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SERUM IMMUNOGLOBULIN LEVELS IN HEALTHY CHILDREN AND ADULTS

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SUMMARY

Serum levels of IgM, IgG and IgA were determined in 270 healthy children, 4–12 years old, and in thirty healthy adults by the radial immunodiffusion method of Mancini. There were nine 1-year age groups of thirty children each (fifteen boys and fifteen girls); the adult group consisted of fifteen males and fifteen females.

The difference in values between adults and children was statistically significant. The IgG and IgA concentrations showed a gradual rise with increasing age; the IgM concentration remained constant at a distinctly lower level than that in adults. The IgA level was about the same in both sexes. Girls had significantly higher IgM and IgG levels than boys. A consistent seasonal influence on the three serum immunoglobulin concentrations could not be demonstrated. A very wide variation in serum levels of each immunoglobulin in each age group was found. Very low values were by no means exceptional. The consequence of this finding for the diagnosis of immunological incompetency is discussed.

INTRODUCTION

Specific antibodies are proteins which, in view of their importance for immunity, are called immunoglobulins; they are localized in the β- and γ-globulin fractions of the serum. On the basis of differences in physico-chemical properties and antigenic structure, five different immunoglobulin classes can be distinguished today, viz. IgM, IgG, IgA, IgD and IgE (*Bull. Wld Hlth Org.*, 1964); these immunoglobulin classes differ also in biological activity (Cohen & Milstein, 1967).

In patients suffering from recurrent infections, a study of immunological reactivity is required when other causes explaining these infections have been ruled out. An analysis of the immunoglobulin pattern forms part of such a study. This can be accomplished with the aid of immunoelectrophoresis, which supplies semiquantitative information on serum IgM, IgG and IgA concentrations. However, the estimate of IgM, IgG and IgA levels is

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not very reliable when low or high concentrations are concerned (Zegers *et al.*, 1968). The IgD and IgE concentrations in normal human serum are so low that their determination calls for more sensitive techniques.

These considerations prompted us to use in the study of serum immunoglobulins, besides immunoelectrophoresis, the method described by Mancini and his co-workers (Mancini *et al.*, 1964; Mancini, Carbonara & Heremans, 1965) and modified by van Munster & Stoelinga (1965); this single radial immunodiffusion method permits of an exact quantitative determination of the various immunoglobulin classes.

It is an established fact that normal serum immunoglobulin concentrations in young children are considerably lower than those in adults (West, Hong & Holland, 1962; Fulginiti *et al.*, 1966; Stiehm & Fudenberg, 1966; Collins-Williams *et al.*, 1967; Johansson & Berg, 1967; Allansmith *et al.*, 1968).

To ensure correct evaluation of serum immunoglobulin levels found in young patients, IgM, IgG and IgA concentrations in serum from normal children and adults were determined.

MATERIALS AND METHODS

The study included 270 children and thirty adults. The children ranged in age from 4 to 13 years. There were nine 1-year age groups of thirty children (fifteen boys and fifteen girls). All children attended either kindergarten or primary school. The adult group also consisted of fifteen males and fifteen females; all were personnel of the University Hospital, but not directly involved in treating patients.

In order to study seasonal influences on the immunoglobulin levels, in the age groups 7–11 years five boys and five girls were studied in November 1966, five boys and five girls in February 1967 and five boys and five girls in May 1967. Both boys and girls were always chosen so that their ages were distributed as evenly as possible over the age group to which they belonged. Both children and adults fulfilled the following requirements. They were in good health, free from infection at the time of the study and in the recent past, not suffering from recurrent infections, without relatives suffering from recurrent infections, without allergic conditions or relatives with such conditions; all were caucasians; all were fasting at the time of venipuncture.

Of blood samples obtained by venipuncture, the serum was divided into portions of 0·5 ml and immediately frozen at −80°C. The serum sample in which immunoglobulin determination was carried out (an average of 2 months after freezing) was thawed immediately before starting determination.

