formed by the interaction of antiserum and γ D, had developed.

A third immunological difference between atopic and nonatopic persons has been proposed relative to antibody synthesis in response to antigens encountered on mucous membranes. While the quantitative antibody response of atopic and nonatopic persons appears to be the same following parenteral antigenic stimulation,²³ it appears to differ in respect to antigenic stimulation occurring via respiratory or gastrointestinal mucous membranes. Atopic persons developed greater skin reactivity after intranasal immunization with aerosolized protein²⁴ and polysaccharide antigens²⁵ and higher levels of nonreaginic bentonite flocculating antipolysaccharide antibody²⁵ than did nonatopic individuals. The incidence of nonreaginic antibody to a dietary protein, bovine serum albumin, has been shown to be three to five times greater in atopic adults as compared to adults having negative skin tests and no clinical evidence of atopy.²⁶ Although the significant increase of γ D in reaginic individuals may be related to this "route of immunization" phenomenon, further investigations of the quantitative and qualitative aspects of antibody synthesis invoked by antigens encountered on mucous membranes is required before such an interpretation can be directly supported.

The observation that the immunoglobulin concentrations in the 118 sera studied herein followed a log gaussian rather than gaussian frequency distribution is similar to the findings of Claman and Merrill²⁷ and is in accord with studies concerning the antibody response of experimental animals to standard amounts of antigen.^{28, 29} The quantitative antibody response of rabbits immunized with single injections of sheep red blood cells,²⁸ key-hole limpet hemocyanin, and bovine serum albumin²⁹ has been shown to follow a log normal frequency distribution.

Table IV. "Normal adult" immunoglobulin concentrations determined by antibody in agar quantitation

Investigator(s)	No.		Immunoglobulin (mg. per 100 ml.)		
			γ G	γ A	γ M
Heremans ³²	25* 22†	Mean $\pm$ 1 S. D.		112 $\pm$ 41	72 $\pm$ 20
Fahey and Mac-Kelvey ⁵	20		1,240 $\pm$ 220	280 $\pm$ 70	120 $\pm$ 35
Stiehm and Fudenburg ⁹	30		1,158 $\pm$ 305	200 $\pm$ 61	99 $\pm$ 27
Kohler and Farr	24‡		1,350 $\pm$ 311	236 $\pm$ 104	83 $\pm$ 28
Claman and Merrill ³⁰	66	Mean (5th and 95th percentile)	995 (720-1,400)	177 (84-314)	187 (93-320)
Allansmith ³³	315§	Geometric mean ($\pm$ 2 S. D.)	1,045 (711-1,536)	169 (60-489)	89 (37-212)

* γ A.

† γ M.

‡These individuals had negative allergic histories but were not skin tested.

§Allansmith's data are expressed as the geometric mean $\pm$ 2 log S. D., with the antilog values in parentheses.

Immunoglobulin quantitation in normal individuals and in persons with a wide variety of diseases has been the subject of several recent reports.^{8, 30, 31} The relative lack of agreement between various investigators concerning normal immunoglobulin concentrations is illustrated in Table IV. While meaningful quantitative results can be obtained within a single laboratory, it is impossible to compare data obtained in different laboratories. This problem could be resolved, in large part, if the same reference standard containing specified concentrations of immunoglobulins was available for use by interested investigators so that results obtained in different laboratories could be compared directly.

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