# NORMAL SERUM IMMUNOGLOBULIN G, A AND M LEVELS IN FULL TERM MALAYSIAN NEWBORNS*

M. YADAV & F.H. SHAH.

## INTRODUCTION

NEWBORNS, have large amounts of IgG antibody almost all of which is passively derived from the mother via the placenta; sometimes IgM and IgA is also present in low concentration in the cord sera but these immunoglobulins are synthesised by the fetus (Solomon, 1971). The other immunoglobulins, i.e. IgD and IgE, appear in the circulation after birth. The level of the immunoglobulins in the newborn varies with several factors which may include gestation age, mother's health and congenital infections (Alford *et al.*, 1969; Hardy *et al.*, 1969). The IgG levels in the fetal circulation increase slowly initially and more rapidly in the third semester or pregnancy, and at term the IgG level in the cord serum may, in some instances, exceed the levels in the mother (Dancis *et al.* 1960, 1961, Gitlin *et al.*, 1964). The immune protection provided by maternal antibody lasts in the infants for over six months but this protection is directed against organisms to which the mother is immune and there is no protection to microorganisms which chiefly evoke IgM antibody (Vahlquist, 1958).

There are few studies on the cord serum immunoglobulins of tropical populations outside the African region (Michaux *et al.*, 1966; Edozein et al., 1962; McFarlane & Udeozo, 1968). Since Malaysia is situated in the tropical zone and the population is subject to regular incidence of protozoal and helminth infections it was desirable to conduct this study in order to obtain basic information on the immunoglobulins of newborns in the four Malaysian races.

Department of Genetics and Cellular Biology,
University of Malaya, Kuala Lumpur, MALAYSIA

M. YADAV & F.H. SHAH,

* Paper presented at the XV International Congress of Pediatrics, New Delhi, October, 1977.

## MATERIALS AND METHODS

### Maternal and cord sera

Blood samples from the three Malaysian races (Malays, Chinese and Indians) were obtained from the Department of Obstetrics and Gynaecology at the University Hospital and the Orang Asli (Malaysia aborigines) samples from the Gombak Orang Asli Hospital. Maternal blood was taken by antecurbital venepuncture one to two weeks prior to delivery and cord serum was withdrawn from the placental side of the umblical cord within a few minutes of delivery. Blood samples were taken only from healthy mothers and newborns who under went an uneventful gestation and normal birth. The gestation age range from 35 to 43 weeks. The blood samples were kept at 4 C overnight after which the serum was separated by centrifugation and stored at -20 C until assayed for immunoglobulins.

### Quantitative determination of immunoglobulins

Immunoglobulin A, G and M and specific antisera to them were prepared according to previously described methods (Vaerman *et al.*, 1963; Fahey and McLaughlin, 1963). The anti-IgA and anti-IgM rabbit sera was made specific for the heavy chain by removing contaminants with pooled cord serum immunoabsorbents (Avrameas and Ternynck, 1969) low in IgM and IgA.

The antibody-agar diffusion plates were prepared and the immunoglobulins quantitated by the radial agar-immunodiffusion technique of Mancini *et al.* (1965). The anti-IgG agar plates were incubated for 3 days while the anti-IgM and anti-IgA agar plates were incubated for 5 days before measurement of the diameters of the precipitin rings. Immunoglobulin standards from World Health Organisation were used. Observations for standard immunoglobulin were plotted on semi-logarithmic scale and the concentration of immunoglobulins in test serum were determined from standard curves and expressed in mg/100 ml

and also in International Units per ml. The accuracy of the method in our hands using different batches of antisera was tested by incorporating a control serum in each plate made. The coefficient of variation for the assay of IgG, IgA and IgM were 3.5, 6 and 6.8 per cent, respectively. Thus, the day to day variation in our tests was small.

## RESULTS

### Immunoglobulin G, A and M levels

Table 1 summarises the data on the immunoglobulin levels in cord sera of full term newborns and maternal sera. Cord serum mean IgG levels of Indians, Malays and Orang Asli newborn are of similar magnitude and these levels are significantly higher ($p < 0.01$) than the cord serum IgG levels of the Chinese newborns. The Indian and Malay mothers have serum IgG of comparable magnitude but the levels in the Chinese mothers are significantly lower than those observed in the Indians ($p < 0.01$) and Malays ($p < 0.05 > 0.01$). The IgG levels in the Orang Asli mothers are similar in magnitude to those in the other three races.