Antisera against IgG, IgA and IgM were obtained in rabbits by footpad inoculation with, respectively, a preparation of heavy chains of pool IgG, a sample of pool serum IgA (kindly supplied by Dr P. J. van Munster, Nijmegen) and an isolated monoclonal M-immunoglobulin from a patient with Waldenström's macroglobulinaemia. The anti-γ antiserum reacted weakly with κ-chain; these cross-reacting antibodies were absorbed with a type K Bence Jones protein.

The anti-α antiserum was absorbed with a preparation of pure pool human IgG and the anti-μ antiserum with serum from a patient without any detectable IgM.

The specificity of antisera was tested in immunoelectrophoresis according to Scheidegger 1955) and immunodiffusion experiments (Ouchterlony, 1958), using several antigens, in particular H- and L-chains and papain-produced subunits of immunoglobulins G and A.

Quantitative determination of immunoglobulins by the radial immunodiffusion method of Mancini *et al.* (1964, 1965) was carried out largely as described by van Munster & Stoelinga (1965). On each plate with twenty-five antigen reservoirs, five dilutions of a reference serum (made up of serum samples from some forty donors) were included in order to obtain a standard curve. The test sera were applied to the plate in two dilutions. After completion of diffusion the ring area was determined and with the use of the standard curve the amount of IgM, IgG or IgA of the test sera was expressed in percents of the reference serum. To determine its absolute IgM, IgG and IgA concentrations, this serum was tested against several standards, viz. a standard serum from Behringwerke, the standard sera supplied with Hyland's immunoplates and, so far as IgA was concerned, a standard serum of known IgA concentration made available by Dr P. J. van Munster. Results obtained with these different standards were evaluated and it was decided to accept as absolute values of the reference serum used: 1002 mg IgG/100 ml, 180 mg IgA/100 ml and 83 mg IgM/100 ml (Zegers *et al.*, 1968).

Statistical analysis of results is reported in the Appendix (page 109).

RESULTS

The results of individual determinations are presented in Figs. 1–3. Tables 1 and 2 and Figs. 4 and 5 present the mean values per age group for males and females together and separately.

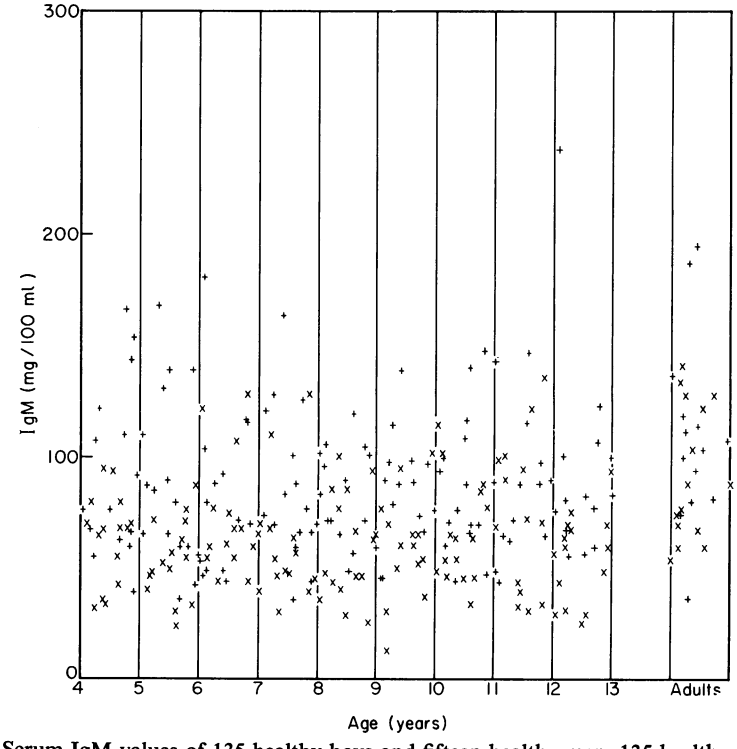

FIG. 1. Serum IgM values of 135 healthy boys and fifteen healthy men, 135 healthy girls and fifteen healthy women. ×, Males; +, females.

