Fetomaternal Passage of Leukocytes and Platelets in Erythroblastosis Fetalis

By R. G. Desai, E. McCutcheon, B. Little and S. C. Driscoll

MATERNOFETAL PASSAGE of leukocytes and platelets has been demonstrated in man during health1 and disease.2,3 However, no direct evidence of fetomaternal passage of these elements has so far been recorded in the literature. The recent use of intrauterine transfusions in the management of erythroblastosis fetalis has afforded the opportunity to study the transfer of the formed elements of blood from fetus to mother in humans.

MATERIALS AND METHODS

Fresh O Rh-negative blood or bank blood less than 24 hours old was used. It was labeled employing atabrine-fluorescent method previously described in detail.4 Seventy-five to 150 ml. of packed cells with hematocrit value between 70–80 per cent were injected into the fetal abdominal cavity by direct visualization, using fluoroscopy with image-intensifier Westinghouse Televeex equipment.4 Details of the selection of Rh-incompatible cases by amniotic fluid examination, the procedure of intra-abdominal fetal transfusion and various immunologic tests will be described elsewhere.5 Following amniotic fluid examination and iso sensitization studies, the fetuses of seven women were transfused intraperitoneally at gestational ages which ranged between 26–34 weeks. Two fetuses received three, and five fetuses received one, intra-abdominal transfusion (Table 1). Twenty ml. samples of maternal blood were withdrawn at ½, 1, 2, and 4 hours in all cases, whereas in a few cases samples were also obtained at 12, 24, 48, and 72 hours. Siliconized glassware and ACD anticoagulant were used throughout the study. White cells and platelets were separated by differential centrifugation and severaluffy coat smears were made to observe fluorescent cells. The Leitz fluorescent microscopic equipment was used and photomicrography was done with Ektachrome Type B film with 5–10 minute exposure times.

Placentas, membranes and umbilical cords were examined fresh, and gross observations were recorded. Portions were fixed in Bouin’s fixative; paraffin sections were stained with hematoxylin and eosin; and slides were then examined by means of light microscopy.

RESULTS

A small number of atabrine-labeled donor blood leukocytes and platelets were demonstrable in the maternal blood samples in all the cases except Case 3 and Case 6. In Case 3, it was subsequently found that most of the blood had

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Table 1.—Obstetrical Data in Subjects with Intrauterine Transfusions

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Previous Pregnancies</th>
<th>History of Fetal Loss</th>
<th>Mother's Blood Group</th>
<th>Duration of Present Gestation (weeks)</th>
<th>Amount of Intra-abdominal Transfusion (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38</td>
<td>5</td>
<td>2</td>
<td>O Neg.</td>
<td>33 1/2</td>
<td>150</td>
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<tr>
<td>2A</td>
<td>29</td>
<td>6</td>
<td>3</td>
<td>O Neg.</td>
<td>31</td>
<td>120</td>
</tr>
<tr>
<td>2B</td>
<td>29</td>
<td>6</td>
<td>3</td>
<td>O Neg.</td>
<td>33</td>
<td>150</td>
</tr>
<tr>
<td>2C</td>
<td>29</td>
<td>6</td>
<td>3</td>
<td>O Neg.</td>
<td>34</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>7</td>
<td>4</td>
<td>O Neg.</td>
<td>33 1/2</td>
<td>150</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>4</td>
<td>1</td>
<td>O Neg.</td>
<td>30</td>
<td>130</td>
</tr>
<tr>
<td>5A</td>
<td>37</td>
<td>6</td>
<td>4</td>
<td>O Neg.</td>
<td>26 5/7</td>
<td>75</td>
</tr>
<tr>
<td>5B</td>
<td>37</td>
<td>6</td>
<td>4</td>
<td>O Neg.</td>
<td>28 6/7</td>
<td>120</td>
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<tr>
<td>5C</td>
<td>37</td>
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<td>6</td>
<td>21</td>
<td>2</td>
<td>1</td>
<td>A Neg.</td>
<td>32</td>
<td>140</td>
</tr>
<tr>
<td>7</td>
<td>29</td>
<td>4</td>
<td>0</td>
<td>O Neg.</td>
<td>31</td>
<td>125</td>
</tr>
</tbody>
</table>

been infused into the amniotic sac, because after a few hours following the transfusion, the patient ruptured her membranes and the amniotic fluid was grossly bloody. In Case 6, fetal distress was diagnosed at the end of the transfusion. No fetal heart was heard the following day. In those cases where the blood was successfully injected, a varying number of leukocytes (15–40 polymorphonuclear cells and mononuclear leukocytes) and 50–100 platelets were seen in each case during examination of a total of 35–40 slide smears by fluorescent microscopy. An occasional leukocyte or platelet was found in the ½-hour sample, but the peak number of cells were seen in the 2-hour sample of maternal blood. No cells were demonstrable after 4 hours. The results show that the fetomaternal passage of intact leukocytes and platelets seems to occur in every case of erythroblastosis fetalis as early as at 26 weeks of gestation.

