Immunoglobulins in Umbilical Cord Plasma

II: Congenital Deformities, Other Abnormalities, and Multiple Pregnancies

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The association between maternal rubella infection in the first trimester of pregnancy and congenital malformations of the infant is well recognized. In the case of other virus infections of early pregnancy, the relation is less certain, and though suspicion has been attached to certain viruses, particularly mumps (Ylinen and Järvinen, 1953) and influenza (Coffey and Jessop, 1959, 1963), some series of influenza cases have shown no such relation (Walker and McKee, 1959; Wilson, Heins, Imagawa, and Adams, 1959). Other infections of the foetus may also cause structural abnormalities of the foetus, e.g. toxoplasmosis infection may be associated with hydrocephalus and microphthalmia.

Maternal variola during pregnancy can cause abortion, stillbirth, or neonatal death (Plotz, 1965), and even the infants of healthy immune mothers may be infected *in utero* and may die from congenital variola (Schick, 1949; Rao, Ratnakannan, Balasubramaniam, and Gopalan, 1954). A similar situation exists with rubella (Schick, 1949).

A high incidence of premature births has been recorded with maternal viral hepatitis (Zondek and Bromberg, 1947), though only a small percentage of infants born of mothers with this infection show overt disease (Plotz, 1965). In the case of Cox-sackie virus, clinical manifestations in the mother may be slight, yet the infant may be fatally affected (Benirschke and Pendleton, 1958; Sussman, Strauss, and Hodes, 1959).

These facts suggest that maternal and perhaps foetal infection may be an unrecognized cause of a number of congenital malformations, abortions, stillbirths, premature births, and neonatal deaths.

The foetus infected by syphilis or toxoplasmosis will produce antibodies *in utero* (Silverstein, 1962; Eichenwald and Shinefield, 1963). Similarly, in cases of antenatal infection by rubella virus and cytomegalic inclusion virus, there is evidence that the foetus is producing antibodies, and raised

concentrations of immunoglobulins Ig A and Ig M can be demonstrated in the umbilical cord blood in a number of such cases (Alford, 1965; Bellanti, Artenstein, Olson, Buescher, Luhrs, and Milstead, 1965; McCracken and Shinefield, 1965; Soothill, Hayes, and Dudgeon, 1966).

If other infections persist in the foetus as do rubella, toxoplasmosis, syphilis, and cytomegalic inclusion disease, it may be possible to recognize these infants on the basis of abnormal concentrations of immunoglobulins in their umbilical cord blood, and for this reason the results of estimations in a series of congenitally deformed and other abnormal infants are reported.

Material and Methods

Quantitation of proteins. The methods used for the quantitation of individual proteins have been described previously, and the results in the present series are compared with those found in previously healthy infants (Thom, McKay, and Gray, 1967). The estimations were carried out using as standard a pool of mixed plasma from 12 healthy adults. Tested with Hyland immunoplates (Hyland Laboratories, Los Angeles, California), this plasma pool contained 1670 mg. Ig G/100 ml., 204 mg. Ig A/100 ml., and 101 mg. Ig M/100 ml. The concentrations of the immunoglobulins in cord plasma specimens were calculated initially as percentages of the concentrations in the reference plasma. These values were then converted to absolute units on the basis of the Hyland immunoplate readings for the reference plasma.

Selection of Cases and Collection of Blood Specimens

A. Congenital malformations. A group of 37 infants was studied on the basis of congenital malformations being recognized at or within a few hours of birth. Although it seemed likely that infants with the more gross lesions would be more likely to show abnormalities of their immunoglobulins, the series included both major and minor structural abnormalities as shown in Table I. For comparison with the previous study of immunoglobulins in the umbilical venous plasma of normal

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TABLE I
Immunoglobulin and Albumin Concentrations in 37 Infants with Congenital Malformations