The range of values was very wide for IgM, IgG and IgA alike. This applies to all groups studied. Three children were found to have a very low serum IgA level (a 5-year-old boy and a 10-year-old boy with 0 mg IgA/100 ml, and a 7-year-old girl with <5 mg IgA/100 ml). The other immunoglobulin values in these children were: 10-year-old boy 45 mg IgM/100 ml and 1650 mg IgG/100 ml; 5-year-old boy 53 mg IgM/100 ml and 1068 IgG/100 ml; 7-year-old girl 63 mg IgM/100 ml and 905 mg IgG/100 ml. In a 9-year-old boy we found 16 mg IgA/100 ml, 15 mg IgM/100 ml and 550 mg IgG/100 ml.

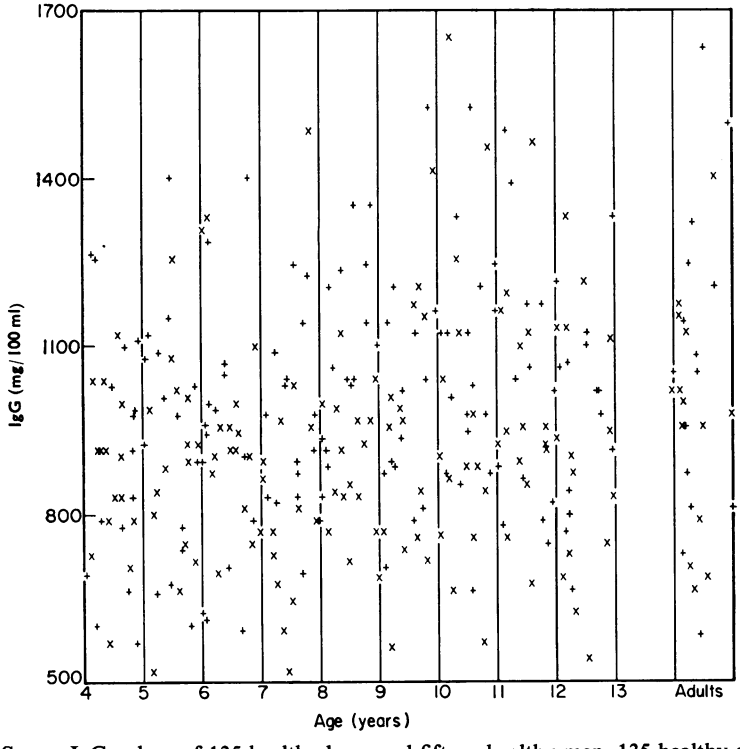

FIG. 2. Serum IgG values of 135 healthy boys and fifteen healthy men, 135 healthy girls and fifteen healthy women. ×, Males; +, females.

Statistical analysis of all data obtained disclosed the following facts.

(1) There was an unmistakable influence of age on the three serum immunoglobulin levels determined. The difference in values between adults and children was statistically significant (Table 2). The IgG and IgA concentrations showed a gradual rise with increasing age. The IgG concentration attained the adult level at about age 10 (in girls slightly earlier than in boys). The IgA concentration was still far from the adult level at age 11–13. At age 4–13, the IgM concentration remained virtually constant at a distinctly lower level than in adults.

(2) There was an unequivocal influence of sex on the concentration of two of the three serum immunoglobulins. The IgA level was about the same in both sexes. The IgG level was significantly lower in boys than in girls. The IgM level significantly differed in the two sexes in all age groups.

