Transplacental passage of blood cells

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Passage of erythrocytes between mother and fetus is a well-documented phenomenon. In 1946, Naeslund and his collaborators (Naeslund and Nylin; Hedenstedt and Naeslund) showed that radioactively labelled erythrocytes, and elliptocytes, when injected into the maternal circulation before delivery, could be found postnatally in the blood of the infant. Similar results have been obtained with various agglutination tests (von Mural, 1967; Eimer and Weiland, 1969) and immunofluorescence (Fischer and Müller, 1967).

Since the introduction of the acid elution method for recognizing cells carrying fetal haemoglobin (Kleihauer *et al*, 1957), the occurrence of fetal erythrocytes in maternal blood has been studied in detail (Zipursky *et al*, 1959; Wimböfer *et al*, 1962; Zilliacus, 1963; Clayton *et al*, 1964; Cohen *et al*, 1964; Cohen and Zuelzer, 1967; Betke and Nierhaus, 1968; Bartsch, 1972). Today, it is generally known that Rh immunization is caused by fetal erythrocytes from Rh-positive fetuses which enter the circulation of Rh-negative mothers (Woodrow and Finn, 1966; Clarke, 1967; Bartsch, 1972), and that this immunization can be prevented by administration of anti-D gammaglobulin of Rh⁻ mothers carrying Rh⁺ fetuses (Clarke, 1967; Pollack *et al*, 1971a; 1971b; Bartsch, 1972; Eklund and Nevanlinna, 1973).

During the last 10 years attempts have been made to demonstrate the passage of leucocytes between mother and fetus. Desai and Creger (1963) reported maternal leucocytes in the cord blood after normal pregnancies. Their results were later challenged by others, who found maternal leucocytes in the umbilical cord only in connection with malformations of fetus and placenta (Kadowaki *et al*, 1965; Turner *et al*, 1966; Olding, 1972). However, quinacrine mustard fluorescence of human chro-

mosomes has been used to show that such a passage may occur in normal pregnancies (Schröder, 1974).

Fetal leucocytes seem to find their way into the maternal blood in most pregnancies (Walknowska *et al*, 1969; de Grouchy and Trébuchet, 1971; Schröder and de la Chapelle, 1972; Grosset *et al*, 1974), but seldom provoke the production of HL-A antibodies in the mother (Schröder *et al*, 1974).

In the mouse maternal erythrocytes and lymphocytes have also been demonstrated in the circulation of the offspring by several different methods (Finegold and Michie, 1961; Tuffrey *et al*, 1969a; 1969b; Barnes and Tuffrey, 1970).

It is my intention in this paper to summarize what is currently known of the passage of blood cells between mother and fetus in man, and to discuss whether there is any difference in the mechanisms by which erythrocytes and leucocytes cross the placenta. Moreover, I shall present results on the same subject in an animal system, the mouse.

Man

Erythrocytes

Fetus to mother. The occurrence of fetal erythrocytes in maternal blood during pregnancy and after delivery has been studied by the acid elution technique of Kleihauer *et al* (1957) and by immunofluorescence (Cohen *et al*, 1964). Both methods are reliable, and allow recognition of a low proportion of fetal cells among the maternal cells. The method of Kleihauer is based on the fact that cells containing Hb A (the haemoglobin of adults) become ghosted and unstainable in acid buffer, while cells containing Hb F (fetal haemoglobin) are resistant to this treatment and remain strongly stainable (Fig. 1). The use of immunofluorescence in this connection is based on the incubation of maternal cells with fluorescein-conjugated antisera directed against the ABO antigens of the fetus. Fetal erythrocytes

FIG. 1. Blood smear from a pregnant women showing one Hb F positive cell.

