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Immune Globulins in Maternal, Cord, and Infant Blood

By

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The presence and levels of IgA, IgM and IgG in maternal, cord and infant sera have been examined by immunodiffusion, paper electrophoresis and immunoelectrophoresis. (i) There was no significant difference in the concentration of IgG between maternal, cord and infant sera. (ii) The occurrence and concentration of IgM and IgA in cord and infant blood are independent of their maternal levels. (iii) While IgM concentration is similar in venous cord blood and in infant serum, IgM concentration is much lower in arterial cord blood. IgA occurs more rarely than IgM in cord and infant sera and there is no significant difference in IgA concentration between venous and arterial cord blood. In accordance with the data of others, the present results suggest that while IgG in the foetus is purely of maternal origin, IgM is formed mostly by the foetus itself. The small amount of IgA in the newborn is probably of maternal origin, though its production by the newborn starts earlier than that of IgG.

There are three possible ways for maternal immune globulins (IgG, IgA, IgM) to be transferred into the foetus, viz.

(i) transplacentally, during pregnancy;

(ii) by ingestion of homologous breast milk or colostrum; and

(iii) by intestinal absorption of amniotic fluid swallowed by the foetus.

In the human foetus the transplacental way is considered to be practically the only route of transfer [22]. While IgG has proved liable to transfer across the placenta [9, 30, 31, 45], IgA, being of a similar molecular size, and IgM, having a molecular weight of approximately one million, are transferred only in traces [6, 7, 15, 21, 22, 25, 35, 39, 40, 46, 48]. In fact, the the first immunoelectrophoretic assays failed to reveal their presence — especially that of IgA — either in infant or in cord blood. More recently, however, several data have suggested the presence of one or both of these two immune globulins in the newborn's serum [6, 7, 15, 16, 19, 23, 25, 26, 34, 39, 44, 48] and in some cases the amounts detected even surpassed those expected on the basis of selectivity of the placental transfer for maternal IgA and IgM. This fact raised the idea of a possible intrauterine formation of beta$_2$ globulins by the foetal organism itself [6, 11, 17, 20, 26, 39, 41, 43].

Since immune globulins have generally been considered to have antibody

character, and since the presence or absence of some antibodies may be of practical importance in the infant, attention has increasingly been focussed on the foeto-maternal relations of the three main immune globulins. The present study has been undertaken to examine this problem.

Materials and Methods

A comparative analysis of the serum proteins of maternal, infant and cord blood was performed. Samples were obtained from 21 pregnant women, a few hours before delivery. Mixed cord blood was collected after transection of the umbilical cord, and blood was drawn from infants at 24 hours of age. In 30 subsequent cases cord blood was obtained separately from the umbilical artery and vein.

Total serum proteins were determined by the micro-Kjeldahl method. Paper electrophoretic and immunoelectrophoretic analyses of the proteins — the latter according to Backhausz, Veres and Vető [3] — were performed as described in a previous paper [6]. By means of a calibration curve [6] the sizes of the bands of precipitates were expressed in centimetres, thus providing semiquantitative values that could be compared. Immunodiffusion was performed by the simple comparative method of Oudin [37], as described by Kávai and Bátory [24]. Accordingly, the surface of a glass-plate of 5×5 cm was divided into two halves. On the upper part maternal (I), cord (II) and infant (III) sera were mounted as separate antigens. The antigens were diluted with equal amounts of a 2% solution of agar dissolved in a pH 8.2 buffer, the same as used for immunoelectrophoresis. On the lower part of the glass plate was mounted the antiserum in a dilution of 1 : 4, previously having been mixed with aliquot amounts of agar. The plates were incubated for 3 to 5 days in a wet chamber at room temperature, thereafter they were washed and stained as for immunoelectrophoresis.

The antibodies (prepared by Human, Budapest) were: antihuman horse serum, antihuman IgG immune serum, antihuman IgA immune serum, antihuman IgM immune serum.

This assay allowed to compare different antigen complexes mounted against a common antiserum. If the different antigens are of similar composition, the bands of precipitates interfuse. If the two antigens are of similar concentration, the precipitation line is straight; in the case of a difference in concentration the line bends toward the spot with the lower concentration, and if one of the components is absent, the band of precipitate will even enter the spot representing the deficient component.

