SERUM IMMUNOGLOBULIN LEVELS IN WHITE, ASIATIC AND BANTU BLOOD DONORS*

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SUMMARY

Healthy Bantu male adults have about 40% more IgG, 30% more IgA and 32% more IgM than comparable Whites. Healthy Asiatic male adults have about 20% more IgG, 23% more IgA and 7% more IgM than comparable Whites. This must be borne in mind when interpreting immunoglobulin assays on individual patients. We agree with other authors[7] that the diagnostic value of individual immunoglobulin levels becomes questionable, in view of the wide range of values found even in a normal population.

It has been reported several times that gammaglobulin levels (and more recently the specific immunoglobulin levels) are higher in persons with pigmented skins than in White people.[1,2] Furthermore, differences have also been reported between different races with pigmented skins[3] and between a group of dark-skinned persons of various non-negroid races and British Caucasians.[4]

Since the normal values quoted by most textbooks are the normal values of immunoglobulins of Caucasians living either in Europe or North America, it seemed wise to determine the normal values of the Bantu and Asiatics living in Natal, in order to interpret quantitative immunoglobulin estimations on patients of these races, performed as a clinical service.

Various factors other than race, such as sex, season of the year in which specimens are taken, age of donor, disease, and certain other factors are known to influence immunoglobulin levels.[5-8] Kalff,[1] in the summary of his paper concludes: 'Control groups used in the study of the immunoglobulin levels must be matched not only for age, sex and race, but also for several environmental factors, both changing and persistent. For any given individual living under particular environmental conditions, it is possible only to say whether his immunoglobulin levels fall within the average range of the region in which he lives. It does not yet seem justified to speak of "normal" human immunoglobulin levels.'

MATERIALS AND METHODS

Serum Samples

In order to eliminate as many variables as possible, 100 serum samples were collected from each of the 3 race groups being investigated. All specimens were from healthy male donors living in Durban and its immediate environs. The specimens were collected in sets of 3, 1 from a White, 1 from a Bantu, and 1 from an Asiatic, which were taken on the same day from donors born in the same year and of the same ABO group. Only specimens from Bantu whose physical features were consistent with the date of birth given were included. An effort was made to use donors with ages distributed between 18 and 65 years, which are the legal limits for blood donors in South Africa.

Control Serum

This was a stored pooled group AB serum from several White donors, Seitz-filtered before testing was commenced, to render it clear.

METHOD OF ASSAY

The assay method was based on the radial immunodiffusion principles of Mancini *et al.*[9] The anti-IgG and anti-IgA were prepared in rabbits and were rendered H-chain specific by absorption before use. They were mixed with 1·3% agar Noble in 0·05M Veronal buffer pH 8·6 in a ratio of 1 part antiserum to 19 parts agar. This dilution ensured optimum precipitation when using 5 μlitres of serum per test. Three ml of the agar/antisera mixture were poured into empty Hyland immunoplates* on a levelling table and were refrigerated at 4°C for at least 2 days before use. Eleven holes were punched on each slide, allowing 3 sets of test samples and 2 controls to be set up on each slide.

Hyland immunoplates were used for the IgM determinations. The holes in the plate were not used and 11 other holes were punched using the same pattern used for the IgG and IgA plates. Five lambda of serum was used for the IgG and IgA, and 8 lambda for the IgM determinations. The serum used for the IgG determinations was first diluted 1 in 20 with normal saline. All sera were measured into the holes using a 50-μlitre Hamilton microsyringe,** held in a device to keep it upright and steady, and which enabled the tip of the needle to be lowered into the hole in the gel. After incubation at room temperature (about 20°C) for 48 hours, the diameters of the precipitation rings were measured using a Hyland reader. Where the edge of the precipitation was too faint to read accurately, the slides were either washed in saline for 24 hours, stained while wet with Ponceau S stain, and decolourized with acetic acid, or treated with 1% tannic acid[10] to enable them to be read more accurately. To control variables from day to day, and from plate to plate, all assays were calculated as a percentage of the mean of

TABLE I. RESULTS OF IMMUNOGLOBULIN ASSAYS OF 100 WHITE, 100 ASIATIC AND 100 BANTU DONORS, EXPRESSED AS A PERCENTAGE OF A CONTROL SERUM

