Med. J. Malaysia Vol. XXXIII No. 1, September, 1978

VARIATION IN SERUM IMMUNOGLOBULIN G, A AND M LEVELS IN MALAYSIAN BLOOD DONORS

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INTRODUCTION

GAMMA-GLOBULINS consist of 5 major class of immunoglobulins (Ig) which are distinguished from each other by their Heavy chain immune specificity. In serum, the IgG makes up about 70 percent, IgA about 1–20 percent and IgM about 1–10 percent of the total gamma-globulins; IgD and IgE are present in trace amounts. The physiochemical characteristics and the biological properties of these immunoglobulins are now fairly well understood and have been discussed in several reviews (Cohen & Milstein, 1967; Tomasi & Bienenstock, 1968; Franklin & Frangiore, 1970; Bennich & Johansson, 1970).

There is a trend to study the levels of the immunoglobulins in various populations in order to gain a better understanding of the biological role of the immunoglobulins and also to derive the normal limits of the serum immunoglobulin levels. It is widely reported, chiefly as a consequence of results derived from work on ethnic Africans, that hypergamma-globulinemia is a common feature of populations in the developing countries of the tropics (Turner & Voller, 1966; Michaux, 1966; McFarlane & Voller, 1966; Rowe *et al.*, 1968; Samuel *et al.*, 1970; Higashi & Chowdury, 1971; McFarlane, 1973; Zegers *et al.*, 1973). Implicit in these observations is the suggestion that elevated immunoglobulin levels in the tropical populations is due to the prevalence of viral and bacterial infections and other parasitic infestation. A comparison of serum immunoglobulin levels in health (Claman & Merrill, 1964; Fahey & McKelvey, 1965; Lichtman *et al.*, 1967; Allansmith *et al.*, 1968; Rowe *et al.*, 1968) and disease (Ngu *et al.*, 1966; Steihm & Fudenberg, 1966; Palma-Carlos *et al.*, 1971; Blaese *et al.*, 1971; Scott & Rasbridge, 1972; Lai & Van Furth, 1974; Dasgupta, 1974) for the same populations show that the immunoglobulin concentrations are raised in certain infections and diseases.

Apart from the reports on Africans, there has been little interest in the level of serum immunoglobulins in populations living in the tropics. Indeed, this blood parameter had not been investigated in normal Malaysians prior to our preliminary studies (Yadav & Shah, 1973, 1977; Shah & Yadav, 1973) although the population, especially in the rural areas, is subject to various endemic tropical diseases and parasitic infestations. Thus, the aim of this study was to determine the normal serum levels of IgG, IgA and IgM of Malaysians from the urban region (Chinese, Indian and Malays) and compare the data with that from the Orang Asli (Malaysian aborigines) who live mainly in the forest and are of a different socio-economic milieu.

MATERIALS AND METHODS

Blood Samples

Blood from normal young adults aged 11 to 20 years was obtained from male and female students at two schools in Petaling Jaya. One ml of blood was drawn by antecurbital venepuncture into a 2 ml disposable syringe and allowed to clot overnight at 4°C in the syringe. Next morning, the serum was separated from the clot by centrifugation. Sera from adults aged 21 to 50 years were obtained from healthy blood donors through the courtesy of the Blood Bank at the University Hospital, Petaling

Jaya. The serum samples from Orang Asli were obtained via courtesy of the Gombak Orang Asli Hospital, Selangor. The sera were from non-patients who normally accompany the sick member of the family to the hospital. The 1846 sera used in this study were kept frozen at −20°C until required.

Preparation of immunoglobulins

The rabbit H-chain specific antisera to IgG, IgA and IgM were prepared in our laboratories following previously described techniques (Vaerman *et al.*, 1963; Fahey and McLaughlin, 1963). Briefly, the IgG was obtained as follows. The precipitate obtained at 40 percent Ammonium sulphate saturation of human serum was dialysed against 0.01 M phosphate buffer (pH 8.0) overnight at 4°C. A 10 ml aliquot of the dialysate protein was applied to a fractionation column (2.6 × 30 cm) containing DEAE-cellulose which had been equilibrated with 0.01 M phosphate buffer (pH 8.0) at 4°C. The same buffer was applied to the column at a flow rate of 15 ml per hour and the eluants collected in 4 ml fractions (LKB Ultra Rac Fraction Collecter No. 7000A). The IgG, the purity of which was attested by Ouchterlony's (1968) double diffusion and immunoclectrophoresis (Scheeidegger, 1955), was eluted from the column as a single peak at optical density 280 nm with 0.01 M phosphate buffer. The fractions containing the IgG were pooled, then concentrated to a final volume of 4 ml by negative pressure ultrafiltration using visking cellulose tubing (8/32" pore diameter less than 10,000 daltons) and stored at −20°C in aliquots of 1 ml.

For preparation of IgA, 30 mls of pooled human serum was diluted nine times its volume with 0.02 M phosphate buffer (pH 6.0) and dialysed at room temperature against 2 litres of the same buffer which was continuously stirred. At the end of 2 hours, the buffer was renewed and dialysis continued for another 4 hours. The fine precipitate formed in the tube was removed by centrifugation and saved for isolation of IgM. The supernatant was concentrated to 30 mls by negative pressure ultrafiltration. Then an equal volume of 0.1 M $ZnSO_4$ was added with continuous stirring, and precipitation was induced by raising the pH to 6.85 by dropwise addition of 1M Na_2CO_3 at room temperature. The precipitate was removed by centrifugation, and the supernatant was concentrated to 4 mls at 4°C by negative pressure ultrafiltration. The solution was freed from zinc by addition of 1 percent (w/v) trisodium EDTA salt and dialysed overnight against 0.01M phosphate buffer (pH 8.0) at 4°C. Then a 2 ml sample of the solution was applied to a DEAE-cellulose column (1.6 × 23 cm) which had been equilibrated with 0.01M phosphate buffer (pH 8.0). The column was eluted by gradient elution, made up of 200 ml of initial buffer (0.01M, pH 8.0) stirred by magnetic stirrer in a beaker connected by a siphon to another vessel containing 125 ml final phosphate buffer (0.3M, pH 8.0). A flow rate of 15 mls per hour was maintained through the column and 3.4 ml fractions effluents were collected with the aid of a fraction collecter. The protein concentration was estimated by measuring the optical density at 280 nm in a Beckman Spectrophotometer. Two protein peaks were obtained and the fractions under the first peak were pooled and concentrated to a final volume of 10 ml. The precipitate obtained by 40% ammonium sulphate saturation of the solution was dissolved in 2 ml saline. Further IgG contaminants were removed by treatment with 0.5 mg of rabbit anti-IgG immunoadsorbent (Avrameas & Ternynck, 1969). The IgA, purity of which was checked by immunodiffusion tests, was dialysed against saline at 4°C and kept at −20°C in aliquots of 1 ml.

