Immunoglobulins in Umbilical Cord Plasma

I: Healthy Infants

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Ig G is selectively transferred across the placenta from mother to foetus (Gitlin, Kumate, Urrusti, and Morales, 1963, 1964a, b), and both by electrophoretic study of \( \gamma \)-globulins (Sternberg, Dagenais-Perusse, and Dreyfuss, 1956) and by specific estimation of Ig G by immunological methods (Kohler and Farr, 1966), it appears that this protein is normally in higher concentration in foetal than maternal serum at term. Only very small amounts of other plasma proteins are transferred from mother to foetus, and of the large molecules, such as fibrinogen and the macroglobulins, little or none is transferred (Gitlin, 1964). Ig A was at first considered to be completely absent from umbilical cord serum (Hitzig, 1957; Scheidegger and Martin du Pan, 1957; Roth, 1962; West, Hong, and Holland, 1962), but small amounts have been demonstrated in many sera (de Muralt and Roulet, 1962; Haworth, Norris, and Dilling, 1965; Stiehm and Fudenberg, 1966). Early tests for Ig M likewise failed to show it in umbilical cord serum (Hitzig, 1957; Scheidegger and Martin du Pan, 1957), though it is now known to be present in concentrations of from 5 to 25% of normal adult values (Franklin and Kunkel, 1958; West et al., 1962; Roth, 1962; Stiehm and Fudenberg, 1966).

Although the above series have differentiated between mature and premature infants by the dividing standard of 2500 g, birthweight, no account has been taken of birthweight differences within the groups, except that Roth (1962) divided the premature infants into two subgroups, and in later studies of Ig A, Haworth and Dilling (1966) divided them into three subgroups. The present study was carried out in order to investigate the relation between the concentrations of individual immunoglobulins in umbilical cord blood and birthweight of the infant, and the interrelationships between the concentrations of the separate immunoglobulins.

Methods

Collection of specimens. Umbilical venous blood was collected via a wide-bore needle into heparinized tubes during the third stage of labour after the umbilical cord had been clamped. The blood was stored at 4° C. for up to 24 hours, when the plasma was separated and kept at approximately — 20° C. until tested. Specimens were obtained from 23 normal full-term infants and 19 apparently healthy premature infants of birthweight less than 2500 g. All infants with congenital abnormalities or haemolytic disease of the newborn were excluded from the series, as were stillbirths and premature infants who died within 24 hours of birth or who later developed respiratory distress syndrome. The results in those groups will be reported later. Also excluded were infants born of mothers who had threatened abortion in the early weeks of pregnancy, but those from pregnancies characterized by toxæmia were included in the series.

Quantitation of immunoglobulins Ig G, Ig A, and Ig M, and albumin. Individual proteins were estimated by single radial diffusion in agar using a modification of the method described by Fahey and McKelvey (1965). The agar was prepared as described by these authors with antiserum added to give final concentrations of 1 in 40 for albumin, 1 in 40 for Ig G, 1 in 100 for Ig M, and 1 in 100 for Ig A. Antiserum from Hyland Laboratories (Los Angeles, California) was used for Ig M and Ig A estimations, and rabbit antiserum prepared in this laboratory for albumin and Ig G. The agar was poured on a perspex plate to give a film measuring 95 × 95 × 1·8 mm. This accommodated 36 wells which were of 2·3 mm. diameter for the Ig M estimations and 1·7 mm. diameter for the other proteins.

All diffusion plates contained 5 duplicate sets of standard solutions made as dilutions in saline of a reference plasma which was stored at — 20° C. and consisted of pooled plasma from 12 healthy adults. Standard dilutions of from 30% to 7·5% reference plasma were used for Ig G estimations, 20% to 5% for Ig M, 20% to 2·5% for Ig A, and 25% to 7·5% for albumin. The unknown plasma specimens were used neat for estimations of Ig A and Ig M, but diluted 1 in 5 in saline for Ig G and albumin and, if necessary, repeat estimations were made with further dilution of the specimens.

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Thom, McKay, and Gray

Results

Ig A. The concentration of Ig A in all specimens of umbilical venous plasma from healthy infants was below the critical testing level of 2.6 mg./100 ml. except in one full-term infant where the concentration was 23.3 mg./100 ml.