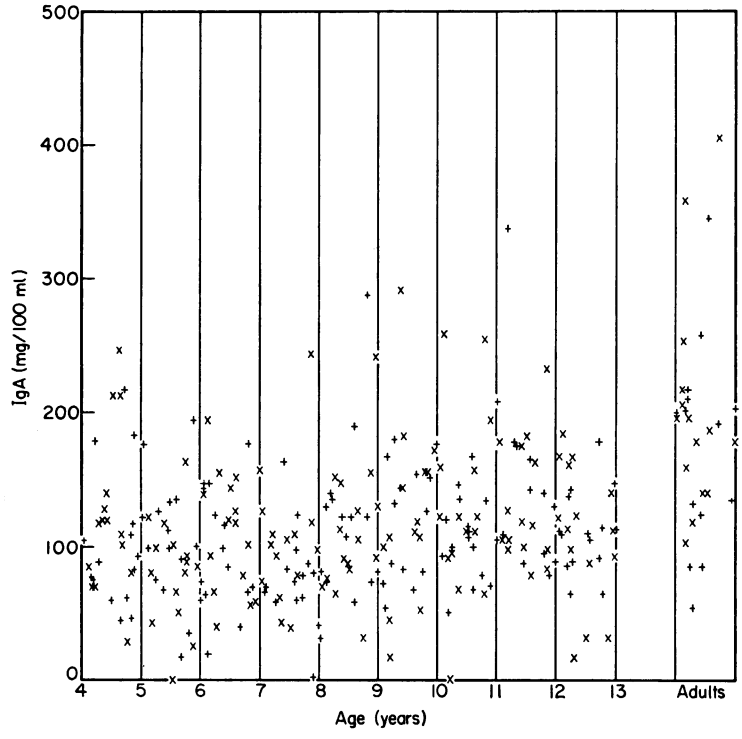

FIG. 3. Serum IgA values of 135 healthy boys and fifteen healthy men, 135 healthy girls and fifteen healthy women. ×, Males; +, females.

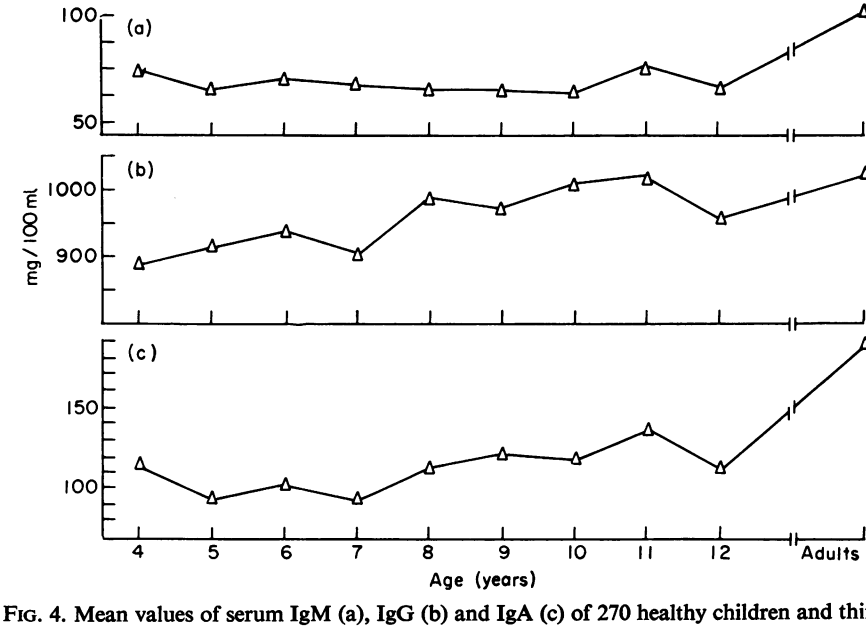

FIG. 4. Mean values of serum IgM (a), IgG (b) and IgA (c) of 270 healthy children and thirty healthy adults.

TABLE 1. Immunoglobulin levels in mg/100 ml in 270 healthy children and thirty healthy adults

	IgM				IgG				IgA			
	Male		Female		Male		Female		Male		Female	
	Mean value range	SD	Mean value range	SD	Mean value range	SD	Mean value range	SD	Mean value range	SD	Mean value range	SD
4–5 years	64 32–95	20	94 40–166	39	872 571–1100	145	904 571–1266	224	123 70–246	60	103 45–217	52
5–6 years	54 24–88	18	91 37–168	39	884 519–1255	182	942 602–1401	220	81 0–163	41	106 17–194	47
6–7 years	74 44–128	26	81 44–181	37	956 695–1329	176	921 592–1401	235	109 39–194	44	96 20–177	45
7–8 years	61 31–128	26	87 36–164	35	832 519–1484	230	972 695–1245	158	104 39–243	50	81 < 5–163	35
8–9 years	59 26–100	24	84 49–120	21	901 716–1121	113	1069 789–1349	180	109 31–241	50	114 31–287	64
9–10 years	60 13–102	23	84 46–139	25	928 560–1412	235	1013 705–1525	207	126 45–291	66	117 54–180	44
10–11 years	66 34–115	23	87 45–148	31	975 571–1650	292	1041 664–1525	210	129 0–251	69	106 50–167	32
11–12 years	75 31–136	33	87 44–147	30	989 675–1463	194	1041 747–1484	229	130 78–232	45	143 78–336	66
12–13 years	55 26–111	20	92 56–238	45	915 540–1329	231	993 664–1214	175	109 16–225	52	111 64–177	31
Adults	93 55–141	30	109 37–195	41	975 664–1401	201	1064 581–1630	287	202 103–404	83	174 54–343	74