The euglobulin precipitate obtained from the preparation of IgA was dissolved in 10 ml saline, and dialysed against 0.1M Tris-HCl buffer (pH 8.0) at 4°C overnight. Precipitate formed during the dialysis was discarded by centrifugation and the supernatant used for the purification of IgM. The protein sample was applied to a Sephadex G-200 column (2.6 × 190 cm) which was equilibrated with 0.1M Tris-HCl buffer (pH 8.0). Eluates were collected at a flow rate of 15 ml/hr. in 5 ml fractions and the protein concentration estimated by reading the optical density at 280 nm. The fractions under the first peak were pooled and concentrated to 10 mls by negative pressure ultrafiltration. The proteins precipitated with 40% ammonium sulphate were dissolved in 2 ml saline and dialysed against 0.01M phosphate buffer at 4°C overnight. The 2 ml sample was then applied to a DEAE-cellulose column (1.6 × 24 cm) which was equilibrated with 0.01M phosphate buffer (pH 8.0). The column was eluted by gradient elution with 160 ml, 0.01M phosphate buffer (pH 8.0) as initial buffer and 80 ml 0.3 M phosphate (pH 8.0) as final buffer. Three ml fractions were collected at a flow rate of 15 ml/hr and the protein concentration was estimated at optical density at 280 nm. The fractions under the second peak were pooled and concentrated to 7 mls by ultrafiltration. Then, Potassium bromide crystals were added (24 gm/100 ml) to give a density of 1.2. The solution was centrifuged at 105,000 g for 24 hours in a Ultracentrifuge (Beckman Spinco Model L, Rotor Type 40). The lipoproteins contaminants which formed a top layer were pippetted off and the remaining solution dialysed against saline. The

purity of IgM was attested by immunodiffusion and aliquots of 1 ml kept at −20°C.

Preparation of antisera

The antisera was made in New Zealand white rabbits (2–3 kg) obtained from Central Animal House of the University Hospital. The rabbits were maintained on animal feed pellets and water *ad libitum* and green vegetables provided twice weekly.

An emulsion made with 0.5 mg of purified immunoglobulin in 1 ml saline was mixed with equal volume of complete Freund's Adjuvant (Difco Co., Detroit, U.S.A.) was injected subcutaneously at 6 to 8 sites on the dorsum and flanks of the rabbit. Subsequent multisite injections were routinely done weekly, in incomplete Freund's Adjuvant. After the 7th injection the rabbits were bled regularly from the marginal vein of the ear and the serum prepared from the blood was stored at −20°C in 5 ml aliquots.

Anti-IgG sera specific to the heavy chain was prepared as follows. To a 50 ml aliquot of anti-IgG rabbit serum was added 1 ml of pooled Sephadex G-200 Fractions of peak I (chiefly IgM and some IgA) and precipitate formed after 30 minutes at room temperature and overnight at 4°C were removed by centrifugation. The procedure to remove light chain cross-reactivity was repeated, usually two to three times, until no further precipitate occurred. The anti-IgG so obtained did not not cross-react with IgM and IgA and was used in the Mancini's (1965) quantitation procedure.

Anti-IgA and anti-IgM were made specific for the heavy chain by the addition of cord serum immunoadsorbents (Avrameas & Ternynck, 1969). One gram of the insoluble immunoadsorbent was added to 10 ml each of anti-IgA and anti-IgM rabbit serum. The mixture was gently stirred and incubated at room temperature for 1 hour and at 4°C overnight. The procedure had to be repeated once or twice, with a fresh batch of immunoadsorbent to make the antisera H-chain specific.

Quantitative Determination of Immunoglobulins

The sera was assayed for immunoglobulin G, A and M by the single radial immunodiffusion method using agar diffusion plates incorporating immunoglobulin H-chain specific rabbit antisera (Mancini *et al.*, 1965). To 100 ml of barbiturate buffer (pH 8.6, ionic strength 0.1) was added 0.5 gm sodium azide to prevent bacterial growth, 6 gms polyethylene glycol to stabilise the agar and enhance precipitation, and 3 gms of agarose (Sigma Chemical Co., St. Louis, U.S.A.). The agar was heated with constant stirring and upon going in solution it was distributed into 20 ml McCartney bottles and stored at 4°C. The required amount of solidified agar was melted in a boiling waterbath and then cooled to 55°C. The antiserum after appropriate dilution with barbiturate buffer (1:6 for anti-IgG, 1:4 for anti-IgA and anti-IgM sera) was brought to 55°C in a water bath. Equal volumes of the agar and anti-serum were mixed and quickly poured using preheated pipettes into special moulds consisting of two glass plates (7 × 10 cm) held 1.8 mm apart; the moulds were placed in the vertical position until the agar solidified. Circular wells were punched in the gel using a gel-cutter of 3 mm bore. In each agar plate, 10 μl of appropriate standards (obtained from World Health Organisation), control and test sera were placed in the wells and allowed to diffuse 3 days in anti-IgG and anti-IgA agar plates and 5 days in anti-IgM agar plates. The average reading of the diameters of the precipitation rings were taken at 90° with each other. Observations for standard immunoglobulin were plotted on semi-logarithmic scale and the concentration of the immunoglobulin in test serum was determined from the standard curve and expressed in mg/100 ml and also in International Units (I.U.) per ml.

Estimate of the reproducibility of the technique for the quantitative determination of immunoglobulins in our laboratory was obtained from the values observed on one control serum included in each antibody-agar plate prepared on different days using different batches of antisera. The coefficient of variation found was 3.5 percent for IgG, 6 percent for IgA and 6.8 percent for IgM. Thus, the variation was small and comparable to those reported by others (Kalff, 1970; Maddison *et al.*, 1975).

RESULTS

Immunoglobulin G levels in serum

Normal levels and distribution: The mean serum IgG levels of 445 Chinese, 409 Indians, 635 Malays and 217 Orang Asli of various ages is summarised in Table I. The mean serum IgG concentration of females is not significantly different from that of the males for each of the age groups tabulated except that of the 11 to 15 years age group. In Chinese, Indian and Malays the mean IgG level of females is significantly higher ($P < 0.01$, $P < 0.05$, $P < 0.01$, respectively) than the mean IgG level of the males; in the Orang Asli there is no difference in the serum IgG levels in the sexes of this age group.