The serum mean IgM levels of cord sera are of equivalent magnitude in the Chinese, Malays and Indians and these levels are significantly lower ($p < 0.01$) than those observed in the Orang Asli. Similarly, the maternal mean IgM level of the 3 races are of similar magnitude but these levels are significantly lower ($p < 0.01$) than those recorded for the Orang Asli. Cord sera IgA was detected in 34.6 percent of Chinese, 40.5 percent of Indians, 31.6 percent of Malays and 62.5 percent of Orang Asli. The mean cord serum IgA levels in infants who possessed IgA ranged from 2.9 to 3.7 mg/100 ml in the four races. The maternal IgA level was of equal magnitude in the four races.

**Table 1**

**Paired maternal and cord sera of full term infants of urban-dwelling Chinese, Indians and Malays, and forest-dwelling Orang Asli.**

| Race | Immunoglobulin levels mg/100 ml | | | | | |
|---|---|---|---|---|---|---|
| | G | | A | | M | |
| | Cord serum | Maternal serum | Cord serum | Maternal serum | Cord serum | Maternal serum |
| Malay | 1169 ± 286 (165) | 1257 ± 321 (165) F/M = 0.92 | 3.7 1.35 - 12 (25/79) | 270 + 84 (154) | 10.9 ± 5.8 (161) | 160 ± 65 (161) |
| Chinese | 1092 ± 270 (168) | 1181 ± 293 (168) F/M = 0.93 | 2.9 1.35 - 10 (27/79) | 286 ± 105 (175) | 11.6 ± 6.3 183) | 177 ± 60 (183) |
| Indian | 1211 ± 282 (210) | 1310 ± 270 (210) F/M = 092 | 3.1 1.35 - 17 (32/79) | 230 ± 73 (196) | 12.5 ± 7.3 (202) | 168 ± 61 (202) |
| Orang Asli | 1254 ± 441 (44) | 1256 ± 321 (44) F/M = 1.0 | 3.1 1.35 - 17 (30/48) | 263 ± 114 (40) | 16.7 ± 6.9 (43) | 268 ± 137 (43) |

a mean ± standard deviation in mg/100 ml; to convert to I.U./ml multiply value by 0.12 for IgG, 1.43 for IgM and 0.66 for IgA. Parenthesis indicates number of observations.

b mean and range; parenthesis indicates the ratio of samples with detectable IgA levels over number tested.

F/M Fetal-maternal ratio of IgG level in cord serum and maternal serum.

Since most of the IgG in the newborn is derived from the mother the fetal-maternal ratio are of interest. In the Orang Asli the ratio was 1 and in the other three races it was just less than 1 (Table 1).

**Correlation between immunoglobulin levels and birth weight and getation**

Table II summarises the multiple regression analysis of the cord serum IgG on the birthweight and gestation age of the newborns and the maternal serum IgG concentration. The statistical analysis indicates a significant dependence of cord serum IgG level on maternal serum IgG level in the Chinese, Indians and Malays. In addition, in Indians the cord serum IgG was significantly dependent at 5 per cent level on the gestation age.

## DISCUSSION

In the four Malaysian races, and also the Taiwanese (Yang *et al.*, 1971) and the Africans Edozien *et al.*, 1962, Michaux, 1966; McFarlane & Udeozo, 1968) the mean feto-maternal ratio was equal to one or lower than one but in contrast, the feto-maternal ratio was greater than one in normal deliveries of Caucasians from the temperate regions (McCracken & Shinefield 1965, Gusdon 1969, Kohlar & Farr 1966, Cockran and Good 1974). The biological reasons for the wide variations in the IgG level of the newborn relative to the maternal serum level is not known. There is a suggestion that in the tropics maternal IgG in the foetus may be catabolically removed at an accelerated rate (Schultze & Heremens, 1966; McFarlane, 1973) but, there is as yet no experimental evidence for this proposal. We feel other factors, such as nutritional status, rate of infections and health, may be important in interferring in the transmission of IgG from mother to foetus. Although, high IgG levels in the newborns provide immune protection for long periods, they also have a suppressive effect on the normal development of the antibody-producing capacity (Graf & Uhr, 1969, Osborn *et al.*, 1952; Perkins, 1959). Maternally derived IgG in the newborn declines at an exponential rate suggesting the absence of new synthesis to replace the catabolised IgG; the half-life of maternally derived IgG is 20 to 30 days (Orlandini *et al.* 1955).