TABLE 2. Statistical data on the serum immunoglobulins in 270 healthy children and in thirty healthy adults*†

	IgM mean value		Significance of difference of adult level		Significance of difference between sexes	IgG mean value		Significance of difference of adult level		Significance of difference between sexes	IgA mean value		Significance of difference of adult level	
	Male	Female	Male	Female		Male	Female	Male	Female		Male	Female	Male	Female
4–5 years	64	94	$P = 0{\cdot}005$	P NS	$P = 0{\cdot}015$	872	904	P NS	$P = 0{\cdot}100$	P NS	123	103	$P = 0{\cdot}007$	$P = 0{\cdot}006$
5–6 years	54	91	$P = 0{\cdot}003$	P NS	$P = 0{\cdot}002$	884	942	P NS	P NS	P NS	81	106	$P = 0{\cdot}004$	$P = 0{\cdot}006$
6–7 years	74	81	$P = 0{\cdot}073$	$P = 0{\cdot}063$	P NS	956	921	P NS	P NS	P NS	109	96	$P = 0{\cdot}001$	$P = 0{\cdot}001$
7–8 years	61	87	$P = 0{\cdot}005$	P NS	P NS	832	972	$P = 0{\cdot}080$	P NS	$P = 0{\cdot}062$	104	81	$P = 0{\cdot}002$	$P = 0{\cdot}003$
8–9 years	59	84	$P = 0{\cdot}002$	$P = 0{\cdot}043$	$P = 0{\cdot}007$	901	1069	P NS	P NS	$P = 0{\cdot}006$	109	114	$P = 0{\cdot}001$	$P = 0{\cdot}026$
9–10 years	60	84	$P = 0{\cdot}002$	$P = 0{\cdot}054$	$P = 0{\cdot}010$	928	1013	P NS	P NS	P NS	126	117	$P = 0{\cdot}010$	$P = 0{\cdot}018$
10–11 years	66	87	$P = 0{\cdot}011$	P NS	$P = 0{\cdot}044$	975	1041	P NS	P NS	P NS	129	106	$P = 0{\cdot}015$	$P = 0{\cdot}003$
11–12 years	75	87	P NS	P NS	P NS	989	1041	P NS	P NS	P NS	130	143	$P = 0{\cdot}007$	P NS
12–13 years	55	92	$P = 0{\cdot}002$	P NS	$P = 0{\cdot}008$	915	993	P NS	P NS	P NS	109	111	$P = 0{\cdot}000$	$P = 0{\cdot}006$
Adults	93	109			P NS	975	1064			P NS	202	174		

* To facilitate comparison the organization of this table is adopted from Allansmith *et al.* (1968).
† With the sign test (Fig. 5) statistically significant differences were found for IgG: boys *versus* girls; IgG: boys *versus* men; IgG: girls *versus* women and IgM: girls *versus* women.

(3) A consistent seasonal influence on the three serum immunoglobulin concentrations could not be demonstrated.

(4) The IgM, IgG and IgA levels in 12-year-old boys were lower than those in younger boys (not significant). The same applied to girls only for IgG and IgA levels.