The placental pathology was studied in detail in each instance and showed a fairly consistent pattern. On gross examination, the placentas weighed between 410–620 Gm. and were morphologically typical of severe erythroblastosis fetalis. They were enlarged, friable, pale, and moist, exhibiting variable degrees of edema. There were occasional intervillous thrombi and, less often, placental infarcts. Histologically, the placentas appeared relatively "immature" (Fig. 1). The villi were large and edematous, with cellular stroma clothed in bilaminate trophoblast; syncytial buds were few; villous vessels contained an unusual number of nucleated erythrocyte precursors (Fig. 2). No appreciable histologic changes were detected between the placentas associated with one as compared to those with multiple fetal intra-abdominal transfusions.

DISCUSSION

Earlier studies on the permeability of the placenta suggested that this organ was impermeable to large particles such as the formed elements of blood; however, recent work on the placental transfer of antibodies14 as well as on transplacental passage of red cells, white cells, and platelets in animals15 and man16 has shown that such transfer does occur in both health and disease. As early as 1943, Levine6 speculated that fetomaternal transfer of erythrocytes must occur if the erythroblastosis fetalis was to be explained as an isoimmune
Fig. 1.—Photomicrograph of placenta. Chorionic villi are large, edematous and cellular, resembling a very immature placenta. (H & E, ×100)

Fig. 2.—Photomicrograph of placenta. Villi are covered by cytotrophoblast and syncytiotrophoblast; stroma is cellular; numerous nucleated erythrocyte precursors lie within fetal vessels. (H & E, ×320)
phenomenon. Since then, several investigators have provided direct evidence of the presence of fetal red cells in the maternal circulation, using various technics such as differential agglutination,\textsuperscript{7} serologic methods,\textsuperscript{8} immunofluorescence,\textsuperscript{9} and more recently the acid elution technic.\textsuperscript{10} In the latter method, the differences in the solubility of adult and fetal hemoglobin of the erythrocyte in acid solution allow the identification of fetal red cells. To the best of our knowledge, direct demonstration of the fetomaternal passage of leukocytes and platelets has not been reported. However, isoimmunization due to both leukocytes and platelets and the occurrence of leukoagglutinins\textsuperscript{12} and platelet antibodies\textsuperscript{18} following multiple transfusions and pregnancy are well established. It is conceivable that fetomaternal passage of varying degrees occurs more frequently than maternofetal transfer.\textsuperscript{1} However, experience in humans is limited to this preliminary report. At the same time, if there is an excessive transfer of cells such as trophoblastic elements, red cells, white cells (lymphocytes), or platelets which may act as antigens, the early rejection of the graft could occur in cases of repeated pregnancy resulting in abortions.

In Case 3 and Case 6, there were no labeled donor cells detected in the maternal blood. The fetuses were subsequently shown to have died in utero. In these cases, the failure of cell passage across the placenta may only indicate fetal death; but as the fetuses were alive at the time of transfusion, the diminished passage of cells may be an indication of impending fetal death.

In our experience at postmortem examination of fetuses which have recently been transfused, infused blood was shown to have disappeared from the peritoneal cavity, presumably absorbed, in 48 to 72 hours. This indicates that red cell absorption is more rapid from the fetal peritoneal cavity than from the adult.\textsuperscript{16}

Less than 100 mg. atabrine is used to label the cells. Studies of the absorption, distribution and placental transfer of atabrine in pregnant and non-pregnant animals and in man indicate that at this dose it is harmless to the fetus at this late stage of organogenesis.\textsuperscript{17,19}

The mechanism of the transport of administered cells from the fetal peritoneal cavity across the placental barrier to the maternal circulation is not resolved by this study. Pathologic alterations of the placenta in erythroblastosis or diabetes mellitus\textsuperscript{2} as shown earlier may be responsible for this demonstration.

**Summary**

Leukocytes and platelets labeled by the atabrine fluorescent technic have been demonstrated in the maternal circulation from $\frac{1}{2}$ to 4 hours following intraperitoneal transfusion of the fetus in utero. The peak concentration of labeled cells was observed 2 hours after transfusion. The fetomaternal transfer of cells has been demonstrated in cases of erythroblastosis fetalis. It is not known whether this occurs in normal fetuses and the mechanism is not understood.
SUMMARIO IN INTERLINGUA

Leucocytos e plachettas marcate per medio del technica a atabnina fluones-cente esseva demonstrate in le circulation materne inter un medic hora e 4 horas post le transfusion intraperitonee del fet in utero. Le concentration maxime de marcate cellulas esseva observate 2 horas post le transfusion. Le transferimento feto-matern de cellulas esseva demonstrate in casos de erythroblastosis fetal. Non es cognoscite si iste phenomeno occurre in fetos normal, e le mechanismo subjacente non es clar.

REFERENCES

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