Case No.	Diagnosis	Birthweight (g.)	Time of Blood Collection if Not Cord Blood	Plasma Protein Concentration			
				mg./100 ml.			% Reference Plasma
				Ig G	Ig A*	Ig M	Albumin
B1	Anencephaly	1732		880		8.3	51.0
B2	Hydrocephalus	2457		860	13.1	6.9	72.0
B3	Hydrocephalus	3720		1790		5.7	81.0
B4	Meningomyelocele and hydrocephalus	1896		710		6.7	51.5
B5		1945		1240		13.0	67.5
B6		3493		1170		8.5	61.0
B7	Meningomyelocele	3380	6 hr.	1960		11.3	70.5
B8	Hare-lip	4004		1230		10.8	73.0
B9	Cleft palate	2840	5 dy.	1440		13.1	62.0
B10	Oesophageal atresia; porencephaly	3181		1750		8.5	67.5
B11	Oesophageal atresia	2655	2 dy.	1640		6.6	70.5
B12	Diaphragmatic hernia	2499	2 hr.	1800		12.9	67.5
B13	Exomphalos	3053		2420		13.5	97.0
B14	Imperforate anus	2570	1 dy.	1250		13.1	53.5
B15	Transposition of great vessels	2684		1340		13.1	72.0
B16	Ventricular septal defect	2797		1480		4.4	59.0
B17	Congenital heart disease—? type	1747		2760		16.4	103.5
B18	Renal agenesis	1711		1550		13.5	61.0
B19	Hypospadias	2130	12 hr.	1290		9.4	66.0
B20		3515	1 dy.	1920		8.1	75.0
B21		2052		1410		9.3	59.0
B22	Mongolism	2670	6 dy.	1370		6.9	59.0
B23		3479	1 dy.	1040		5.8	61.5
B24		2158	1 dy.	1310		7.6	63.0
B25	Ring chromosome 21/22	2315	8 dy.	850		11.4	65.0
B26	Polydactyly; multiple abnormalities; ? trisomy 17/18	1825		1430		6.1	67.5
B27	Micromelia	454		350		8.7	43.5
B28	Achondroplasia	3266		800		3.3	57.0
B29	Syndactyly	2726	5 dy.	1210		15.4	62.5
B30	Dislocated knees and hips	2741		1420		10.8	59.0
B31	Talipes	3202		1250		15.0	47.0
B32	Talipes; micrognathus	3380		1000		4.5	41.0
B33	Talipes; cranio-tabes	3479	4 dy.	1540		15.6	62.5
B34	Cranio-tabes	3429		1210		17.5	74.0
B35	Sacroccygeal teratoma	3124	12 hr.	1210		7.6	69.0
B36	Large pigmented naevus	2315		1800		9.4	66.5
B37	Large capillary naevus	3607		1520		6.3	51.5

* Where no Ig A concentration is recorded the result is less than 2.6 mg./100 ml.

infants, it was clearly desirable to have umbilical cord specimens from the infants with deformities, and, when possible, blood was collected during the third stage of labour via a wide-bore needle in the umbilical vein. It became evident early in the study that a number of interesting cases would be missed if the survey was restricted to those infants seen and diagnosed as abnormal at birth. Some infants with deformities were referred from peripheral hospitals and were not available for study until they were several hours or, occasionally, days old, while even with infants born in the central maternity hospital, certain abnormalities such as oesophageal atresia were not recognizable at birth. It was, therefore, decided to collect blood from a peripheral vein from those cases missed at birth. Table I records the age at which blood was collected, and in all figures the umbilical venous and later peripheral venous blood specimens are differentiated. The implications of the decision are discussed later.

All blood specimens were stored initially at 4° C. for up to 24 hours when the plasma was separated and then stored at approximately -20° C.

B. Miscellaneous abnormalities. Fifteen infants were studied on the basis of abnormalities of the pregnancy or of the infant at birth, excluding congenital malformations. The cases studied are listed in brief in Table II, and for those showing abnormalities of the plasma proteins, a clinical history is given in greater detail below. Umbilical venous blood was obtained in 13 of the cases, while in the remaining 2, peripheral blood was taken at the age of 12 hours (Table II).