There are two postulates which help to explain the mechanism of transmission of IgG across the placenta. In Caucasian cord serum IgG levels are significantly raised relative to maternal serum levels in normal vaginal delivery as opposed to elective Caesarian birth (Jones & Payne, 1967; Cochran, 1972; Cochran & Good, 1974). Thus, these observations led to the suggestion that con-

**Table II**

**Multiple Regression analysis of cord serum IgG concentration on the birthweight, gestation age and maternal serum IgG level of newborns of Chinese, Indian & Malay origin**

| | Chinese | | | | Indians | | | | Malays | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Y | $X_1$ | $X_2$ | $X_3$ | Y | $X_1$ | $X_2$ | $X_3$ | Y | $X_1$ | $X_2$ | $X_3$ |
| Mean | 1072.3 | 3021.9 | 39.5 | 1193.8 | 1189.6 | 3089.9 | 39.7 | 1299.6 | 1164.0 | 3135.4 | 39.6 | 1264.0 |
| Standard deviation | 272.9 | 335.4 | 3.3 | 299.0 | 288.9 | 356.5 | 1.6 | 316.7 | 289.1 | 313.0 | 1.4 | 320.0 |
| Standard error of mean | 20.1 | 25.2 | 0.25 | 22.5 | 18.5 | 16.4 | 0.1 | 20.3 | 21.65 | 23.54 | 0.11 | 50.03 |
| Correlation $X_i$ vs Y(r) | - | 0.074 | 0.097 | 0.388** | - | 0.079 | 0.176 | 0.380** | - | 0.080 | 0.108 | 0.234 |
| Regression coefficient | - | 0.068 | 9.04 | 0.37 | - | 0.075 | 26.88 | 0.372 | - | 0.079 | 15.86 | 0.1016 |
| Standard error of reg. coeff | - | 0.056 | 5.722 | 0.062 | - | 0.049 | 10.926 | 0.053 | - | 0.069 | 15.17 | 0.032 |
| T value | - | 1.218 | 1.579 | 5.882 | - | 1.535 | 2.463 | 6.96 | - | 1.152 | 1.045 | 3.170 |

Y = cord IgG level in newborns in mg%  X1 = weight of newborns in grams  T = t-test value

X2 = gestation age in weeks  X3 = IgG levels of maternal serum in mg%

tractions of labour at parturition are physiologically instrumental in causing increased maternofetal transmission of IgG which then cause an elevation of the immunoglobulin in cord over maternal levels. Brambell (1966) has proposed that maternal transmission of IgG is an active process mediated by IgG-specific Fc receptors on the trophoblast membrane of the placenta. Both these mechanisms however do not help in providing a satisfactory explanation for the variable transmission of maternal IgG to foetus particularly in the lower-than-one feto-maternal IgG ratios observed in Afroasian populations.

Since cord serum IgG is chiefly of maternal origin, a correlation between the serum IgG concentration of mother and newborn would be expected. We confirm in three Malaysian races the observations made in Americans (Allansmith *et al.*, 1968) and Europeans (Berg and Nilson, 1969) that a correlation which was independant of the birthweight and gestation, was present between maternal and fetal serum IgG levels. In full term Caucasian newborns there was no correlation between cord serum IgG level and other parameters like birthweight and gestation age (Berg and Nilsson, 1969, Thom *et al.*, 1967) and our data supports these observations. Cord serum IgA and IgM levels show no correlation to birthweight, gestation age or maternal immunoglobulin level.

In Hawaii (Wang *et al.*, 1973) Taiwan (Yang *et al.*, 1971) and America (Van Furth *et al.*, 1965) the cord serum IgM values range from 5 to 15 per cent of the adult serum levels, and in the Malaysian samples the Igm values are well within this range (6.3 to 7.4 per cent). The cord serum IgM levels in the three urban races are of equal magnitude and similar findings have been made for the Caucasians. In contrast, the relatively higher cord serum IgM and IgA levels in the Orang Asli, is suggestive of greater antigen stimulation of the fetus and this may be as a consequence of high infection rate prevalent in this community (Bolton, 1968). Furthermore, a high per cent of the newborns have IgA present in the serum; we find 34.6, 40.6, 31.6 and 62.5 per cent of the Chinese, Indian, Malays and Orang Asli, respectively, have demonstrable IgA in the cord serum. The absence of IgA in some cord sera may be due to absence of IgA synthesis by fetus because of inadequate antigen stimulation or transient IgA deficiency. Similarly, the low level of IgA level (2 to 10 mg/100 ml) in normal cord sera has been reported for several populations (de Muralt and Roulet 1962, Haworth *et al.*, 1965; Steihm & Fudenberg, 1966; Hobbs & Davies, 1967; Chandra *et al.*, 1970, Yang *et al.*, 1971, Corrodi & Hitzig, 1973, Maroulis *et al.*, 1971).