have been found in the maternal blood even during the first trimester. However, the proportion is usually less than 1 fetal cell per 50 000 maternal cells. Different authors have found somewhat different frequencies of mothers with fetal red cells in their blood at this stage of pregnancy (Clayton *et al*, 1964; Cohen *et al*, 1964; Betke and Nierhaus, 1968; Bartsch, 1972), but the values generally seem to be below 10%. During the second and third trimesters there is a rise in the frequency, since about 20–40% of mothers are positive immediately before delivery (Table I). At this stage more than 1 fetal cell per 50 000 maternal cells is found in 5–10% of the cases. The presence of fetal erythrocytes in the maternal circulation seems to be related to the blood groups of mother and fetus, since in ABO-compatible pregnancies fetal cells are detected twice as often as in ABO-incompatible ones (Clayton *et al*, 1964; Cohen *et al*, 1964; Woodrow and Finn, 1966; Cohen and Zuelzer, 1967; Betke and Nierhaus, 1968; Bartsch, 1972). Immediately after delivery fetal erythrocytes are found in 20–50% of all women (Table I). The difference in the frequency of mothers with fetal erythrocytes between ABO-compatible and ABO-incompatible mother/child pairs persists after delivery (Clayton *et al*, 1964; Cohen *et al*, 1964; Cohen and Zuelzer, 1967). Fetal erythrocytes are also found more often in the

TABLE I
OCCURRENCE OF FETAL ERYTHROCYTES IN THE
MATERNAL CIRCULATION

Reference	% of Mothers with Fetal Erythrocytes in their Blood	
	During Pregnancy	At Term or after Delivery
Zipursky <i>et al</i> (1959)	—	21.0
Wimhöfer <i>et al</i> (1962)	—	15.4
Clayton <i>et al</i> (1964)	15.0	47.0
Cohen <i>et al</i> (1964)	17.0	50.0
Woodrow and Finn (1966)	3.5	19.8
Cohen and Zuelzer (1967)	—	47.0
Betke and Nierhaus (1968)	—	19.0
Bartsch (1972)	13.6	40.6

mother's blood in Rh-compatible than in Rh-incompatible pregnancies (Cohen and Zuelzer, 1967; Betke and Nierhaus, 1968).

Erythrocytes have a life span of about 100–200 days. Where fetal erythrocytes are present in the maternal blood at delivery, and the blood groups of mother and child are incompatible, these cells are rapidly eliminated from the maternal blood (Cohen and Zuelzer, 1967). Where such incompatibility exists, agglutinated fetal erythrocytes have been found in the maternal blood after delivery (Zilliacus, 1963). In homospecific pregnancies (Fig. 2), on the other hand, fetal erythrocytes that have entered

FIG. 2. Survival of fetal erythrocytes in the maternal blood after delivery in four homospecific pregnancies (Cohen *et al*, 1964).

the maternal circulation seem to show a survival time that is in accordance with the normal life span of RBC (Cohen *et al*, 1964).

In complicated pregnancies, eg, toxæmia, vaginal bleeding, etc, the proportion of fetal red cells in the maternal blood is considerably higher than in uncomplicated ones (Bartsch, 1972). Higher proportions are also found after abortions, amniocentesis, and Caesarean sections (Wimhöfer *et al*, 1962; Bartsch, 1972).

Thus fetal erythrocytes occur not infrequently in the maternal circulation during pregnancy. In most cases, however, the number of these cells is less than 1 per 50 000 maternal cells. The frequency of mothers with fetal erythrocytes and the proportion of such cells rise somewhat at delivery, and the survival time of fetal RBC in the maternal blood seems to be closely related to the ABO and Rh compatibility between mother and fetus. In some homospecific pregnancies fetal erythrocytes can still be found in the maternal blood up to 3 months after delivery.