C. Multiple pregnancies. Specimens of plasma from 8 pairs of twins, one single twin, and one set of triplets were studied. In all but one of the infants, blood was obtained from the umbilical vein. In this one case the first twin (Case D6a) was normal and the second (Case D6b) was stillborn, macerated, and hydrocephalic. From it blood was obtained by cardiac puncture immediately after birth. The remaining twins and triplets were apparently healthy apart from haemolytic disease of the newborn in Cases D8 (a and b) and D9 (a and b) and extreme prematurity with early neonatal death in Cases D1 (a and b).

TABLE II

Immunoglobulin and Albumin Concentrations in 15 Infants with Various Abnormalities of the Perinatal Period or Associated Pregnancy

Case No.	Diagnosis	Birth-weight (g.)	Ig G (mg./100 ml.)	Ig A† (mg./100 ml.)	Ig M (mg./100 ml.)	Albumin (% Reference Plasma)	Interpretation
C1	Threatened abortion	2364	680		21.8	46.5	High Ig M; ? increased production
C2	Threatened abortion	1441	450		18.2	48.5	High Ig M; ? increased production
C3	Threatened abortion; cataracts; early NND*	930	780	84.7	72.7	50.0	High Ig M and Ig A; ? increased production
C4	Acute maternal hydramnios; stillbirth	2215	1170		10.9	68.0	Normal
C5	Stillbirth	795	620		9.9	48.5	Probably normal
C6	Stillbirth	1108	530		5.4	53.5	Probably normal
C7	Stillbirth; intrauterine sepsis	2655	1530		23.0	79.5	High Ig M; ? increased production
C8	Early NND*	1101	820		7.4	61.0	Probably normal
C9	Early NND*	895	410		2.5	39.5	Probably normal
C10	Frequent apnoeic attacks; NND 13 days	1051	510		3.1	53.5	Probably normal
C11	Maternal viral pneumonia and myocarditis	1945	970		7.2	63.5	Normal
C12	Maternal hydramnios; odd facies; early NND*	2350	620		10.5	41.5	Low albumin; slightly low Ig G; ? normal
C13	Fifth early NND in succession*	1590	680		6.1	51.5	Normal
C14	Growth retardation; hepatosplenomegaly; purpura	2158	1490		31.1	74.0	High Ig M; ? increased production
C15	Meconium peritonitis	3536	970		32.6	58.0	{ High Ig M; ? increased production Low Ig G and albumin ? haemodilution ? decreased production

* Early NND = neonatal death before 12 hours of age.

† Where no Ig A concentration is recorded, the result is less than 2.6 mg./100 ml.

Note: The estimations were carried out on umbilical venous plasma except in cases C14 and C15 when venous plasma collected approximately 12 hours after birth was used.

FIG. 1.—Ig G concentration in 37 infants with congenital malformations. Regression of Ig G concentration on birthweight with 95% confidence limits for healthy infants is illustrated as a continuous line, and regression line for congenital malformations as a broken line. ● Umbilical venous plasma. ○ Postnatal venous plasma.

Results

A. Congenital Deformities

Ig A. In all but one of the infants in this group, the concentration of Ig A in umbilical cord serum fell below the critical testing level of 2.6 mg./100 ml. The one exception was infant B2 with congenital hydrocephalus whose cord plasma concentration was 13.1 mg./100 ml.

Ig G. The concentrations of Ig G found in the infants with congenital abnormalities mainly fell within the 95% confidence limits for healthy infants, though particularly in the higher weight groups the majority of the results are below the normal mean (see regression line in Fig. 1). Since the initial hypotheses of the investigation did not include the possibility of the concentrations of the plasma proteins studied being below the mean values for healthy infants, a statistical analysis was not carried out to confirm the apparent lowering of concentrations in the larger infants, but the results certainly suggest that further data should be collected to confirm the present observations.

There was no clear difference in the scatter of

results from cord blood and postnatal specimens, and since haemoconcentration most commonly occurs in the first few hours after birth (Gairdner, Marks, Roscoe, and Brettell, 1958), it is likely that inclusion of these specimens in the series would have raised rather than lowered the protein concentration in plasma. The one high result was from an infant whose plasma albumin and Ig M concentrations were at the upper limit of normal, and it was thought that the specimen might have been haemoconcentrated.