Results

Fig. 1 demonstrates IgG levels obtained by paper electrophoresis; their mean level amounted in maternal sera (I) to 980 mg per 100 ml, and in infant sera (III) to 1040 mg per 100 ml. Somewhat higher values were

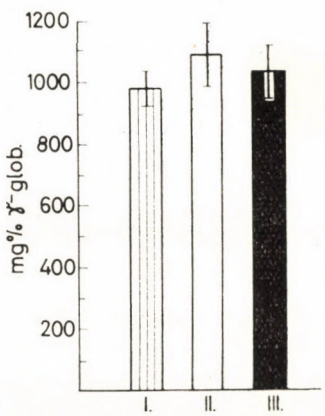


Fig. 1. Gamma globulin levels in maternal (I), infant (III) and cord (II) sera as obtained by paper electrophoresis

revealed in mixed cord blood (II), but the difference was not significant statistically.

The presence of IgA and IgM could be established by paper electrophoresis in all of the maternal sera and in about half of the cord blood samples. In infant sera these globulins — especially IgM — occurred more frequently, but the absence of one or both of the beta$_2$ globulins could be observed also in these cases (Electrophoresis figs. a, b, c, d, e).

IgA and IgM levels in either cord or infant sera were mostly negligible when compared to maternal values. In some newborns, however, a level markedly exceeding the mean value was revealed. Quantitative relationships are represented by the semiquantitative values of Fig. 2. As for IgG, while its maternal level remained below that of the mixed adult sera, the latter was surpassed by the values obtained in cord and infant sera. These differences were, however, not significant statistically.

IgA and IgM in maternal sera were above control levels, while in infant and cord sera they remained under the control values. Especially marked was this reduction in the case of IgA, interfering even with its reliable determination.

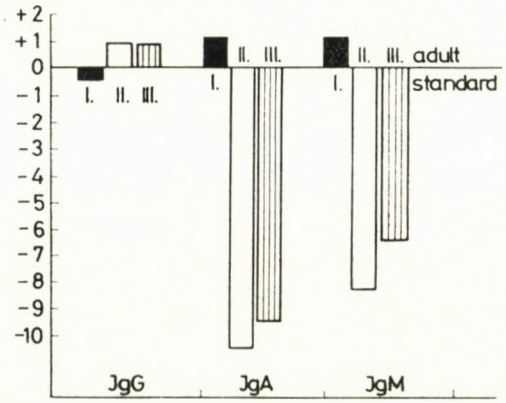


FIG. 2. Semiquantitative immune globulin values in maternal (I), mixed cord (II) and infant (III) sera, as compared to standard adult values. Abscissa: length of bands of precipitate in cm, as obtained from the calibration curve constructed for immunoelectrophoretic evaluation. The values are linearly related to the concentration. (See: references 6 and 7)

In view of the increased levels of IgM in the infant sera as compared to mixed cord blood, IgA and IgM concentrations in arterial and venous cord blood were examined separately. It was found that IgM occurred more frequently in venous cord blood and its concentration was also superior to that found in the umbilical artery (Fig. 3). Considering the low levels, no significant differences in IgA concentration could be established between arterial and venous cord blood.

The results obtained by immunoelectrophoresis were checked by immunodiffusion assay. Both methods revealed a similar incidence of IgA and IgM in the infant sera, while in cord blood IgM was detected less frequently when tested by immunodiffusion. Nevertheless, the presence of beta$_2$ globulins in both cord and infant sera could be established also by this method. The absence of one or both of these globulins was more frequent in cord blood. (See Figs. a, b, c, d, e, f).

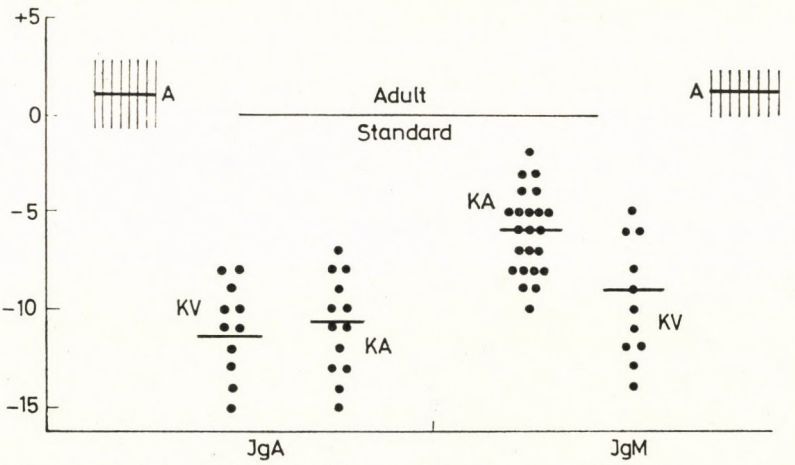


FIG. 3. IgA and IgM concentration in umbilical vein (KV) and artery (KA) as compared to adult and maternal (A) values. Points mark single values