Table I: Serum IgG levels in Malaysians of four racial origins

Race & Sex	Age (years)	Young adults			Adults				Total
		11–15	16–20	21–25	26–30	31–35	36–40	41–50	11–50
CHINESE	Males	1084 ± 322† (24)	1286 ± 554 (147)	1340 ± 382 (84)	1361 ± 326 (68)	1216 ± 408 (52)	1106 ± 265 (22)	1394 ± 479 (7)	1280 ± 449 (404)
	Females	1340 ± 581** (13)	1146 ± 196 (10)	1365 ± 295 (11)	1367 ± 201 (3)	1228 ± 239 (4)	–	–	1291 ± 385 (41)
	Males & Females	1174 ± 435 (37)	1277 ± 540 (157)	1343 ± 373 (95)	1361 ± 321 (71)	1218 ± 396 (56)	1106 ± 265 (22)	1394 ± 479 (7)	1281 ± 443 (445)
INDIAN	Males	1079 ± 436† (24)	1336 ± 360 (75)	1363 ± 384 (104)	1271 ± 286 (80)	1271 ± 457 (64)	1176 ± 340 (16)	1126 ± 321 (16)	1287 ± 382 (379)
	Females	1323 ± 194* (13)	1229 ± 180 (6)	1233 ± 111 (6)	1167 ± 120 (3)	1115 ± 235 (2)	–	–	1257 ± 186 (30)
	Males & Females	1165 ± 387 (37)	1328 ± 351 (81)	1357 ± 376 (110)	1267 ± 282 (83)	1265 ± 452 (66)	1176 ± 340 (16)	1126 ± 321 (16)	1284 ± 316 (409)
MALAY	Males	1236 ± 279† (7)	1362 ± 282 (203)	1297 ± 471 (196)	1318 ± 292 (92)	1243 ± 316 (50)	1525 ± 314 (26)	1625 ± 305 (4)	1330 ± 284 (578)
	Females	1587 ± 192** (12)	1280 ± 172 (20)	1211 ± 215 (15)	1342 ± 157 (6)	1250 ± 36 (4)	–	–	1332 ± 228 (57)
	Males & Females	1458 ± 284 (19)	1355 ± 275 (223)	1291 ± 384 (211)	1320 ± 285 (98)	1243 ± 304 (54)	1525 ± 314 (26)	1625 ± 305 (4)	1331 ± 280 (635)
ORANG ASLI	Males	1720 ± 480† (5)	1759 ± 440 (33)	1836 ± 898 (78)	1911 ± 234 (7)	1872 ± 323 (6)	2042 ± 850 (7)	1717 ± 413 (15)	1818 ± 727 (151)
	Females	1583 ± 768 (6)	1818 ± 472 (20)	1770 ± 740 (12)	1825 ± 159 (8)	2111 ± 224 (6)	1816 ± 450 (9)	1470 ± 248 (5)	1788 ± 484 (66)
	Males & Females	1646 ± 674 (11)	1781 ± 453 (53)	1827 ± 859 (90)	1865 ± 201 (15)	1991 ± 320 (12)	1914 ± 665 (16)	1656 ± 393 (20)	1809 ± 662 (217)

† Mean ± standard deviation in mg per 100 ml; multiply by 0.12 to convert to I.U./ml. Parenthesis indicates number of observations.

Significant difference between males and females of each group * $P < 0.05$, ** $P < 0.01$

The frequency distribution profiles of the serum IgG levels for Chinese (Fig. 1), Indian (Fig. 2) and Malays (Fig. 3) aged 11 to 50 years show a similar range (700 to 2350 mg/100 ml) and mode (1151 to 1300 mg/100 ml). In the Orang Asli (Fig. 4) the IgG levels are more broadly distributed, with a wider range (851 to 3250 mg/100 ml) and the mode is widely spread (1451 to 1900 mg/100 ml). About 13 percent of the Orang Asli have IgG levels greater than the highest level observed in the other 3 races (Fig. 4).

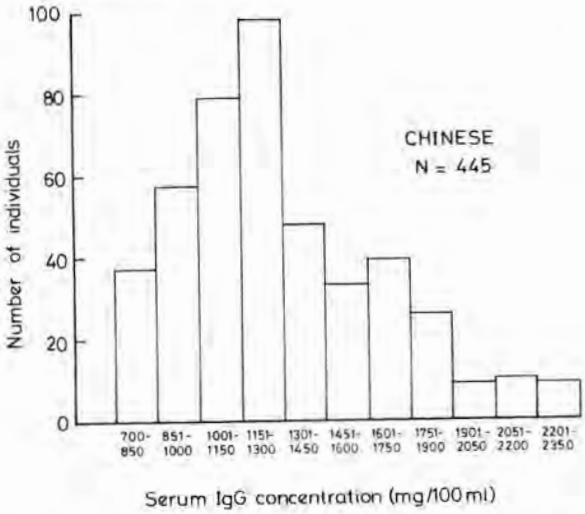

Figure 1: Frequency histogram profile of the serum IgG levels in the Chinese.

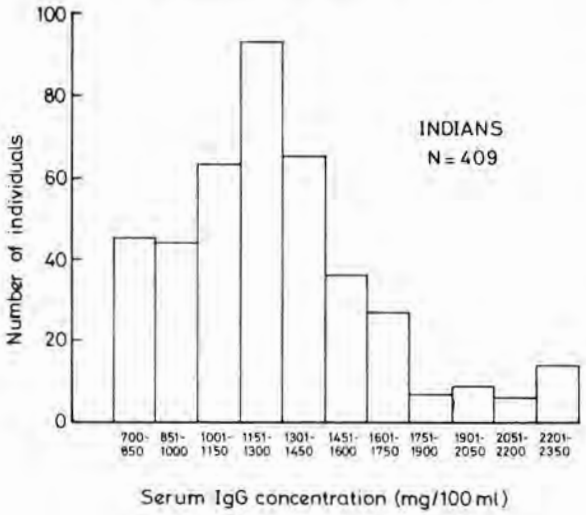

Figure 2: Frequency histogram profile of the serum IgG levels in the Indians.

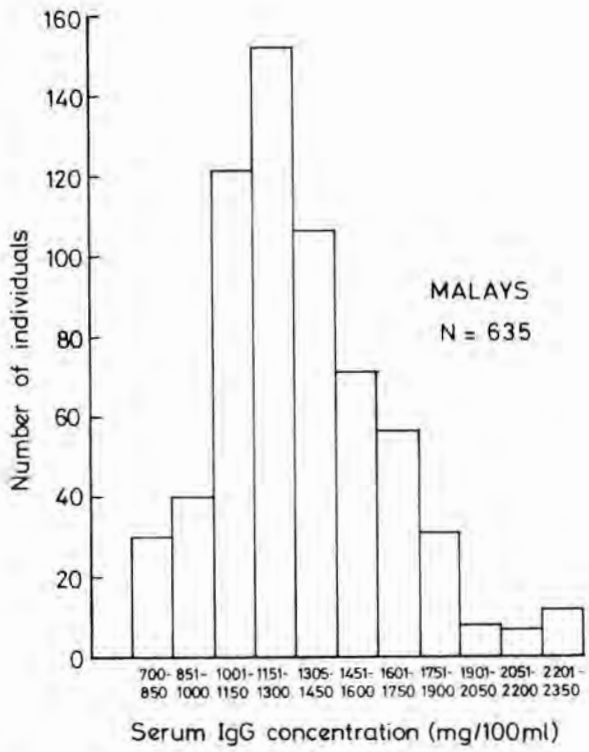

Figure 3: Frequency histogram profile of the serum IgG levels in the Malays.

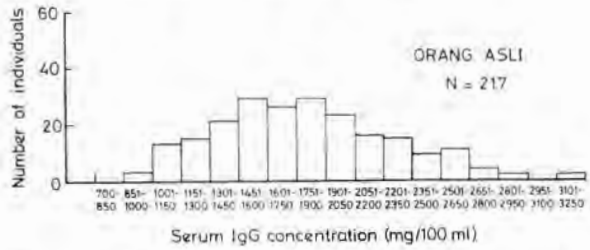

Figure 4: . Frequency histogram profile of the serum IgG levels in the Orang Asli.