The close correlation between birthweight and Ig G found in healthy infants (Thom *et al.*, 1967) was not evident ($r = 0.217$; $p > 0.1$); again this did not appear to be due to use of postnatal blood specimens, since the correlation for the 23 umbilical cord specimens remained below the level of statistical significance ($r = 0.178$; $p > 0.1$).

Ig M. The Ig M concentrations in all but one of the congenital deformity infants fell within the 95% confidence limits for healthy infants (Fig. 2). The mean concentration in all 37 plasma specimens was 9.9 mg./100 ml. and in the 23 umbilical cord specimens it was 9.6 mg./100 ml. There was no statistically significant difference between the concentrations in umbilical cord and postnatal plasma specimens ($t = 0.606$; $0.6 > p > 0.5$), and no significant difference between these results and the mean concentration of 9.8 mg./100 ml. found in healthy infants.

Albumin. Although most of the concentrations of albumin found in the deformed infants fell within the 95% confidence limits for healthy infants, only 7 of the 37 were above the normal mean, and especially in the heavier infants the values were low

FIG. 2.—Ig M concentration in 37 infants with congenital malformations. Mean concentration and 95% confidence limits for healthy infants are illustrated. See Fig. 1 for key.

FIG. 3.—Albumin concentration in 37 infants with congenital malformations. Regression of albumin concentration on birthweight with 95% confidence limits for healthy infants is illustrated as a continuous line, and regression line for congenital malformations as a broken line. See Fig. 1 for key.

(see regression line in Fig. 3). As was found with Ig G, the normal correlation between albumin concentration and birthweight was lacking ($r = 0.141$; $p > 0.1$), again apparently not as a result of inclusion of postnatal specimens (correlation coefficient for albumin and birthweight for umbilical cord specimens only, $r = 0.126$; $p > 0.1$).

Relationships between concentrations of Ig M, Ig G, and albumin.

FIG. 4.—Relation of Ig M concentration to Ig G concentration in 37 infants with congenital malformations. Regression of Ig M concentration on Ig G concentration with 95% confidence limits for healthy children is illustrated as a continuous line, and regression line for congenital malformations as a broken line. See Fig. 1 for key.

FIG. 5.—Relation of Ig M concentration to albumin concentration in 37 infants with congenital malformations. Regression of Ig M concentration on albumin concentration with 95% confidence limits for healthy infants is illustrated as a continuous line, and regression line for congenital malformations as a broken line. See Fig. 1 for key.

shows no evidence of discrepancy between the concentrations of Ig M and those of Ig G or albumin. The regression lines of Ig M concentration on Ig G concentration and Ig M concentration on albumin concentration are closely similar to the corresponding lines for healthy infants.

B. Fifteen miscellaneous abnormalities.

The results of plasma protein estimations in 15 specimens of umbilical venous blood from infants studied on the basis of various abnormalities of

FIG. 6.—Ig G concentration in 15 infants with various abnormalities of the perinatal period or of the associated pregnancy. Case numbers refer to Table II. Regression of Ig G concentration on birthweight with 95% confidence limits for healthy infants is illustrated. See Fig. 1 for key.

pregnancy or neonatal period are given in Table II. The Table includes an interpretation of the results in terms of normality or abnormality, and what was considered to be the most likely immediate cause of any deviation from normal, such as by fluid shift or by under- or over-production, or increased or diminished katabolism of a protein, or abnormality of placental transfer. The information available does not permit precise diagnosis in these terms, and the interpretations made are based on probabilities. It is more likely, for example, that high concentrations of the individual proteins, albumin, Ig G, and Ig M together with normal ratios of Ig M to Ig G and albumin would indicate haemoconcentration rather than excessive production or diminished katabolism of all the proteins.

As in the series of congenital abnormalities, the range of birthweights in the miscellaneous group of abnormalities extended well below the minimum studied in healthy infants, and precise comparison of results was possible in only 9 of the 15 cases.