DISCUSSION

Although comparative analyses of maternal and infantile serum globulins have repeatedly been performed, the data reported are rather inconsistent. The problem of immune globulins has been a major source of disagreement. As regards the ratio of IgG level in maternal and cord sera, while some authors advocated a definite prevalance of cord blood [1, 4, 10, 12, 13, 14, 23, 27, 28, 29, 33, 36, 38], others denied any significant difference [2, 21a, 34, 35, 49]. These discrepancies gave rise to the question, whether IgG is provided for the foetus exclusively by the mother, or it has some other source as well.

Increased levels found in cord blood pointed to the existence of an extramaternal source of IgG. Active protein synthesis in the placenta or in the foetus itself has been suggested. The former possibility has been stressed mainly by ZAPP who demonstrated the composition of the newborn's serum protein system to be more closely related to that of the washed placenta than to the maternal serum

protein structure [50, 51]. In the opinion of others the placenta is capable of resynthesizing broken drown IgG, providing thus the foetus with additional amounts. EWERBECK and LEVENS [13] favour the possibility of IgG synthesis in the foetus itself, considering that alterations in the maternal serum protein system leave the foetal serum proteins uninfluenced.

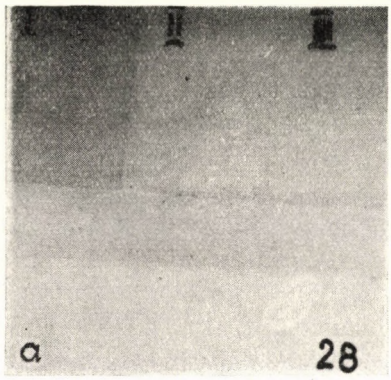
Against these suggestion speaks not only the late appearance of IgG synthesizing plasma cells in the foetus [35, 42, 47], but also the course of IgG production in children born to mothers with agammaglobulinaemia [18, 49]. Experiments performed with labelled IgG are also suggestive of the maternal origin of infantile IgG.

The fact that the IgG level is often higher in cord blood than in the mother is in itself yet insufficient evidence of the extramaternal source hypothesis. The difference may result from the fact that in the majority of reports the amount of IgG has been expressed in terms of relative percentage, although IgG has a larger share of total serum proteins in the newborn at delivery than in adults. This possibility has been pointed out by von MURALT and GUGLER [35] who were unable to demonstrate any significant difference in the absolute IgG value between the mother and her newborn.

Our results are consistent with the data of those who reported similar amounts of IgM in mother and newborn. The differences observed may have been due to haemoconcentration resulting from an insufficient fluid uptake by the newborn. This fact may explain also the larger scatter in the values for infants as compared to those for the mothers. The similarity of the IgG levels in the mother and the newborn has been demonstrated also by immunochemical methods [49].

The possibility that the placenta would synthesize gamma globulins, seems to be invalidated by the results of DANCIS et al. [8], who showed by means of ^{14}C-glycine that the placenta was capable of forming alpha and beta but no gamma globulin.