Age variations: In the age groups between 16 to 35 years there is little difference in the serum IgG levels of the 3 urban races, Malays, Chinese and Indians (Fig. 5). The mean IgG levels of the Malays aged 11 to 15 years is higher than the Chinese and Indians of the same age group. Among people aged 35 years and older, the Malays have higher mean IgG level than the Indians and Chinese. In contrast to the urban races, the Orang Asli have the highest IgG levels at all ages between 16 to 40 years; in the 11 to 15 years and 41 to 50 years age groups, the IgG levels of the Orang Asli are of equal magnitude to those of Malays of the same age

group but these levels are significantly higher ($P < 0.05$) when compared to the levels in Chinese or Indians of the same age group.

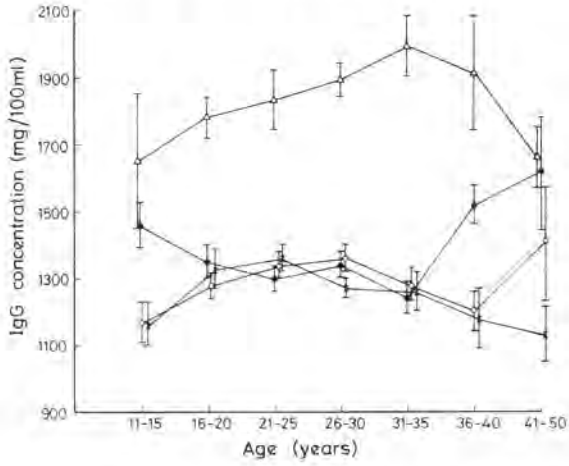

Figure 5: Changes in serum IgG levels with age in Chinese (○), Indians (x), Malays (△) and Orang Asli (●). Bars indicate standard error of the mean.

The mean serum IgG levels of young adults (11 to 20 years) is not significantly ($P < 0.05$) different from the mean levels of adults (21 to 50 years) in Chinese, Indian and Orang Asli. However, in Malays, the mean serum IgG concentration of young adults is significantly higher ($P < 0.01$) than those of adults (Table II).

Table II

Mean Serum IgG levels in Chinese, Indians, Malays and Orang Asli

Age (years) / Race	Young adults (11–20 years)	Adults (21–50 years)	Total (11–50 years)
Chinese	1257 ± 523† (194)	1301 ± 369 (251)	1282 ± 443 (445)
Indian	1277 ± 373 (118)	1287 ± 290 (291)	1284 ± 316 (409)
Malay	1363 ± 277* (242)	1311 ± 323 (393)	1331 ± 280 (635)
Orang Asli	1758 ± 496 (64)	1830 ± 719 (153)	1809 ± 662 (217)

† Mean ± standard deviation in mg per 100 ml. Parenthesis indicates number of observations.

* Significant difference ($P < 0.01$) between young adults and adults of each race.

Racial differences: Analysis of variance of the total mean serum IgG level for the four races shows that there is a significant difference ($P < 0.01$) between the IgG concentration of the Orang Asli and the IgG concentration of the 3 urban races. The total mean serum IgG levels do not differ significantly ($P < 0.01$) between the Malays, Chinese and Indians, but the total mean serum IgG of the Malays is significantly higher compared to the Chinese at the 5 percent probability level. There is no significant difference in the mean serum IgG levels of the Malays and Chinese adults but a significant difference ($P < 0.01$) exists between the mean serum IgG levels of the Chinese and Malay young adults. Thus, the significant difference, observed in the total mean serum IgG levels of Malays and Chinese is due to the variance of the mean serum IgG concentrations in young adults.

Immunoglobulin A levels in serum

Normal levels and distribution: In the Indians and the Orang Asli the mean serum IgA level is not significantly different in males and females of the age groups studied. In Chinese and Malays the females have significantly higher ($P < 0.01$) serum IgA levels than males when all age groups are considered; in Chinese this sex difference is mainly due to the age group 16–20 years and in Malays it is due to the age group 11 to 15 years (Table III). The frequency distribution profiles of the serum IgA for the three urban races of the age 11 to 50 show the range between 80 to 440 mg/100 ml; the mode is at 161–200 mg/100 ml for Chinese and Indians and at 121–160 mg/100 ml for Malays (Figs. 6, 7, 8). In the Orang Asli, the IgA levels are broadly distributed with a range of 161 to 680 mg/100 ml and a mode of 321–360 mg/100 ml. in 18 percent of the Orang Asli the serum IgA levels are greater than the highest level observed for the 3 urban races (Fig. 9).

Age variations: The mean serum IgA level in the Orang Asli is higher than the levels observed in the three urban races for all age groups investigated (Fig. 10). The mean IgA levels of the Indians aged 16 to 35 years is higher than the IgA levels of the Chinese and Maalys of the same age group. Of the four races, only in Malay and Chinese young adults have a significantly higher ($P < 0.01$) mean IgA level than the levels of adults of each race; the mean IgA levels in young and old adults are of equal magnitude in Indians and Orang Asli (Table IV).

Racial differences: Analysis of variance of the data shows that a significant difference ($P < 0.01$) exists between the serum IgA levels of the Orang Asli on one hand and the serum IgA levels in Chinese,

Table III: Serum IgA levels in Malaysians of four racial origins

Race & Sex	Age (years)	Young adults 11–15	16–20	21–25	Adults 26–30	31–35	36–40	41–50	Total 11–50
CHINESE	Males	218±59† (25)	190±63 (103)	180±70 (138)	177±70 (68)	192±74 (50)	187±76 (24)	189±87 (16)	187±72 (424)
	Females	257±83 (16)	260±65** (9)	206±65 (17)	–	–	–	–	237±77** (42)
	Males & Females	233±72 (41)	196±66 (112)	183±69 (155)	177±70 (69)	192±74 (50)	187±76 (24)	189±87 (16)	191±72 (466)
INDIAN	Males	232±66† (27)	217±79 (60)	228±80 (130)	222±71 (66)	222±102 (40)	203±52 (12)	199±71 (22)	222±79 (357)
	Females	247±57 (12)	206±65 (11)	190±57 (10)	–	–	–	–	216±62 (33)
	Males & Females	237±64 (39)	215±77 (71)	225±79 (140)	222±71 (66)	222±102 (40)	203±53 (12)	199±71 (22)	222±78 (390)
MALAY	Males	200±61† (36)	189±59 (60)	187±91 (100)	174±68 (60)	169±70 (22)	168±36 (4)	170±38 (12)	184±74 (294)
	Females	257±72* (13)	222±77 (7)	183±94 (12)	–	–	–	–	221±88* (32)
	Males & Females	215±69 (49)	192±62 (67)	187±92 (112)	174±68 (60)	169±70 (22)	168±36 (4)	170±38 (12)	188±77 (326)
ORANG ASLI	Males	396±104† (17)	388±94 (24)	393±116 (79)	417±88 (7)	394±18 (4)	407±95 (4)	413±98 (17)	396±107 (152)
	Females	350±101 (13)	392±98 (14)	390±125 (16)	486±93 (8)	415±35 (4)	361±84 (6)	356±98 (6)	390±109 (67)
	Males & Females	376±105 (30)	389±96 (38)	392±118 (95)	454±97 (15)	404±37 (8)	379±90 (10)	388±101 (23)	394±107 (219)

† Mean ± standard deviation in mg per 100 ml; multiply by 0.66 to convert to I.U./ml. Parenthesis indicates number of observations.