Ig M. The infant noted above to have a high Ig A concentration also had an Ig M concentration clearly above that in all other infants in the group (Fig. 1). These differences were so definite that it was decided to remove the case from the series of healthy infants. His results are shown on the graphs for individual protein concentrations but are not used for statistical analysis including mean values.

Although the concentration of Ig M in umbilical cord plasma rises from a mean of 9.1 mg./100 ml. in the premature infants to 10.4 mg./100 ml. in the full-term infants, there is not a statistically significant correlation between Ig M concentration and birthweight (r = 0.181; p > 0.1). The over-all mean is 9.8 mg./100 ml. for the 22 full-term and 19 premature infants in the series, with a fairly wide scatter (standard deviation 3.6 mg./100 ml.). Individual results are recorded in Fig. 1.

Ig G. The mean concentration of Ig G in the remaining 22 healthy full-term infants was 1759 mg./100 ml., and that in the 19 healthy premature infants, 1317 mg./100 ml. Fairly close correlation exists between Ig G concentration and birthweight (r = 0.518, p < 0.001), Ig G increasing with rising birthweight as expressed in the regression equation

\[ Y = 0.421X + 484, \]

where \( X \) = birthweight in grams and \( Y \) = Ig G concentration in mg./100 ml. Taking the birthweight of the average full-term infant to be 3250 g., the mean Ig G concentration at term calculated from the regression equation is 1852 mg./100 ml. or 111% of the adult reference plasma. The scatter of individual results in the series may be seen in Fig. 2.

Albumin. Umbilical cord albumin concentration is closely correlated to birthweight (r = 0.627; p < 0.001), and the concentration shows a steady rise with increasing birthweight (Fig. 3) as expressed in the regression equation

\[ Y = 0.0146X + 35.6, \]

where \( X \) = birthweight in grams, and \( Y \) = albumin concentration expressed as a percentage of the concentration present in the reference plasma used. This gives a calculated concentration of 83.0% of the reference plasma albumin concentration per 100 ml. plasma for an infant of 3250 g. birthweight. The actual mean concentration for the 22 infants of
Immunoglobulins in Umbilical Cord Plasma

Fig. 2.—Regression of umbilical venous plasma Ig G concentration on birthweight, with 95% confidence limits for Ig G concentration. Case not included in statistical analysis (see text).

Birthweight over 2500 grams was 82.4% of the reference plasma albumin concentration, and for the 19 infants under 2500 g. was 63.1% of the reference plasma albumin concentration.

Relationships between individual immunoglobulin concentrations. Fig. 4 illustrates the interrelationships between the concentrations of the individual plasma proteins studied. The concentrations of albumin and Ig G are closely correlated, and both correlate with birthweight, but Ig M concentration is not significantly related to birthweight. There is, however, a significant relation between the concentration of both Ig G and albumin and that of Ig M. Fig. 5 and 6 shows the individual relations and the regression lines and 95% confidence limits relating Ig M and Ig G and albumin. The graphs provide a useful means of showing discrepancies between the concentrations of the proteins in pathological states.

Fig. 3.—Regression of umbilical venous plasma albumin concentration on birthweight, with 95% confidence limits for albumin concentration. Case not included in statistical analysis (see text).

Fig. 4.—Relationships between the concentrations of Ig M, Ig G, and albumin in umbilical venous plasma, and birthweight of the infant.

Fig. 5.—Regression of Ig M concentration on Ig G concentration in umbilical venous plasma, with 95% confidence limits for Ig M concentration. Case not included in statistical analysis (see text).
**Discussion**

As in several previous studies which have been referred to in the introductory paragraphs, Ig A was not detectable by the method used in most specimens of umbilical cord plasma in the present series. Only one infant gave a positive test, with a concentration of 23.3 mg. Ig A/100 ml. plasma. Detailed comparison of the case notes of this mother and infant with those of the others in the 'normal' group showed no abnormality to account for the result.