Fig. 6 and 7 show that in the majority of cases the Ig G and albumin concentrations were within the 95% confidence limits for healthy infants, though, as in the case of the congenital deformity group, the concentrations, particularly of Ig G, tended to be low. In the case of Ig G, only 1 of the 9 results was above the normal mean, this being in an infant (Case C14) who showed abnormalities of other plasma proteins, as discussed below.

Six infants can be accepted as having high Ig M concentrations (Fig. 8). One of these fell below the

FIG. 7.—Albumin concentration in 15 infants with various abnormalities of the perinatal period or of the associated pregnancy. Case numbers refer to Table II. Regression of albumin concentration on birthweight with 95% confidence limits for healthy infants is illustrated. See Fig. 1 for key.

FIG. 8.—Ig M concentration in 15 infants with various abnormalities of the perinatal period or of the associated pregnancy. Case numbers refer to Table II. Mean concentration and 95% confidence limits for healthy infants are illustrated. See Fig. 1 for key.

weight range of normals studied, but the result was clearly pathological. In all 6 of these infants, simultaneous consideration of their concentrations of Ig M, albumin, and Ig G (Fig. 9 and 10) suggested that the raised Ig M levels were primary disturbances of this protein and not due to haemoconcentration.

The infant with the highest Ig M concentration in the series (Case C3) also had the highest Ig A concentration with a value of 84.7 mg./100 ml. This was the only infant in the 'miscellaneous' group giving a positive test for Ig A.

The case histories of the infants considered to show probable immunoglobulin abnormalities are considered below.

Case No. C15. Birthweight 3536 g.; gestation period 40 weeks (mother sure of dates). Spontaneous vertex delivery. There was no abnormality of pregnancy or maternal health, and the infant was well at birth but was noted at the age of 1 hour to have a distended abdomen. This distension steadily increased, and laparotomy was

FIG. 9.—Relation of Ig M concentration to albumin concentration in 15 infants with various abnormalities of the perinatal period or of the associated pregnancy. Case numbers refer to Table II. Regression of Ig M concentration on albumin concentration and 95% confidence limits for healthy infants are illustrated. See Fig. 1 for key.

carried out at the age of 16 hours when a large perforation of the splenic flexure of the colon was found, with an extensive meconium peritonitis considered by the surgeon to have been present for several days before birth. The distal bowel appeared normal. The perforation was closed surgically, a temporary colostomy was performed, and the infant made an uninterrupted recovery.

The reason for the perforation of the colon is not known. The high Ig M concentration in the infant could be a response to some infection which led to the perforation but, if so, it is not clear what type of infection would cause such a lesion. Alternatively, the Ig M could be a response to the presence of meconium in the peritoneal cavity. Double diffusion in agar tests between the infant's serum and extracts of meconium from other infants have shown no precipitin lines, but it is felt that the infant could have responded to a protein of maternal origin in the meconium, though this would have had to originate in the liquor amnii component, and Derrington and Soothill (1961) failed to detect other than normal serum proteins in the supernatant from centrifuged amniotic fluid.

FIG. 10.—Relation of Ig M concentration to Ig G concentration in 15 infants with various abnormalities of the perinatal period or of the associated pregnancy. Case numbers refer to Table II. Regression of Ig M concentration on Ig G concentration and 95% confidence limits for healthy infants are illustrated. See Fig. 1 for key.

Case No. C14. Birthweight 2158 g.; gestation period, 40 weeks (mother sure of dates). Spontaneous vertex delivery.

This infant was considerably underweight for the period of gestation, and was a long, thin, wasted baby. He had moderate enlargement of liver and spleen, and developed a transient purpuric rash. Platelet count was 89,000/c.mm. on the second day of life. The blood count was otherwise normal, and chest x-ray film was negative.

It is felt that this infant had been infected *in utero*, probably by a virus, to produce a clinical picture with some of the features of the rubella syndrome. Virological studies are in progress.

Case No. C3. Birthweight 930 g.; gestation period, 31 weeks (mother sure of dates).