Despite earlier contradictions, the purely maternal origin of IgG now seems to be well established, the newborn being capable of IgG production only after the second week of life. However, as far as beta$_2$ globulins are concerned, opinions still differ. Before the presence of these proteins during the first postnatal weeks had been shown, it was assumed that the placenta was impermeable for them and, consequently, their appearance in the infant's serum coincided with the beginning of formation in the newborn. Since improved techniques have made it possible to detect traces of beta$_2$ globulin, the hypothesis of a transplacental transfer of maternal IgA and IgM has emerged. Especially strong is the evidence in the case of IgM, the early appearance of which has almost unvariably been demonstrated. The sometimes unusually high concentration of IgM, and a further rise in this concentration in the early postnatal period lent, however, further support to the self-production hypothesis [6, 7, 11, 17, 20, 26, 39, 41, 43]. IgM levels found in foe-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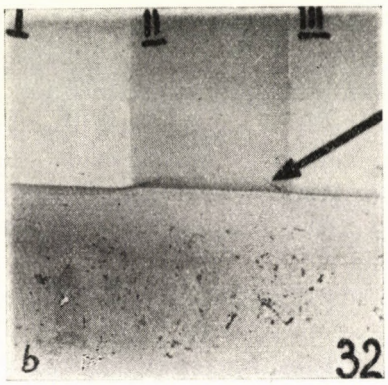
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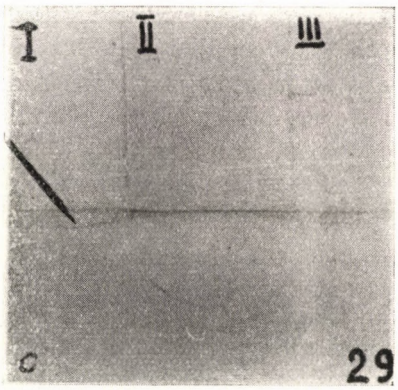
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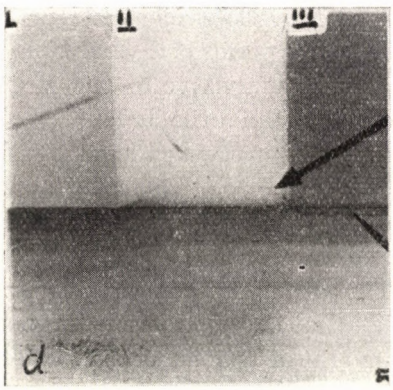
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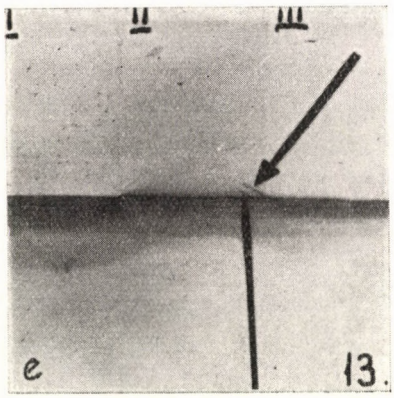
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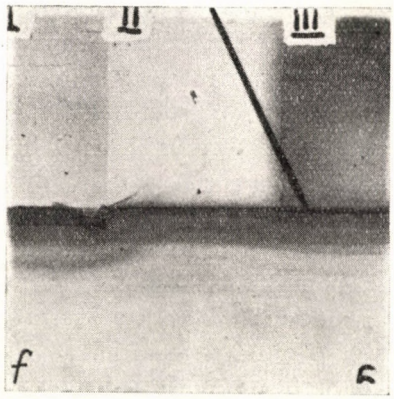
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tuses and infants with intrauterine infection are also favouring this assumption [11, 17, 43]. In support of it is also the higher incidence of IgM in infant serum than in cord blood, when examined by immunodiffusion, and the IgM level in arterial cord blood being lower than in infant and in venous cord blood.

These results, as well as that of others [26], underline the possibility of an active participation of the foetus in the formation of its own immunological state.

Immunodiffusion figures —— IgA
—→ IgM

a) Monovalent antihuman IgG immune serum tested against IgG of maternal (I), cord (II) and infant (III) sera gives a single straight band of precipitate, indicating the similar IgG content of the antigens.

b) When monovalent antihuman IgG + IgM immune serum is used as antibody the band of IgG is located similarly as before. The band of precipitate of IgM is bending from both the maternal (I) and infant (III) sera angularly towards cord serum, indicating the absence in cord serum, but the presence in maternal and infant sera, of IgM.

c) When the three sera are tested by immunodiffusion against monovalent antihuman IgG + IgA immune sera, the band of precipitate of IgA shows a wavery course, which is widest below the container of maternal serum (I), much smaller at infant serum (III) and almost negative at cord-serum (II).

d) Bands of precipitates formed against polyvalent antihuman immune serum reveal that IgA, though at different concentrations, is present in all three sera. IgM is absent from cord serum (middle).

e) IgA and IgM are present both in maternal and infant sera, but are lacking in cord serum.

f) Although at different concentrations, IgA is present observed in all three sera; IgM occurs solely in the maternal sample.

IgA in mixed cord blood and in infant serum is less common than IgM, and the concentration of IgA is also lower than that of IgM. Furthermore, no significant difference could be revealed between venous and arterial cord blood samples, as far as IgA is concerned. All this is suggestive of the purely maternal origin of foetal IgA.

Although FURTH et al. [17] were unable to find signs of foetal IgA formation, our previous observations of the early postnatal increase in IgA levels indicate that the self-production of even this globulin may precede that of IgG in the infant.

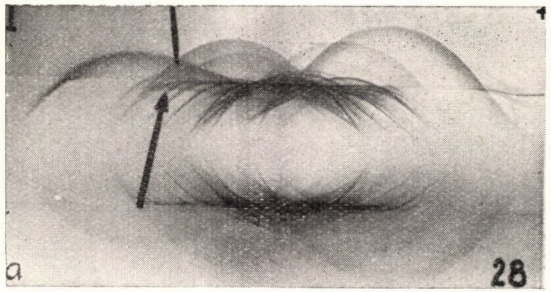
IgA and IgM levels in infant and in cord blood are independent from those in the mother. High levels in maternal blood may be associated with low values or a complete deficiency of the two $beta_2$ globulins in the infant, and vice versa.