	IgG			*IgM*			*IgA*		
Parameter	*Whites*	*Asiatics*	*Bantu*	*Whites*	*Asiatics*	*Bantu*	*Whites*	*Asiatics*	*Bantu*
	60·74 -	85·98 -	100·0 -	23·49 -	9·73 -	23·14 -	64·38 -	47·66 -	58·60 -
Range	264·69	234·94	234·94	241·55	244·26	288·5	301·26	276·60	417·25
Mean	103·47	123·74	141·94	99·1	122·59	129·06	120·71	127·64	152·62
Variance	584·19	704·9	1 084·39	1 024·00	1 441·49	1 817·32	1 307·4	1 317·0	3 855·5
Standard deviation	24·17	26·55	32·93	32·00	37·96	42·63	36·15	36·29	62·09
Coeff. of variation	23·36	21·46	23·20	32·29	30·96	33·03	29·94	28·43	40·68

*Date received: 9 March 1971.

*Hyland Laboratories, Los Angeles, California, USA.
**Hamilton Company Inc., Whittier, California, USA.

the two control assays in the following manner:

$$\frac{\text{Area of test precipitation ring—area of hole}}{\text{Area of control precipitation ring including area of hole—area of hole}} \times 100$$

The area of the hole was subtracted because it is a constant which is always added, making a proportionately greater difference to low values than to high ones. All statistical analysis was done on the 'percentage of control' figures thus obtained.

As a matter of interest, the IgG, IgM and IgA levels of the control serum, obtained from the mean of 6 assays using Hyland immunoplates were 1 260 mg, 80 mg and 247 mg/ml respectively. As will be seen in the Tables, the IgG and IgA levels in the control were almost identical with the means of the White test samples (as would be expected), though for some reason, the IgM level was about 20% lower than the mean of the test samples.

RESULTS

Table I gives the data obtained in the IgG, IgA and IgM assays. In all 3 classes the Bantu have the highest levels. They have approximately 40% more IgG than Whites and about 18% more than Asiatics who, in turn, have about 20% more than the Whites. The coefficient of variation is about the same in all 3 race groups (approximately 23%) although the highest and lowest values recorded both occurred in Whites. The Bantu have about 30% more IgA than Whites, and the Asiatics about 23% more. The deviation about the mean is larger than that found for IgG, but is similar at about 32%.

IgM levels in Bantu are about 32% higher than in Whites, and Asiatics are about 7% higher. The coefficient of variation of the Whites and Asiatics is similar (28 - 29%), but that in Bantu is much higher: 40 - 68%.

STATISTICAL ANALYSIS

The observations for each race group were now divided by age and blood group, and the number of observations for each race group in each category was:

Year of birth	*A*	*B*	*O*
1911 - 1920	0	2	3
1921 - 1930	3	4	5
1931 - 1935	8	4	5
1936 - 1940	6	10	8
1941 - 1945	7	5	7
1946 - 1950	7	8	8
Total	31	33	36 (100)

It was decided to do a complete three-way analysis of variance, which requires equal numbers of observations in each sub-group. To retain as many observations as possible, the age groups were amended to 1921 - 1932, 1933 - 1936, 1937 - 1940, 1941 - 1945 and 1946 - 1949, with 5 observations in each sub-group (a total of 225 observations). The last observations made in any sub-group with a total of more than 5, were discarded. The analysis of variance is given in Tables II, III and IV.

TABLE II. THREE-WAY VARIANCE ANALYSIS OF IgG LEVELS

Variation	*d.f*	*S.S.*	*M.S.S.*	*F*
Between races	2	60017·095600	30008·547800	60·625915
Between blood groups	2	1819·455200	909·727600	1·837911
Between age groups	4	1325·287000	331·321750	·669365
Interaction race/blood	4	1662·348200	415·587050	·839605
Interaction race/age	8	3212·994600	401·624325	·811396
Interaction blood/age	8	7880·078600	985·009825	1·990003
2nd order interaction	16	3721·672300	232·604518	·469928
Residual	180	89096·198200	494·978878	
Total	224	168735·129700		