The mother of this infant was admitted to hospital with a threatened abortion at 22 weeks' gestation. She continued to have intermittent brown vaginal discharge until the 31st week when further fresh bleeding occurred and she delivered a 930 g. infant with bilateral cataracts who died a few hours later. Necropsy showed only the features of prematurity and atelectasis.

It is postulated that this infant probably had a virus infection, but unfortunately the results of the immunoglobulin estimations were not available in time to carry out virus studies. (On the basis of the congenital cataracts, this infant could be classified with the congenital deformities, but has been included in the miscellaneous group on the basis of unexplained very premature birth and early neonatal death.)

Case No. C7. Birthweight 2655 g.; gestation period, 39 weeks by x-ray film (mother not sure of dates). The mother was well throughout the pregnancy.

Labour was induced by artificial rupture of the membranes and syntocinon infusion 26 hours before spontaneous vertex delivery of a stillborn infant. Necropsy showed asphyxial changes with a small intraventricular haemorrhage. The umbilical cord showed evidence of infection with polymorphonuclear infiltration involving the three vessels, and the mother had been febrile for several hours before delivery. The placenta was not examined histologically, though it had been noted to have an offensive odour at delivery.

Unless one postulates an asymptomatic infection of the foetus earlier in the pregnancy and unrelated to the perinatal infection, one must consider the possibility that infection of the placenta may have altered its permeability and permitted the passage of maternal Ig M to the foetus, or that the foetus produced Ig M rapidly in response to the antenatal infection.

Case No. C1. Birthweight 2364 g. This infant had a slightly raised Ig M concentration but fairly low Ig G and albumin, so that relative to these proteins Ig M was considerably above the normal range. This mother had a threatened abortion at the 16th week of pregnancy and later hydramnios, but no abnormality of the infant was found. It is possible that the threatened abortion was due to a maternal (? viral) infection, and that the foetus acquired and retained the infecting organism, showing no ill effect but forming antibodies at some stage of the pregnancy.

In addition to Cases C1 and C3, one other pregnancy in the group was characterized by threatened abortion, Case No. C2 (birthweight 1441 g.), where slight bleeding occurred at the 6th and 10th weeks. The absolute concentration of Ig M was raised in this infant also, and it was disproportionately high in relation to albumin and probably to Ig G (Fig. 9 and 10), and it is possible that haemodilution was masking to some extent the raised Ig M level. This infant showed no abnormalities at birth.

Clearly, a large series of pregnancies characterized by threatened abortion should be studied by this method.

C. Multiple pregnancies. Interpretation of the results in this group of infants has not taken into account differences between the mean weights of twins and singletons for a given period of gestation. It was felt that no valid allowance could be made for this factor, and its omission does not materially affect the conclusions.

FIG. 11.—Ig M concentration in umbilical venous plasma in multiple pregnancies. Case numbers refer to text. Mean concentration and 95% confidence limits for healthy singletons are illustrated. X Plasma from cardiac puncture.

Ig A and Ig M. Ig A was present in detectable amount in three infants—twin D1a (5.9 mg./100 ml.), twin D1b (4.3 mg./100 ml.), and twin D5a (64.5 mg./100 ml.). The former twins were 25-week abortions who died a few hours after birth and whose Ig M concentrations of 11.9 mg. and 8.0 mg./100 ml. may also be high for that period of gestation but no normal values are available for comparison. Twin D5a, from whose sib blood was not obtained, had a clearly raised Ig M concentration of 28.6 mg./100 ml. These twins were healthy at birth but the mother had repeated small vaginal haemorrhages during the 3rd and 4th months of pregnancy.

A further pair of twins (Cases D8a and b) had high concentrations of Ig M. They were healthy infants born after an uneventful pregnancy except that the mother had rhesus antibodies. Both infants had strongly positive Coombs tests at birth but umbilical cord haemoglobin concentrations of approximately 15 g./100 ml., and replacement transfusion was not required. In a series of cases of haemolytic disease of the newborn studied in this laboratory, abnormalities of immunoglobulin concentration in the umbilical cord blood were rarely encountered (to be published), and no explanation has been found for the high Ig M value in the twins.

Fig. 11 shows the close similarity which exists between Ig M concentrations of individual members of a set of twins or triplets.