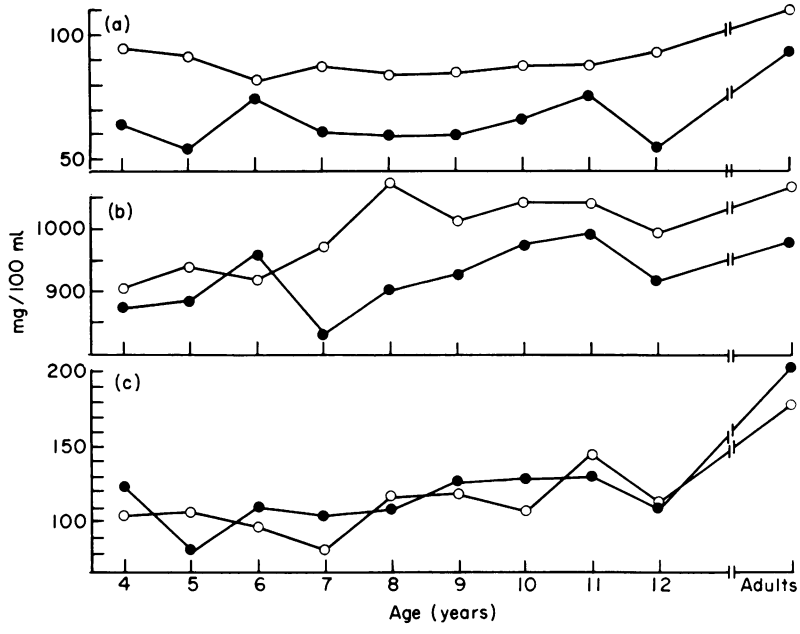

FIG. 5. Mean values of serum IgM (a), IgG (b) and IgA (c) of 150 healthy males (●) and 150 healthy females (○).

DISCUSSION

The data obtained in this study on immunoglobulin levels in normal children cannot be compared without qualification with the values published by other investigators (West *et al.*, 1962; Fulginiti *et al.*, 1966; Stiehm & Fudenberg, 1966; Collins-Williams *et al.*, 1967; Johansson & Berg, 1967; Allansmith *et al.*, 1968). In part, this is probably a consequence of the use of different methods of determination (Fahey & Lawrence, 1963; Fahey & McKelvey, 1965).

Another factor which must have affected results is a difference in the composition of the material, e.g. due to application of different criteria in selecting test subjects.

However, the principal factor responsible for differences in results of studies of normal children of comparable age groups probably lies in the lack of an established international reference serum for immunoglobulin determination (Rowe, 1968).

With the reference serum used, the values obtained in this study were consistent with those reported by Stiehm & Fudenberg (1966) for children, and with those published by Norberg (1967) and Rowe *et al.* (1968) for adults.

Our study has shown that serum IgG and IgA levels gradually rise with increasing age. No such increase was detectable in the IgM level.

A striking feature was that the 12-year-olds were still far from attaining the IgA level found in adults. This finding demonstrates once again how long it takes before the serum IgA level reaches the adult value. The IgG level did not attain the adult value until about age 10, i.e. later than is generally assumed. The most surprising were the serum IgM levels, which showed no clearly detectable changes between age 4 and age 13, but were distinctly lower than the adult level. This finding contradicts the general assumption that the serum IgM level should attain adult values as early as about age 1. In this respect our observations are corroborated by Johansson & Berg (1967), who found a marked increase in IgM until age 1, at which age a plateau was reached that remained constant at a clearly lower value than the adult level. Their observations were confined to the first 6 years of life.

The fall of serum immunoglobulin levels at age 12 might be connected with the beginning of puberty. Stiehm & Fudenberg (1966) observed a decrease of IgM and IgG values between age 12 and age 16. It is important to establish which factors alter immunoglobulin synthesis during this period of life.

Another conspicuous phenomenon was the unmistakable influence of sex on serum IgM and IgG levels. For IgM, this was previously demonstrated by Butterworth, McClellan & Allansmith (1967) and Rowe *et al.* (1968). In our material, girls showed significantly higher IgM and IgG levels than boys. It is not clear whether this expresses a difference in genetic regulation of immunoglobulin synthesis or reflects other influencing factors. An interesting fact in this context is the observation of Washburn, Medearis & Childs (1965) that boys are more susceptible to infections than girls.

The results of the analysis of variance in the year groups 7–11 showed that the relation between the season and the immunoglobulin level was not identical in all year groups. The biological significance of this finding is not clear. A more meaningful assessment of seasonal variation might have been obtained by following the immunoglobulin levels in individual subjects throughout the year.