Significant difference between males and females of each group * $P < 0.05$, ** $P < 0.01$

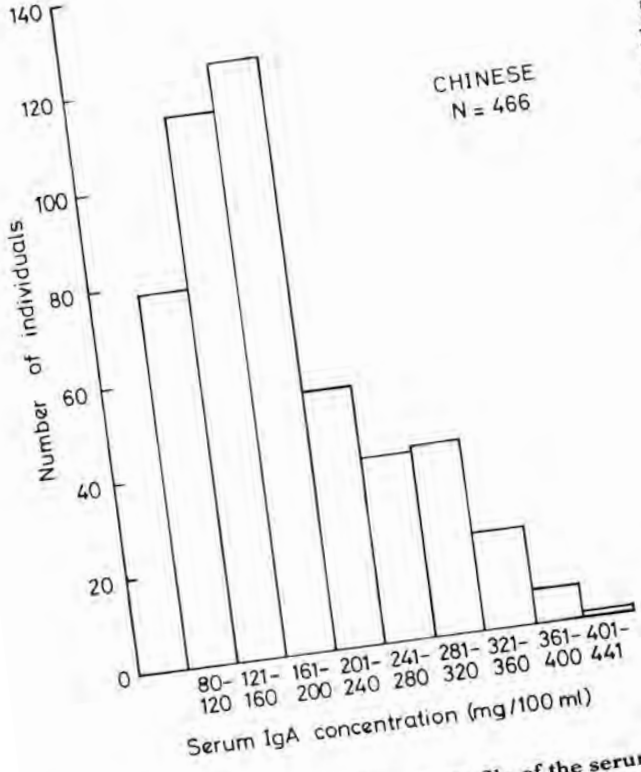

Figure 6: Frequency histogram profile of the serum IgA levels in the Chinese.

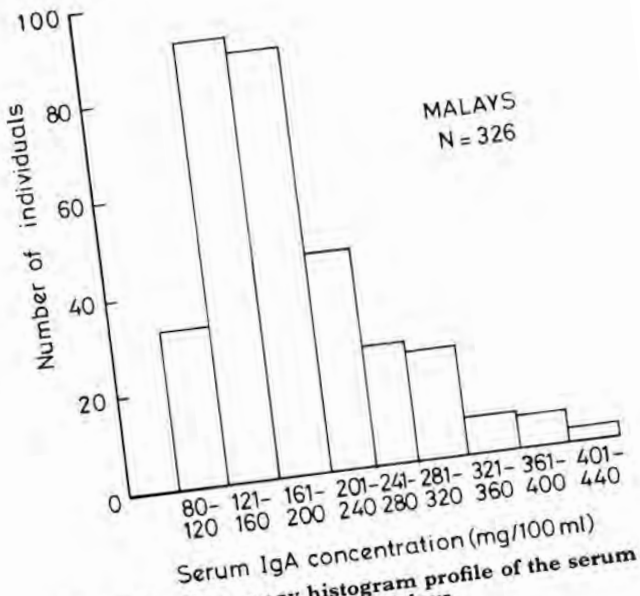

Figure 8: Frequency histogram profile of the serum IgA levels in the Malays.

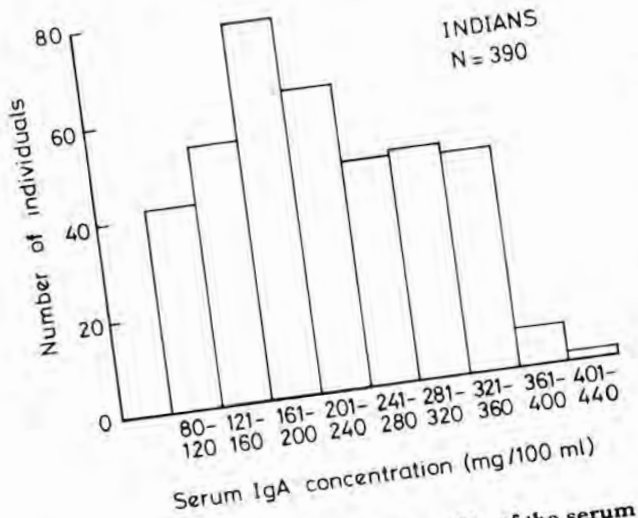

Figure 7: Frequency histogram profile of the serum IgA levels in the Indians.

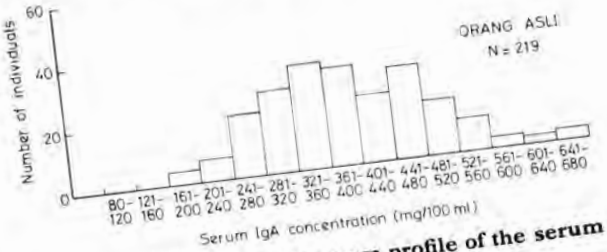

Figure 9: Frequency histogram profile of the serum IgA levels in the Orang Asli.

Table IV
Mean serum IgA levels in Chinese, Indians, Malays and Orang Asli

Age (years) / Race	Young adults (11–20 years)	Adults (21–50 years)	Total (11–50 years)
Chinese	206 ± 70†** (153)	184 ± 72 (313)	191 ± 72 (466)
Indian	223 ± 74 (110)	221 ± 80 (280)	222 ± 78 (390)
Malay	201 ± 67** (116)	180 ± 80 (210)	188 ± 77 (326)
Orang Asli	384 ± 100 (68)	401 ± 101 (151)	396 ± 101 (219)

† Mean ± standard deviation in mg per 100 ml.
Parenthesis indicates number of observations.
** Significant difference ($P < 0.01$) between young adults and adults of each race.