Estimation of other plasma proteins on the specimen showed: albumin: 75.0% of the normal reference plasma concentration; Ig G: 2100 mg./100 ml.; Ig M: 26.0 mg./100 ml. Because the Ig A and Ig M results were clearly above all others in the group, the case was excluded from the statistical analysis. However, it was of interest to find that a specimen of the mother's plasma taken at the time of delivery showed an Ig A concentration of 416 mg./100 ml., which was 204% of the concentration in the pooled reference plasma. It was decided to estimate the concentration of Ig A in a number of mothers at term to discover whether the result in this mother was outside the normal range. In 55 mothers studied (including the case under discussion) the mean concentration of Ig A was 206 mg./100 ml., with a range from 59 to 530 mg./100 ml. and a standard deviation of 86 mg./100 ml. Fig. 7, however, shows that all but two of the results lay between 59 and 347 mg./100 ml. The mother under discussion had the second highest result in the series. The infant of the mother with the highest value of 530 mg./100 ml. had a normal Ig A concentration of below 2.6 mg./100 ml., and it is generally agreed that Ig A does not normally cross the placenta from mother to foetus in appreciable amounts. The reason for the high Ig A concentration in this one child and his mother is not clear.

In comparable studies of serum Ig A concentration in mothers at time of delivery, Haworth and Dilling (1966) found mean concentrations of from 215.3 to 289.8 mg./100 ml. in five groups based on birthweight of the infant and growth retardation. There was no significant difference between the results in the five groups.

The minimal sensitivity of the method used for the estimation of Ig A in umbilical cord plasma specimens was a concentration of 2.6 mg./100 ml., which is just above the mean of 1.0 mg./100 ml. found by Stiehm and Fudenberg (1966) in their series of normal infants, and it seems likely that the techniques used in the present series would readily detect pathological increase in Ig A concentration such as has been recorded in the rubella syndrome (Alford, 1965; Bellanti, Artenstein, Olson, Buescher, Luhrs, and Milstead, 1965; Soothill, Hayes, and Dudgeon, 1966) and in infection by cytomegalic inclusion virus (McCacken and Shinefield, 1965).

The concentration of Ig M in umbilical cord blood does not rise significantly over the weight range studied—from 1450 to 3760 g.—and it is, therefore, permissible to use the mean of the results in a series of normals as a standard for comparison with results in pathological states. In similar comparisons of Ig G, mean values cannot be used unless the groups are carefully controlled for weight, since Ig G concentration rises steeply with increasing birthweight, an increase of 500 g. (1·1 lb.) in birthweight being associated with a rise in Ig G of.
Immunoglobulins in Umbilical Cord Plasma

211 mg./100 ml. Albumin shows a similar steep rise in concentration with increasing birthweight.

The fluid balance of the infant is labile and readily upset both after and before birth as, for example, in the infants of mothers given large volumes of hypotonic solution (Altstatt, 1965). It is, therefore, readily possible for true increase in total circulating immunoglobulins in the infant to be masked by haemodilution or simulated by haemoconcentration. A high concentration of immunoglobulins in the infant's plasma may represent a true increase or haemoconcentration, and normal or low concentrations may be found when increased total circulating immunoglobulins and haemodilution occur together. It is, therefore, necessary to have some criterion of haemocoencentration and haemodilution. Haematocrit readings may be used for this purpose, but in this and later studies albumin concentration has been utilized since it is convenient to measure by the technique used for immunoglobulins, and the test can be postponed until a convenient time after collection of the specimen. By studying together the concentration of the four plasma proteins estimated, it may be possible to differentiate between alterations in fluid balance and changes specifically affecting the immunoglobulins of foetal or maternal origin.

Summary

Ig G, Ig A, Ig M, and albumin concentrations were estimated on umbilical venous plasma from 42 healthy infants, 19 of whom were under 2500 g. birthweight. Ig G and albumin concentrations were positively related to birthweight, whereas Ig M showed an insignificant rise over the weight range studied. A significant positive relation existed between Ig M concentration and both Ig G and albumin concentrations. Ig A concentration was below 2.6 mg./100 ml. in all but one infant, in whom the concomitant presence of high Ig M levels suggested that he should not be considered to be normal.

The concentrations of Ig G, Ig M, and albumin in infants weighing less than 2500 g. at birth were 1317 mg./100 ml., 9.1 mg./100 ml., and 63.1% of the concentration in pooled adult reference plasma respectively, with corresponding values for infants weighing over 2500 g. at birth of 1759 mg./100 ml., 10.4 mg./100 ml., and 82.4% adult reference plasma concentration.

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REFERENCES