In view of the very wide variation in serum levels of each immunoglobulin in each age group, with very low values by no means exceptional, the diagnostic value of individual immunoglobulin determinations becomes questionable. Only the finding of exceptionally low values or of a pattern typical of one of the forms of dysimmunoglobulinaemia will justify correlating the values obtained with the clinical picture. Of course the question may be raised whether the many low values may have been obtained because the children studied had a very low incidence of infectious diseases. It seems likely that these immunologically normal children would have had higher serum immunoglobulin levels if they had been subject to vigorous antigenic stimulation (Hitzig, 1963).

The question whether a patient suffering from recurrent infections produces sufficient immunoglobulins must probably remain unanswered until his serum levels can be compared with those in normal age-mates submitted to vigorous antigenic stimulation.

APPENDIX

Statistical analysis

Logarithmic transformation was applied before starting an analysis of variance, because experience has shown that the logarithms of the immunoglobulin concentrations have an approximately normal distribution.

In terms of a possible seasonal influence, only the group of the 7–11-year-olds showed a

balanced composition. In the first instance, therefore, an analysis of variance was computed exclusively with the data of this group. All possible effects were considered.

TABLE 3. Analysis of variance

Source	SS	df	MS	*F*	*P*
Total	132·927	449	0·2961		
A	113·874	2	56·9369	1455·151	0·001
B	0·383	1	0·3829	9·787	0·005
C	0·484	4	0·1211	3·095	0·02
D	0·091	2	0·0453	1·157	—
AB	0·452	2	0·2260	5·777	0·005
AC	0·360	8	0·0449	1·149	—
AD	0·091	4	0·0228	0·583	—
BC	0·056	4	0·0141	0·360	—
BD	0·044	2	0·0222	0·567	—
CD	0·760	8	0·0950	2·428	0·02
ABC	0·215	8	0·0269	0·688	—
ABD	0·053	4	0·0134	0·342	—
ACD	0·974	16	0·0609	1·555	—
BCD	0·535	8	0·0669	1·709	—
ABCD	0·469	16	0·0293	0·749	—
Error	14·086	360	0·0391		

The following abbreviations are used in the analysis of variance Table 3.

A: Immunoglobulins	main effect
B: Sex	main effect
C: Age	main effect
D: Season	main effect
AB: Immunoglobulins–sex	interactions, etc.

Apart from the unsurprising influence of the immunoglobulin class, the following effects stand out:

Sex	main effect
Age	main effect
Immunoglobulin–sex	interaction
Age–season	interaction

These effects and a seasonal effect, if any, were estimated by determining the means, without logarithmic transformation, in the various groups. The estimates of these effects are presented in Table 4.

The remaining five age classes have been included in further analysis. There would seem to be no major objections to this in view of the fact that: (a) the seasonal influence as main effect is far from significant, and (b) there is no discernible line in the age–season interaction.

Table 2 indicates, among other values, the value for Student's *t*-test at paired comparison of a number of group means.

The sign test indicates a significant difference in comparison of the following groups:

(1) IgG: boys *versus* girls.
(2) IgG: boys *versus* men.
(3) IgG: girls *versus* women.
(4) IgM: girls *versus* women.

TABLE 4. Estimates of a number of effects

	Immunoglobulin		Main effect		
Immunoglobulin	IgM	IgG	IgA		
Effect	−313·8	+587·0	−273·1		
	Sex	Main effect			
Sex	Boys	Girls			
Effect	−19·5	+19·5			
	Age	Main effect			
Age	7-year	8-year	9-year	10-year	11-year
Effect	−32·9	+0·4	−1·0	+11·6	+21·9
	Season	Main effect			
Season	I	II	III		
Effect	+9·7	+8·4	−18·1		
	Immunoglobulin–sex	Interaction			
	IgM	IgG	IgA		
Boys	+8·7	−31·7	+23·0		
Girls	−8·7	+31·7	−23·0		
	Age–season	Interaction			
Season	7-year	8-year	9-year	10-year	11-year
I	+21·0	−15·4	+7·3	+9·7	−22·5
II	−16·6	−10·6	+23·3	−15·8	+19·6
III	−4·4	+26·0	−30·6	+6·1	+2·9

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