TABLE III. THREE-WAY VARIANCE ANALYSIS OF IgA LEVELS

Variation	*d.f.*	*S.S.*	*M.S.S.*	*F*
Between races	2	28407·037000	14203·518500	9·611159
Between blood groups	2	8497·311000	4248·655500	2·874957
Between age groups	4	13855·109100	3463·777275	2·343849
Interaction race/blood	4	7886·414200	1971·603550	1·334133
Interaction race/age	8	5475·016300	684·377037	·463100
Interaction blood/age	8	18571·778500	2321·472312	1·570881
2nd order interaction	16	20694·956000	1293·434750	·875234
Residual	180	266006·749600	1477·815275	
Total	224	369394·371700		

TABLE IV. THREE-WAY VARIANCE ANALYSIS OF IgM LEVELS

Variation	*d.f.*	*S.S.*	*M.S.S.*	*F*
Between races	2	72123·653212	36061·826606	15·363434
Between blood groups	2	4062·279286	2031·139643	·865327
Between age groups	4	15072·818347	3768·204586	1·605369
Interaction race/blood	4	7702·352906	1925·588226	·820359
Interaction race/age	8	11818·680317	1477·335039	·629389
Interaction blood/age	8	12075·200403	1509·400050	·643050
2nd order interaction	16	29162·851965	1822·678247	·776516
Residual	180	422505·065720	2347·250365	
Total	224	574522·902156		

The F values obtained for variation between races are highly significant in all cases. Means were compared by the t-test, with the following results:

	IgG		*IgA*		*IgM*	
Comparison	*t*	*P values*	*t*	*P values*	*t*	*P values*
Whites/Asiatics	3·91	<·01	3·55	<·01	1·82	>·05
Asiatics/Bantu	5·11	<·01	0·45	>·50	3·68	<·01
Whites/Bantu	11·00	<·01	4·00	<·01	5·54	<·01

Thus, for IgG, all 3 means differ significantly from each other, while for IgA, the means of the Asiatics and Bantu differ significantly from the mean for Whites, but not from each other. For IgM, the means for Asiatics and Whites differ significantly from the mean for Bantu, but not greatly from each other.

The F values obtained for the interaction between blood groups and age groups and for the 2nd order interaction between all 3 factors, are both just significant at the 5% level, for IgG. This means that a few of the extreme means differ significantly from each other, but there is no pattern in these differences, and they are not thought to be important. In the case of the interaction between blood groups and age group only 13 of the 105 differences are found to be significant.

With no really significant differences in the interaction, the variations due to interactions may be grouped with

TABLE V. THREE-WAY VARIANCE ANALYSIS OF IgG LEVELS (POOLED DATA—SEE TEXT)

Variance	*d.f.*	*S.S.*	*M.S.S.*	*F*
Between races	2	60017·0956	30008·5478	61·40
Between blood groups	2	1819·4552	909·7276	1·86
Between age groups	4	1325·2870	331·3218	0·68
Residual	216	105573·2919	488·1652	
Total	224	168735·1297		

TABLE VI. THREE-WAY VARIANCE ANALYSIS OF IgA LEVELS (POOLED DATA—SEE TEXT)

Variance	*d.f.*	*S.S.*	*M.S.S.*	*F*
Between races	2	28407·0370	14203·5185	9·63
Between blood groups	2	8497·3110	4248·6555	2·88
Between age groups	4	13855·1091	3463·7773	2·35
Residual	216	318634·9146	1475·1616	
Total	224	369394·3717		

TABLE VII. THREE-WAY VARIANCE ANALYSIS OF IgM LEVELS (POOLED DATA—SEE TEXT)

Variance	*d.f.*	*S.S.*	*M.S.S.*	*F*
Between races	2	72123·653212	36061·826606	16·118213
Between blood groups	2	4062·279286	2031·139643	·907839
Between age groups	4	15072·818347	3768·204586	1·684238
Residual	216	483264·151311	2237·334033	
Total	224	574522·902156		

residual variation, giving the analysis of variance in Tables V - VII. These show the same results as the first 3 Tables.

It is well documented that immunoglobulin levels vary with age, so the reason why no differences could be demonstrated is thought to be that the numbers in each group were too small, and also that the ages of the donors were between 18 and 65, when immunoglobulin levels are relatively stable—very large changes generally only occurring in childhood, and later in life.[1,7,10]

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