FIG. 12.—Ig G concentration in umbilical venous plasma in multiple pregnancies. Case numbers refer to text. Regression of Ig G concentration on birthweight with 95% confidence limits for healthy singletons is illustrated. See Fig. 11 for key.

Ig G and albumin. Comparison of the Ig G birthweight graph (Fig. 12) and the albumin birthweight graph (Fig. 13) shows that they are very alike, and, as in the case of Ig M, there is generally close matching of concentrations in sets of twins or triplets. The only exception is in the case of twins D6a and b, where D6a was a normal infant and D6b a macerated stillborn hydrocephalic. The blood specimen from D6b was grossly haemolysed, and the low concentrations of both Ig G and albumin (in each case, 19% of the concentration in the twin

FIG. 13.—Albumin concentration in umbilical venous plasma in multiple pregnancies. Case numbers refer to text. Regression of albumin concentration on birthweight with 95% confidence limits for healthy singletons is illustrated. See Fig. 11 for key.

sib) were presumably due to dilution. The Ig M concentration in this twin, however, was 9.7 mg./100 ml. which is relatively much closer to the value of 13.9 mg./100 ml. found in the healthy twin, and it is likely that in the hydrocephalic infant marked dilution of the plasma had lowered a high Ig M level to lie within the normal range.

In the case of twin D5a, who also had low Ig G and albumin concentrations (Fig. 12 and 13), a similar mechanism may have operated, and the high concentrations of Ig A and Ig M already noted in this infant are even more striking when this is taken into account.

Discussion

Congenital malformations. The newborn infant responds to antigenic stimulation by the production initially of 19S γ -globulin (Ig M) (Smith, 1960; Uhr, Dancis, Franklin, Finkelstein, and Lewis, 1962; Smith and Eitzman, 1964), and the presence of Ig M antibodies to toxoplasmosis in the umbilical cord serum of infants affected by toxoplasmosis has been demonstrated by Eichenwald and Shinefield (1963) who pointed out that these antibodies were produced by the foetus, as indicated by the higher titre in the foetus than the mother, and the fact that Ig M does not normally cross the placental barrier. The existence of Ig M antibodies in the umbilical cord serum of infants with the congenital rubella syndrome has similarly been interpreted as indicating antibody production by the foetus, though the possibility of passage of maternal Ig M through a damaged placenta cannot entirely be excluded (Alford, 1965). In early pregnancy, Alford (1965) found that the predominating antibody to rubella virus in the foetus was an Ig G and it seemed likely that this had originated in the maternal plasma. The continuing presence of Ig M antibodies throughout the remainder of pregnancy may be occasioned by the persistence of active infection which in turn may be due to failure of Ig M antibodies to clear the virus from infected tissues. Ig G concentration in the serum of small foetuses is very low, and sufficient maternal antibody may not be transferred to ensure eradication of the infection.

Raised concentrations of Ig M may not persist until birth of the infant, if the infection is eradicated early in pregnancy. It may be only in those cases where the virus persists in the tissues throughout pregnancy, as is now known to occur with rubella virus, that the search for raised concentrations of Ig M in umbilical cord blood of infants with congenital malformations, arising from early infection of the foetus, will be successful. In other early infections of the foetus, the infecting agent may be

destroyed by Ig G antibodies passed from the mother, and an Ig M antibody response by the foetus may never have occurred, or may have been so transient or so slight that no trace remains when the infant is born, or the early Ig M antibodies may have been replaced by Ig G antibodies. Furthermore, there must be a critical age before which the foetus cannot produce antibodies, and van Furth and his colleagues (van Furth, Schuit, and Hijmans, 1965) found evidence of synthesis of Ig M by the foetus from only about the 20th week of gestation.

Normal concentrations of Ig M and also of Ig A in the cord blood of infants with congenital malformations, therefore, do not exclude the possibility that the deformities arose because of early infection of the foetus; they indicate only that there is no continuing production of foetal antibodies such as might be expected if the infection had persisted in the foetus. There is also the possibility that, though a state of immunological tolerance is not induced by early infection of the foetus by rubella virus, some other infections at this time may induce this state so that foetal antibodies are not produced.