Mother to fetus. All studies indicate (Table II) passage of maternal erythrocytes into the circulation of the fetus in some normal pregnancies, although the frequency is higher in complicated ones (toxæmia, Caesarean section, etc). Data on the subject are not consistent. The results of Naeslund (1950/1951), using ^{32}P , indicated such passage in 5% of all pregnancies, while his results with ^{59}Fe (1951) gave positive results in over 50% of cases. Cohen and Zuelzer (1965) found maternal cells in only 2.8% of infants when heel blood was studied, but in more than 10% in umbilical cord samples. The results of Mengert *et al* (1955), Fischer and Müller (1967), and Eimer and Weiland (1969) indicate passage in

TABLE II
OCCURRENCE OF MATERNAL ERYTHROCYTES IN THE BLOOD OF THE FETUS

Reference	% of Children with Maternal Erythrocytes
Hedenstedt and Naeslund (1946)	66
Naeslund and Nylin (1946)	16
Naeslund (1950/1951)	60
Mengert <i>et al</i> (1956)	5
Macris <i>et al</i> (1958)	80
Zarou <i>et al</i> (1964)	12
Cohen and Zuelzer (1967)	19
Fischer and Müller (1965)	2.8
von Muralt (1967)	58
Eimer and Weiland (1969)	10
	66

more than 50% of cases. In other studies frequencies of between 10 and 66% were found (Hedenstedt and Naeslund, 1946; Naeslund and Nylin, 1946; Macris *et al*, 1958; Zarou *et al*, 1964; von Muralt, 1967). The wide range of frequencies in the studies cited above may be due to the differences in sensitivity of the methods used or to various pitfalls involved, the criteria for choosing the series or the source of fetal blood. The amount of maternal blood found in the infant forms 1–10% of the total fetal blood volume (Macris *et al*, 1958; Cohen and Zuelzer, 1965; Fischer and Müller, 1967; von Muralt, 1967). Maternal erythrocytes seem to occur in the blood of more than 10% of all infants without giving any clinical symptoms. When there has been copious bleeding from mother to fetus the infant often shows raised haemoglobin and haematocrit values, with decreased Hb F but raised Hb A values, cyanosis, and dyspnoea (von Muralt, 1967). After the elimination of the maternal cells, however, the symptoms subside (von Muralt, 1967).

The maternal red cells have been reported to disappear rapidly from the blood of the infant. With agglutination tests, Eimer and Weiland (1969) failed to detect maternal RBC in the blood of newborns 40 hours after delivery. Von Muralt (1967) found maternal cells 2–3 days after delivery, but none when the test was repeated 3–6 months later.

Leucocytes

Fetus to mother. After 10 years of intensive research the question of fetal erythrocytes in the maternal blood is fairly well documented, while the question whether fetal leucocytes are present in the maternal circulation is still somewhat controversial (Jacobs and Smith, 1969; Walknowska *et al*, 1969; de Grouchy and Trébuchet, 1971; Schröder and de la Chapelle, 1972).

Using chromosome studies, Walknowska *et al* (1969) were able to show that cells with a male

karyotype may be present in the circulation of mothers carrying a male fetus. These cells have been interpreted as fetal lymphocytes that have entered the maternal blood after passage through the placenta, since they have responded to PHA (phytohaemagglutinin) stimulation in 3-day cultures and entered mitosis. Walknowska *et al* (1969) found XY mitoses in blood cultures from 21 out of 30 pregnant women; 19 boys and 2 girls were born (Table III). Of the nine cases in which no male cells were found, six girls and three boys were born. The proportion of fetal mitoses in the 19 cases was between 0.2–1.5% of the mothers' mitoses, with a mean value of 0.5% (Table III, Fig. 3).

Since these values are much higher than those reported for fetal erythrocytes in the maternal circulation, Walknowska *et al* (1969) suggest that they do not reflect the situation *in vivo*. They suggested that preferential stimulation of fetal cells in the blood of mothers with PHA was responsible.