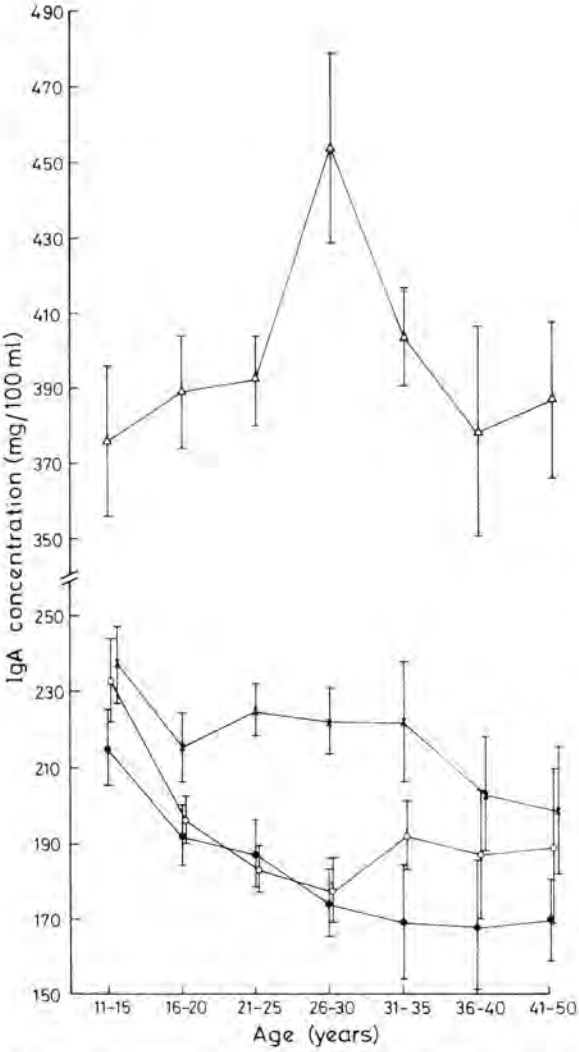

Figure 10: Changes in serum IgA levels with age in Chinese (○), Malays (●), Indians (x) and Orang Asli (△). Bars indicate standard error of mean.

Indians and Malays, respectively, on the other hand. The higher mean serum IgA level of the Indians differ significantly ($P < 0.01$) from the mean serum IgA levels of Chinese and Malays, but there is no significant difference ($P < 0.05$) between the mean IgA levels of the Chinese and Malays.

Immunoglobulin M levels in serum

Normal levels and distribution: In the Chinese, Indians and Malays, the females have significantly higher ($P < 0.01$) serum IgM levels than males, in all the age groups studied. In contrast, the mean serum IgM level of 201 Orang Asli studied, no statistical difference was observed between the values for females and males in each of the age groups listed and the total for all age groups (Table V).

The distribution profiles for serum IgM concentration in Chinese, Indians and Malays show that for the three races, the levels range from 35 to 420 mg/100 ml with the mode at 106 to 140 mg/100 ml. The serum IgM levels for Orang Asli ranges from 71 to 490 mg/100 ml with the mode at 211 to 245 mg/100 ml. In 3 percent of the Orang Asli the serum IgM level is greater than 420 mg/100 ml which is the highest level observed for the urban races (Figs. 11, 12, 13, 14).

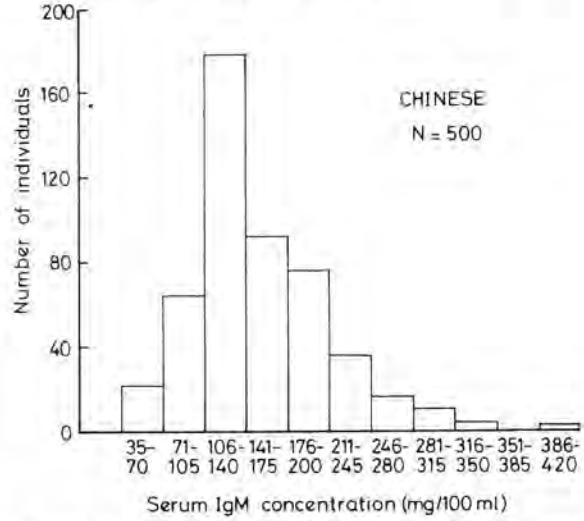

Figure 11: Frequency histogram profile of the serum IgM levels in the Chinese.

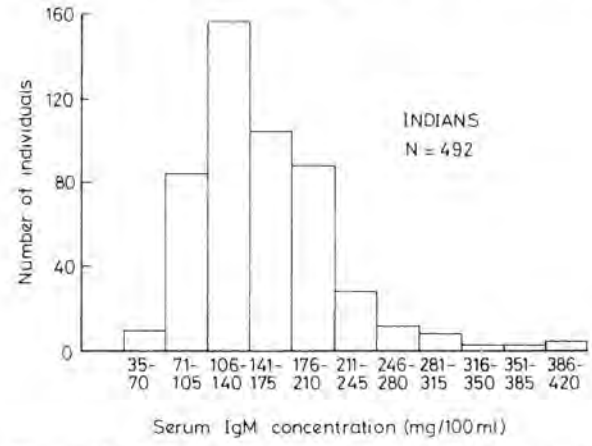

Figure 12: Frequency histogram profile of the serum IgM levels in the Indians.

Table V: Serum IgM levels in Malaysians of four racial origins

Race & Sex	Age (years)	Young adults			Adults				Total
		11–15	16–20	21–25	26–30	31–35	36–40	41–50	11–50
CHINESE	Males	165±39† (20)	147±54 (132)	150±50 (132)	131±42 (62)	130±35 (38)	120±30 (18)	146±48 (28)	143±49 (204)
	Females	218±79** (28)	212±67** (28)	182±43** (6)	178±50** (8)	–	–	–	208±71** (70)
	Males & Females	195±71 (48)	159±62 (160)	148±50 (138)	136±45 (70)	130±35 (38)	120±30 (18)	146±48 (28)	152±57 (500)
INDIAN	Males	166±34† (30)	148±46 (92)	143±45 (130)	131±41 (66)	143±37 (60)	133±41 (25)	130±30 (32)	142±43 (436)
	Females	220±72** (24)	290±111** (6)	245±83** (12)	199±48** (14)	–	–	–	224±77** (56)
	Males & Females	190±61 (54)	155±59 (98)	151±57 (142)	142±50 (80)	143±37 (60)	133±41 (26)	130±30 (32)	152±55 (492)
MALAY	Males	144±34† (14)	153±37 (48)	154±59 (132)	134±38 (84)	141±46 (40)	160±100 (14)	146±47 (10)	147±53 (342)
	Females	228±94** (16)	194±67** (8)	201±74** (16)	249±55* (12)	–	–	–	219±79** (52)
	Males & Females	189±84 (30)	159±45 (56)	159±63 (148)	148±58 (96)	141±46 (40)	160±100 (14)	146±47 (10)	157±62 (394)
ORANG ASLI	Males	212±88† (17)	229±55 (21)	256±82 (74)	235±65 (7)	259±62 (4)	218±49 (4)	205±50 (12)	240±78 (139)
	Females	250±39 (7)	249±72 (15)	264±55 (15)	265±42 (8)	297±76 (5)	284±89 (5)	234±90 (7)	260±72 (62)
	Males & Females	223±86 (24)	237±63 (36)	257±78 (89)	252±55 (15)	280±73 (9)	255±81 (9)	215±69 (19)	246±77 (201)

† Mean ± standard deviation in mg per 100 ml; multiply by 1.43 to convert to I.U./ml. Parenthesis indicates number of observations.