In the present series of infants with congenital malformations, no clear-cut abnormalities of the immunoglobulins were demonstrated, in particular no increase in the concentration of Ig M either *per se* or in relation to albumin or Ig G. Ig A was raised in one infant with congenital hydrocephalus.

The rather low Ig G and albumin concentrations found in the larger infants in the group could be due to some degree of haemodilution, or could arise from deficient placental transfer of Ig G and deficient synthesis of albumin by the foetus. However, the similarity of the regression lines for deformed and healthy infants in Fig. 4 and 5 shows that a parallel alteration has occurred in the concentrations of at least three plasma proteins suggesting that haemodilution is the most likely explanation for the findings.

Miscellaneous abnormalities. The pilot survey reported here of immunoglobulin estimations on 15 infants with various abnormalities of the associated pregnancy or of the neonatal period suggests that this may be a profitable field for further study. Of the 15 cases, 3 showed very high Ig M concentrations and 3 moderately raised levels, and the high Ig M/Ig G and Ig M/albumin relation for these cases confirmed that the raised Ig M concentrations were not the result of haemoconcentration.

In 3 of these infants, including one with congenital cataracts, and also in one twin of the group of twins studied, raised Ig M concentrations followed threatened abortion in the early months of pregnan-

cy, and it is clearly necessary to study a large series of such cases. It is possible that a proportion of such cases are due to an unidentified infection of mother and foetus, or, alternatively, that there is a breakdown of the normal state of tolerance between mother and foetus at this time. On these and other infants with raised umbilical cord immunoglobulins, an attempt must be made to identify the antigen to which antibodies have been formed, and virological studies are in progress on the sera in the present series giving positive results.

Multiple pregnancy. A striking finding in this group of infants is the close correspondence between immunoglobulin and albumin concentrations in infants from the same pregnancy. This is evident both for the proteins of foetal origin and for Ig G transferred from the mother. The only discrepancy occurred in the presence of congenital malformation of one twin, and the results in this infant illustrate the necessity to estimate several plasma proteins and examine their interrelationships, since reliance on Ig M and Ig A estimations alone suggested that this infant had normal immunoglobulins, whereas, as explained above, it is possible that dilution of the plasma was responsible for lowering high Ig M levels. High Ig A concentrations found in 25-week twin abortions who died very shortly after birth were associated with Ig M levels which could be high for this period of gestation, and further studies are being made on unexplained abortions, very early premature births, and unexplained neonatal deaths.

Summary

Ig G, Ig A, Ig M, and albumin were estimated in umbilical venous plasma from 37 infants with congenital malformations, 15 infants with miscellaneous abnormalities of the perinatal period or of the associated pregnancy, and 20 infants from multiple pregnancies.

Apart from a high level of Ig A in an infant with congenital hydrocephalus, no abnormalities of the immunoglobulins, Ig A and Ig M, were found in the group of congenital malformations. The albumin and Ig G concentrations were moderately low in the larger infants in the group.

In the other two groups of infants, abnormal concentrations of immunoglobulins were found in four infants born of mothers who had earlier threatened abortions, though only one of these infants had an obvious abnormality at birth in the form of congenital cataracts. Abnormal concentrations of immunoglobulins were found also in an infant with meconium peritonitis, an infant with

intrauterine growth retardation, neonatal purpura, and hepatosplenomegaly, an infant stillborn following intrauterine sepsis, twin abortions who died soon after birth, twins suffering from mild haemolytic disease of the newborn, and a stillborn hydrocephalic twin. The causes of the immunoglobulin abnormalities are discussed, and the need for further studies on such infants is stressed.

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Addendum

Since these results were submitted for publication, a further series of pregnancies characterized by threatened abortion has been studied. When vaginal bleeding during pregnancy consisted of only slight loss at the time a menstrual period was due, the infants did not show abnormal concentrations of immunoglobulins. Of 9 cases with more definite threatened abortion, 2 showed slightly raised Ig M concentrations, and in none was Ig A detected.