Similar values have been reported by de Grouchy and Trébuchet (1971), who studied the occurrence

of fetal lymphocytes in the maternal blood by the same method as Walknowska *et al* (1969). Of 21 pregnant mothers, 12 had XY mitoses in their blood cultures (Table III). Of these, eight delivered boys and four girls. The remaining nine mothers had no XY mitoses in blood cultures made during pregnancy, and six of them gave birth to girls (Table III). The proportion (0.6%) of fetal mitoses found by de Grouchy and Trébuchet (1971) in maternal blood cultured during pregnancy is in good accord with the figures of Walknowska *et al* (1969) (Fig. 3). Occasional false positive cases were found by both teams and were attributed to earlier pregnancies of male fetuses, with persistence of fetal leucocytes in the maternal blood until the next pregnancy.

Since both studies (Walknowska *et al*, 1969; de Grouchy and Trébuchet, 1971) are based on the finding of a single or a few XY mitosis among hundreds of maternal mitoses, the reliability of the method may be questioned. The criteria of a Y chromosome vary from one laboratory to another, and for adequate identification the morphology of the cell studied has to be clear. Jacobs and Smith (1969) have shown that occasional XY mitoses can be found in the blood even in newborn girls. Furthermore the method is very time-consuming, and only fetal cells capable of responding to PHA by mitotic division can be recognized.

The distal part of the Y chromosome shows bright fluorescence after QM staining in metaphase (Fig. 4) and interphase (Fig. 5) (Caspersson *et al*, 1970; Pearson *et al*, 1970). Male WBC can easily be distinguished from female WBC at both metaphase and interphase without time-consuming chromosome analysis. This method has been used successfully in clinical diagnoses (Caspersson *et al*, 1970; Polani and Mutton, 1971; Forsius *et al*, 1972).

The reliability of the method for detecting a low proportion of male interphase cells among female interphase cells was tested by making artificial mixtures of male and female lymphocytes (Schröder and de la Chapelle, 1972). The proportion of male WBC was 100, 10, 1, and 0%, and the slides were analysed blindly. The results (Table IV) indicate that the method can be used with confidence, provided very stringent criteria are set for a cell to be regarded as Y-positive.

The frequency of lymphocytes containing a Y-body (the Y chromosome at interphase) was studied in 25 cases between the fourth and ninth months of pregnancy. False-positive cases of the type proposed by Walknowska *et al* (1969) and de Grouchy and Trébuchet (1971) were excluded by choosing

TABLE III
NUMBER OF XY MITOSES IN THE CIRCULATION
OF PREGNANT WOMEN

Reference	No. of Women	Sex of Child	No. of Cells Studied	
			Total	No. of XY Cells
Walknowska <i>et al</i> (1969)	19	Male	7399	32 (0.5%)
	3		1771	0
	6	Female	2945	0
	2		1095	2 (0.3%)
De Grouchy and Trébuchet (1971)	8	Male	3766	21 (0.6%)
	3		2409	0
	6	Female	4002	0
	4		2204	5 (0.3%)

FIG. 3. Prevalence of XY mitoses in lymphocyte cultures of maternal blood at various stages of pregnancy.

FIG. 4. A mitosis stained with QM from a blood culture of a mole with a large Y chromosome (arrow).

FIG. 5. A PHA-stimulated lymphocyte of a male showing a Y-body (arrow) after QM staining.

TABLE IV

PREVALENCE OF INTERPHASE NUCLEI WITH A Y-BODY IN ARTIFICIAL MIXTURES OF PHA-STIMULATED LYMPHOCYTES AND UNSTIMULATED LYMPHOCYTES FROM A NORMAL MALE AND A NORMAL FEMALE
(Schröder and de la Chapelle, 1972)

Proportion of Male Cells in Mixture (%)	No. of Cells Studied	Stimulated Lymphocytes		Unstimulated Lymphocytes	
		No. of Cells with Y-body	%	No. of Cells with Y-body	%
100	1000	351	35	410	41
10	1000	32	3.2	37	3.7
1	1000	4	0.4	3	0.3
0	1000	0	0	0	0

TABLE V

PRESUMPTIVE Y-BODIES IN PHA-STIMULATED LYMPHOCYTES FROM 25 WOMEN IN THE 4th-9th MONTH OF PREGNANCY
(Schröder and de la Chapelle, 1972)