Significant difference between males and females of each group * $P < 0.05$, ** $P < 0.01$

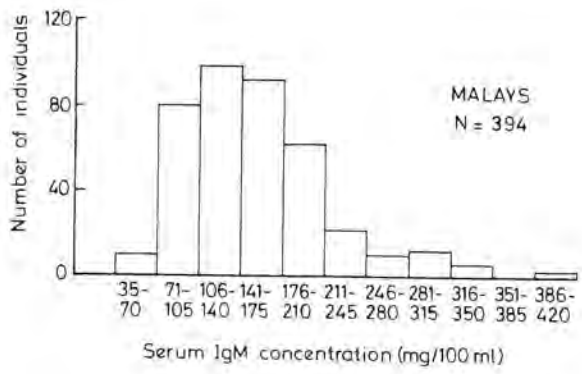

Figure 13: Frequency histogram profile of the serum IgM levels in the Malays.

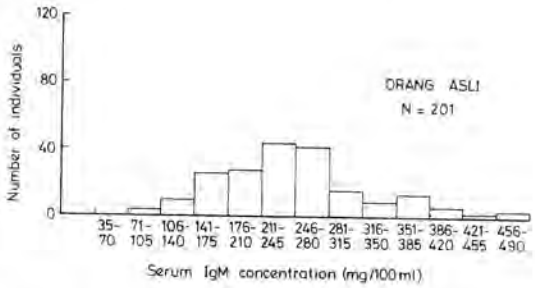

Figure 14: Frequency histogram profile of the serum IgM levels in the Orang Asli.

Age variations: The Chinese, Indians and Malays have almost similar serum levels of IgM for the various age groups studied, but the mean IgM level of the Orang Asli is significantly higher ($P < 0.01$) than those recorded for the urban races. The mean serum IgM levels drop from about 180–200 mg/100 ml in the three urban races at 11–15 years of age to about 140 mg/100 ml at 41–50 years of age (Fig. 15). The young adults of Chinese, Indian and Malay have serum IgM levels which are significantly higher ($P < 0.01$) than those of the adults. In the Orang Asli, the young and old adults have serum IgM levels of similar magnitude (Table VI).

Racial differences: Analysis of variance for the total mean IgM level of the four races shows that although there is no significant difference ($P < 0.01$) between the total mean IgM levels of the Chinese, Indians and Malays, the total mean of each of these races is significantly lower ($P < 0.01$) than that of the total mean of serum IgM levels of the Orang Asli.

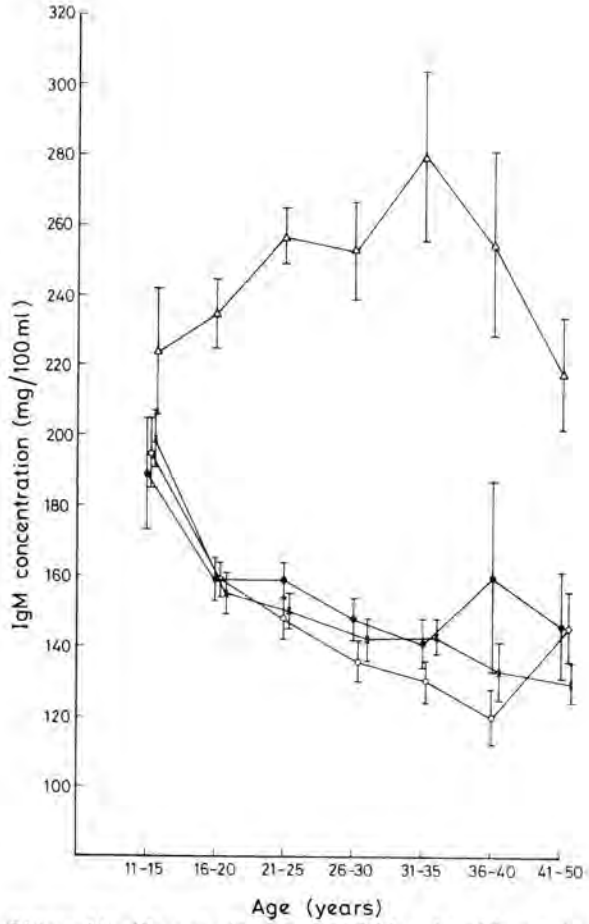

Figure 15: Changes in serum IgM levels with age in the Chinese (○), Indians (x), Malays (●) and Orang Asli (△). Bars indicate standard error of mean.

Table VI
Mean serum IgM levels in Chinese, Indians, Malays and Orang Asli

Age (years) / Race	Young adults (11–20 years)	Adults (21–50 years)	Total (11–50 years)
Chinese	167 ± 66†** (208)	147 ± 47 (292)	152 ± 57 (500)
Indian	168 ± 62** (152)	145 ± 50 (340)	152 ± 55 (492)
Malay	170 ± 63** (86)	154 ± 61 (308)	157 ± 62 (394)
Orang Asli	232 ± 74 (60)	252 ± 77 (141)	246 ± 77 (201)

† Mean ± standard deviation in mg per 100 ml. Parenthesis indicates number of observations.
** Significant difference ($P < 0.01$) between young adults and adults of each race.

DISCUSSION

In adults a correlation between age and serum IgG levels has not been demonstrated in Caucasian Americans (Steihm & Fudenberg, 1966; Lichtman, 1967; Allansmith *et al.*, 1968), and Africans (Rowe *et al.*, 1968a). This lack of correlation between age and immunoglobulin was also noted for IgA and IgM (Lichtman, 1967a; Rowe *et al.*, 1968; Kalff, 1970). Similarly, in the four Malaysian races, each of the three serum immunoglobulins were not correlated with age, for the age group 11 to 40 years. There was no difference in the serum levels of IgG in the young adults (11–20 yrs) and adults (21–50 yrs) in the Chinese, Indians and Orang Asli, but in Malays the young adults had higher levels of serum IgG than adults. In the Orang Asli, there was no difference in the serum IgM and IgA levels of young adults and adults indicating that serum immunoglobulin levels varied little between the age of 10 to 50 years. In the Chinese, the Indians and the Malays, there was a decline of serum IgM and IgA levels in adults but in the Indians the serum IgA levels did not show any variation between the ages 10 to 50 years. Adult serum IgG levels are attained in Caucasian Americans at age 11 to 20 years (Steihm & Fudenberg, 1966; Allansmith *et al.*, 1968) but similar work for Malaysians has not been reported (Yadav & Iyngkaran, in preparation). However, from the foregoing it is clear that adult serum immunoglobulin levels are attained at 11–20 years or earlier.

Females in many populations have been reported to have significantly higher serum IgM levels than males (Butterworth *et al.*, 1967; Grundbacher, 1972; Rowe *et al.*, 1968; Rhodes *et al.*, 1969; Maddison *et al.*, 1975; Allansmith *et al.*, 1969; Kalff, 1970) and this difference becomes apparent in most instances after six years of age (Butterworth *et al.*, 1967; Allansmith *et al.*, 1969). A sex difference in the level of serum IgA or IgG has not been generally observed. In Malaysian females of Chinese, Indian and Malay origin, the serum IgM levels were significantly higher than in males but in the Orang Asli a sex difference in the serum IgM levels was not present. Grundbacher (1972) has suggested that the X-chromosome of man carries genes with an effect on serum IgM concentration but the observations do not exclude the effect of hormones (Washburn *et al.*, 1965) or other factors associated with the sexes (Yadav and Shah, 1977). The lack of difference in serum IgM levels of males and females in Orang Asli and occasionally in other races (Norberg, 1967; Alarcon-Segovia and Fishbein, 1970; Buckley & Dorsey, 1971) has been attributed to high serum IgM levels which masks the difference (Fahley & McKelvey, 1965; Veys & Claessen, 1968) and these high levels may develop as a consequence of regular antigenic exposure to recurrent parasitic infections or other antigenic exposure. The Orang Asli had significantly higher serum IgM levels than the other three urban-residing races.