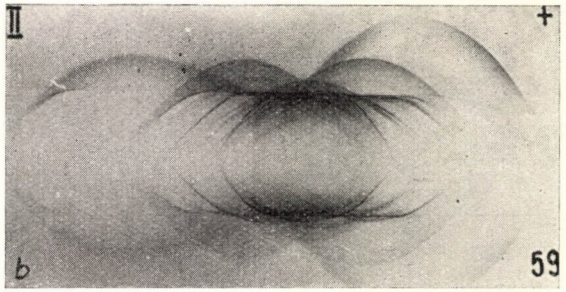
As a conclusion it can be stated that

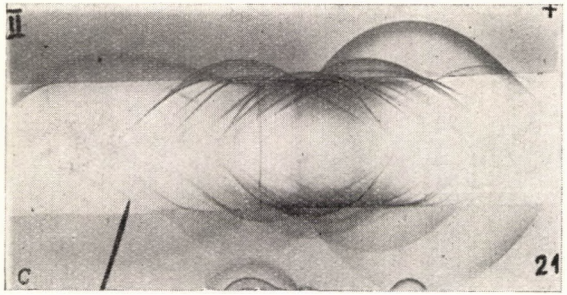
1) there is no significant difference in the IgG level of maternal, cord and infant sera. Our results confirmed the data assuming no gamma globulin synthesis in the placenta, thus supposing their purely maternal origin.

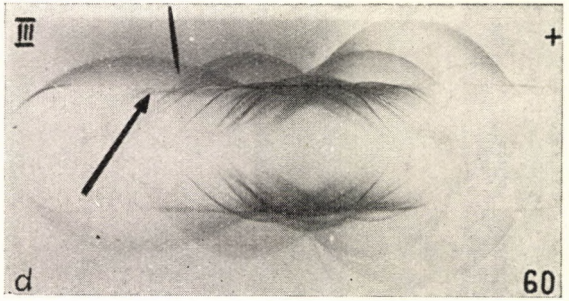
2) IgA and IgM were always present in maternal blood, and despite the haemodilution, their concentration exceeded normal adult values.

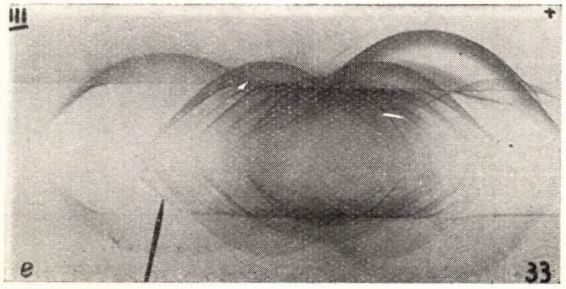
3) The absence of one or both of the $beta_2$ globulins could be established in more than half of the cases, when tested by immunoelectrophoresis. The lack of IgM was even more frequent, when examined by immunodiffusion.











Immunoelectrophoretic analyses

IgA ———
IgM ⟶

a) Immunoelectrophoresis of maternal serum.
b) Immunoelectrophoresis of cord-blood, revealing a lack of IgA and IgM precipitation bands.
c) Immunoelectrophoresis of cord blood, revealing at the higher dilutions the band of IgA in a negative position.
d) Immunoelectrophoresis of infant serum, showing bands of precipitates of both IgA and IgM.
e) Immunoelectrophoresis of infant serum revealing the band of IgA at higher dilutions.

4) The incidence and level of IgA and IgM in the one-day-old newborn significantly surpassed the values found in mixed cord blood. The difference was more striking in the case of IgM.

5) IgM occurs more frequently and in a larger amount in venous, than in arterial cord blood. As regards IgA, a similar difference could not be detected.

The observation that the foetus may synthesize IgM, and that the production of IgA starts earlier than that of IgG, must not lead to the conclusion that the newborn is sufficiently protected against infections. IgM and IgG levels in the infant are rising slowly to attain adult values, while the breakdown of maternal immune globulins is completed more rapidly. Nevertheless, the possibility of the early appearance of 19S macroglobulin, representing the first step in all kinds of antigen-antibody reaction, is suggestive of the fact that the foetus takes an active part in the formation of its own immunological state.

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