## SUMMARY

Immunoglobulin G, A and M were assayed using the Mancini's radial agar-immunodiffusion technique, in paired maternal and cord sera of full-term infants of urban-dwelling Chinese, Indians and Malays, and forest-dwelling aborigines (Orang Asli). The mean serum IgG level of newborns in Orang Asli (1254 $\pm$ 441 mg/100 ml), Indian (1211 $\pm$ 282 mg/100 ml) and Malays (1169 $\pm$ 286 mg/100 ml) were of comparable magnitude but these levels were higher than the levels in the chinese (1092 $\pm$ 270 mg/100 ml). In Malaysians, as reported for other races, there was a significant dependence of cord serum IgG level on maternal serum IgG levels. The mean feto–maternal serum IgG level ratio at term was equal to one or just less. The cord sera IgG was not correlated to the birthweight and gestation age. The cord serum IgM levels in Chinese, Indian, Malay and Orang Asli newborn at term were 11.6 $\pm$ 6.5, 12.5 $\pm$ 7.3, 10.9 $\pm$ 5.8 and 16.7 $\pm$ 6.9 mg/100 ml, respectively. Furthermore, IgA was present in 34.6%, 40.5%, 31.6% and 62.5% in Chinese, Indian, Malay and Orang Asli, respectively. Cord serum IgA and IgM levels show no correlation to birthweight, gestation age or maternal serum immunoglobulin levels. In general, cord serum immunoglobulin A, G & M levels in Malaysians are significantly elevated as compared to levels noted in Caucasians from temperate 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, for blood samples of adult blood donors; Professor T. Sinnathuray of the Department of Obstetrics & Gynaecology, University Hospital and his staff, for the supply of cord and maternal blood from Chinese, Indian and Malays; Dr. B. Brown of the United States Army Medical Research Unit for supply of the Orang Asli cord and maternal sera.

The project was supported by the University of Malaya Research Grants Committee. During the

tenure of the project FHS held a University Kebangsaan Tutorship Award. Some facilities were provided by a grant to M.Y. from the World Health Organization.

## REFERENCES


Alford, C.A., Foft, J.W. Blankenship, W.J., Cassady, G and Benton, J.W. Jr. (1969). Subclinical CNS disease of neonates. A prospective study of infants both with increased levels of IgM. *J. Pediat.* **75,** 1167 — 1178.

Allansmith, M., McClellan, B.H. Butterworth, M. and Maloney, J.R. (1968). Development of immunoglobulin levels in man. *J. Pediat.* **72,** 276 — 290.

Avrameas, S and Ternynck, T (1969). The cross-linking of proteins with glutaraldehyde and its use for the preparation of immunoadsorbents *Immunochemistry* **6,** 53 — 66.

Berg, T. and Nilson, B.A. (1969). Foetal development of serum levels of IgG and IgM. *Acta Paediat. Scand.* **58,** 577 — 583.

Bolton, J.M. (1968). Medical services to the Aborigines in West Malaysia. *Brit. Med. J.* **2,** 818 — 823.

Brambell, F.W.R. (1966). The transmission of immunity from mother to young and the catabolism of immunoglobulin. *Lancet* **ii,** 1087 — 1093.

Brambell, F.W.R. (1970). The transmission of passive immunity from mother to young. *Frontiers of Biology*, vol. 18 (Eds) Neuberger, A. and Tatum, F.L. Amsterdam.

Chandra, R.K., Guha, D.K. and Ghai, O.P. (1970). Serum immunoglobulins in the newborn. *Ind. J. Pediat.*, **37,** 361 — 365.

Cockran, T.E. (1972). Fetal and maternal immunoglobulin concentrations at delivery and post partum. *J. Obst. Gynaec. Brit. Cwlth.* **79,** 238 — 243.

Cochran, T.E. and Good, W. (1974). The distribution of immunoglobulin and albumin between maternal and cord serum at delivery. *J. Obst. Gynaec. Brit. Cwlth.* **81,** 980 — 987.

Corrodi, U. and Hitzig, W.H. (1973). Die pranatale Entwicklung der Immunoglobulin. *Monatsschr. Klinderheilkd* **121,** 1 — 5.

Dancis, J., Lind, J. and Vara, P. (1960). pp. 185 — 187. in *The Placenta and fetal membrances* (Ed) C.A. Villee, Williams and Wilkins, Baltimore.

Dancis, J., Lind, J., Orazt, M., Smolens, J. and Vara, P. (1961). Placental transfer of proteins in human gestation. *Am. J. Obstet. Gynec.* **82,** 167 — 171.

De Muralt, G., Roulet, D.L.A. (1962). Recherches immunologiques sur les proteines fetales, en particular sur une globuline specifique de foetus human. pp. 297. in Protides of Biological *Fluids.* Proc. 9th Colloquina, Briges 1961 (ed) H. Porter.