The urban-living Malaysians, in contrast to other populations living in the tropics, like Liberians (Capucinelli *et al.*, 1972), Nigerians (McFarlane *et al.*, 1970) Bantu and Pygmies from Congo (Simbeye, 1970) and the Africans of Senegal (Lamy, 1966), Tanzanians (Nantulya & Lindquist, 1973) and the Watut and non-Watut aborigines of Papua New Guinea (Wells, 1968), have significantly lower immunoglobulin levels, especially that of IgG and IgM. Indeed, the urban Malaysians possessed immunoglobulin levels comparable to those reported for populations of the temperate region, for example, the British (Rowe *et al.*, 1966), Americans (Fahey & McKelvey, 1965), Belgians (Veys & Claessens, 1968) and resident of Lisbon (Palma-Carlos & Palma-Carlos, 1971). Although Malaysians in general are subject to various tropical diseases, the relatively lower serum immunoglobulins in Malaysians may be attributed to the better hygienic conditions of the urban group studied.

The serum immunoglobulin levels of the Orang Asli are comparable to the levels of the Watut and non-Watut aborigines, the Liberians and Tanzanians, and higher than the levels of the Indian population (Schgal & Aikat, 1970; Samuel *et al.*, 1970; Dasgupta, 1974) and the Caucasians of the temperate regions (Fahey & McKelvey, 1965; Steihm & Fudenberg, 1966; Veys & Claessens, 1968; Palma-Carlos & Palma-Carlos, 1971). It is apparent that tropical populations, especially those living in unsanitary conditions and lacking regular medical care would be subject to high rate of parasitic infections and their serum immunoglobulin levels are likely to be elevated.

The jungle-dwelling Orang Asli, compared to the urban-living Malaysians, have significantly high levels of serum IgG, IgA and IgM. These elevated serum immunoglobulins in the Orang Asli have been attributed to prevalence of parasite infections among this community (Yadav & Shah, 1977) but direct evidence is not available. Consolidated results of surveys conducted in the Orang Asli show the presence of 22 helminth and protozoal parasites (Dunn, 1972). The main parasites in terms of potential pathogenicity and prevalence (percent in parenthesis) are *Entamoeba histolytica* (3.2%), *Giardia lamblia* (11.1%), *Ascaris* (38.1%), *Trichuris trichuria* (55.4%), hookworm (72.8%) and others (32.2%). In addition, filiarisis (Ramachandran *et al.*, 1964), Malaria (Bolton, 1972) and leprosy (Bolton, 1968)

are common in the community. In African races, elevation of serum immunoglobulin G, A and M levels is associated with malaria (Abele *et al.*, 1965; Tobie *et al.*, 1966; Targett, 1970), with filiarisis (Michaux, 1966), with amoebiasis (Abioeye *et al.*, 1972) and with tuberculosis (Malomo *et al.*, 1970; Fahey, 1965). Studies on the changes in the levels of serum immunoglobulins before, during and after infection by human and simian malaria showed a direct correlation between the rise of malaria antibody production with increased serum IgM, IgG and IgA levels (Abele *et al.*, 1965; Tobie *et al.*, 1966). At present there is a need for similar investigations in the Malaysians.

It has been clearly established in animal experiments (McDevitt & Benacerraf, 1969: Benaceraf & Katz, 1975) and there is some evidence from man that genetic factors play an important role in the development and dynamic maintenance of serum immunoglobulin levels. For instance, a higher serum IgG, IgA and IgM levels have been observed in negroes in contrast to white Caucasians living in similar socio-economic environments (Lichtman *et al.*, 1967; Karayalcin *et al.*, 1973; Maddison *et al.*, 1975).

Our studies show that Malays (Yadav *et al.*, 1977) and Orang Asli (Yadav & Shah, 1977, and this communication) living in the rural areas have high serum immunoglobulins because of the prevalence of parasites in their environment but Malays with better hygenic conditions in urban areas have low normal serum immunoglobulin levels. We have no information from Orang Asli who have moved to urban areas but hospitalised pregnant females have lower serum immunoglobulin levels than non-hospitalised females (Shah, 1975; Shah & Yadav, 1977). These observations suggest that in Malaysians, environmental factors are chiefly responsible for the high serum immunoglobulins found in rural Malaysians.

SUMMARY

The serum immunoglobulin levels of urban-residing Chinese, Malays and Indians were compared to those of the forest-dwelling Orang Asli of the age group 11 to 50 years. The serum IgG, IgA and IgM mean levels in the Chinese are 1281 ± 443, 191 ± 72, 152 ± 57 mg/100 ml; Indians are 1284 ± 316, 222 ± 78, 152 ± 55 mg/100 ml; Malays are 1331 ± 280, 188 ± 77, 157 ± 62 mg/100 ml and Orang Asli are 1809 ± 662, 394 ± 107, 246 ± 77 mg/100 ml, respectively. In contrast to the urban-residing group, all these serum immunoglobulin levels were significantly raised in the Orang Asli. The serum IgM levels in the Chinese, Indians and Malays were of equal magnitude for all age groups but the serum IgG levels were raised ($P < 0.05$) in the Malays and the serum IgA levels were raised ($P < 0.001$) in the Indians. For the age groups studied an association between the mean immunoglobulin level and increasing age was not noted. Females had higher serum IgM levels than males in Chinese, Indians and Malays but in the Orang Asli there was no sex difference in the serum IgM level. There was no difference in serum IgA and IgG levels between the sexes in all four races.

In general, the serum IgG, IgA and IgM total mean levels of urban-residing Malaysians are comparable to the levels reported for Caucasians residing in countries of the temperate regions. However, in the Orang Asli, the three serum immunoglobulins were higher than those observed for populations of the temperate regions and the serum levels of immunoglobulin are comparable to those reported for populations normally living in the tropical regions.

ACKNOWLEDGEMENTS

We wish to express our appreciation and thanks to the following: Mr. G. Rajendran, Superintendent of the Blood Bank, University Hospital and his staff; the Principals of the La Salle Secondary School and the Assunta Secondary School, Petaling Jaya for their cooperation in allowing student volunteers to donate blood and Dr. H.T. Ong and staff of the Gombak Orang Asli Hospital, Selangor, for sera from the Orang Asli. We thank Mr. K.H. Chong for the preparation of the drawings and Mrs. Peggy Azavedo for clerical assistance.

The investigation was supported by the University of Malaya Research Grants Committee. Some facilities were provided by a WHO grant to MY. A University Kebangsaan Tutorship Award was held by FHS during the tenure of the research work.

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