Edozien, J.C., Gilles, H.M. and Udeozo, I.O.K. (1962) Adult and cord blood gammaglobulin and immunity to malaria in Nigeria. *Lancet* **ii,** 951 — 955.

Fahey, J.L. and McLaughlin, C. (1963). Preparation of antisera specific for 6.6SY-globulins, $B_{2A}$globulins, $Y_1$-macroglobulin and for Type I and II and globulin determinants. *J. Immunol.* **91,** 484 — 497.

Gitlin, D., Kumate, J., Urrusti, J. and Morales, C. (1964). The marked selectivity of the human placenta in the transfer of proteins from mother to fetus. *J. Clin. Invest.* **43,** 1938 — 1957.

Graf, M.W. and Uhr, J.W. (1969). Regulation of antibody formation by serum antibody I Removal of specific antibody by means of immunoadsorption. *J. Expt. Med.*, **130,** 1175 — 1186.

Gusdon, J.P. (1969). Fetal and maternal immunoglobulin levels during pregnancy. *Am. J. Obstet. Gynaec.* **103,** 895 — 900.

Hardy, J.B., McCraken, G.H. Jr., Mellits, E.D., Gilkeson, M.R. and Sever, J.C. (1969). Serum immunoglobulin levels in newborn infants III some preliminary observations from a survey of cord blood levels in 2600 infants. *J. Pediat.* **75,** 1211 — 1223.

Haworth, J.C. Morris, L.N. and Dullington, A. (1965). A study of immunoglobulin in premature infants. *Arch. Dis. Childh.* **40,** 243 — 250.

Hobbs, J.R. and Davies, J.A. (1967). Serum and G-globulin levels and gestational age in premature babies. *Lacet* **i,** 757 — 759.

Jones, W.R. and Payne, R.B. (1967). Effect of mode of delivery on IgG concentration in newborn. *Amr. J. Obstet. Gynaec.* **109,** 971 — 976.

McCraken, G.H. and Shinefield, H.R. (1965). Immunoglobulin concentrations in newborn infants with congenital cytomegalic inclusion disease. *Pediatrics* **36,** 933 — 937.

McFarlane, H. (1973). Immunoglobulins in populations of subtropical and tropical countries *Adv. Clin. Chem.* **16,** 154 — 238.

McFarlane, H. and Udeozo, I.O.K. (1968). Immunochemical estimation of some proteins in Nigerian paired maternal and fetal blood. *Arch. Dis. Childh.* **43,** 42 — 46.

Michaux, J.L. (1966). Less immunoglobulins des Bantous a letat normal et pathologique. *Ann. Soc. Belge Med. Trop.* **46,** 575 — 583.

Osborn, J.J., Dancis, J. and Julie, F. (1952). Studies on the immunology of the newborn infant II Interference with active immunization by passive transplacental circulating antibody. *Paediatrics* **10,** 328 — 334.

Perkins, F.T., Yetts, R., and Gaisford, W. (1959). Response of infants to a third dose of poliomyelitis vaccine given to 10 to 12 months after primary immunization. *Brit. Med. J.* **1,** 680 — 682.

Schultze, H.E. and Heremans, J.F. (1966). Molecular biology of human proteins with special reference to plasma proteins Vol. I. Nature and metabolism of extracellular proteins p. 533. Elsevier, Amsterdam.

Steihm, E.R. and Fudenberg, H. (1966). Serum levels of immunoglobulin in health and disease. *Pediatrics* **37,** 715 — 727.

Thom, McKay, E. and Gray, D. (1967). Protein concentrations in the umblical cord plasma of premature and mature infants. *Clin. Sci.* **33,** 433 — 444.

Vahlquist, B. (1958). The transfer of antibodies from mother to offspring. *Adv. Pediat.* **10,** 305 — 338.

Vaerman, J.P., Heremans, J.F. and Vaerman, C. (1963). Studies of the immunoglobulins of human serum I Method for simultaneous isolation of the three immunoglobulins from individual serum samples. *J. Immunol.*, **91,** 7 — 10.

Van Furth, R., Schurit, H.R. and Hijman, W. (1965). The immunogical development of the human fetus. *J. Exp. Med.* **122,** 1173 — 1191.

Wang, N., Spraque, C., Yokohama, M., and Park, H.S. (1973). IgM levels of newborn in Hawaii. *Experimentia* **29,** 871 — 872.

Yang, S.L., Kleinman, A.M., Rosenberg, E.B. and Wei, P.Y. (1971). The effect of labor and mode of delivery on immunoglobulin concentration in the neonate. *Amer. J. Obstet. Gynec.* **109,** 78 — 81.