No. of Women	Sex of Child	No. of Cells Studied	
		Total	With Presumptive Y-body
9	Male	49 500	45 (0.09%)
2		10 000	0
9	Female	46 600	0
5		20 500	22 (0.11%)

TABLE VI

POSSIBLE REASONS FOR APPARENT DISCREPANCIES BETWEEN MATERNAL LYMPHOCYTE Y-BODY FINDINGS AND SEX OF CHILD

Presumptive Y-bodies (female child)	Misinterpretation due to exceptionally bright autosomal fluorescence in mother or child Previous abortion of male fetus
No Y-bodies (male child)	Short or non-fluorescent Y Absence or very low incidence of fetal cells in maternal blood

FIG. 6. A metaphase from a female showing brilliant fluorescence in the short arm of one chromosome No. 13 (arrow).

FIG. 7. An interphase cell from the female of Figure 6. Brilliantly fluorescent autosomal spot indicated by arrow.

TABLE VII

OCCURRENCE OF Y-BODY CONTAINING CELLS IN 86 MOTHERS BETWEEN 14th AND 18th WEEK OF PREGNANCY FROM 5- TO 6-DAY LYMPHOCYTE CULTURES

(Grosset *et al*, 1974)

No. of Women	Sex of Child	Y-body Containing Cells
42	Male	Present
3		Absent
9	Female	Present
32		Absent

only primiparae or women who had earlier given birth to a girl. Of 11 mothers who gave birth to a boy, nine had Y-body containing cells in their blood during pregnancy; a mean proportion of 0.09% (Table V). In one mother Y-positive cells were found as early as the fourth month of pregnancy. However, false-positive cases seem to occur. In five out of 14 pregnancies where the sex of the fetus was female, the mothers had 'Y-body' positive cells in their blood (Table V). Retrospective studies suggested reasons for these discrepancies (Table VI).

The most common reason for misinterpretation was brilliant fluorescence of one of the maternal autosomes (Fig. 6), which often looked like a Y-body at interphase (Fig. 7). Grosset *et al* (1974) using an almost identical method have obtained similar results (Table VII).

Since the Y chromosome can be seen as a Y-body in about 30–50% of the interphase cells (Schröder and de la Chapelle, 1972), the values cited above for fetal lymphocytes in the maternal blood during pregnancy are in good accord with those obtained in mitotic studies (Walknowska *et al*, 1969; de Grouchy and Trébuchet, 1971). Zimmerman and Schmickel (1971) have tried to use the brilliant

fluorescence of the Y chromosome at interphase to detect male WBC in the circulation of women during pregnancy, but without success. Polani and Mutton (1971) have shown Y-positive cells in the blood of non-pregnant women. The numbers given by these authors indicate that 1–5% of the interphase WBC of normal women contain a Y-body-like structure during interphase. These values are much higher than we have found in pregnant women (Schröder and de la Chapelle, 1972), and our impression is that Y-body-like structures are very rare in interphase cells of normal women, being found only in women with an autosome that fluoresces brilliantly. The criterion for a Y-body seems to vary between different laboratories. The Y-body can be used as a criterion for a fetal WBC in the maternal circulation if very stringent criteria are adopted for its identification (Fig. 8), and if all mothers with brilliant autosomal fluorescence are excluded. This is also indicated by our later data (Schröder *et al*, 1974).

FIG. 8. An unstimulated lymphocyte from the circulation of a mother immediately after delivery of a boy. The lymphocyte contains a Y-body (arrow).

In a study where the frequency of fetal WBC in the maternal blood was evaluated after delivery, a coded experiment was performed where all mothers with brilliant autosomal fluorescence were excluded to avoid false positive results. Altogether 240 000 cells were studied from 24 mothers, the observer being unaware of the sex of the child, and only one false-positive cell was found (Table VIII).

The different fetal WBC types that occur in the blood of mothers have been evaluated by examining unstimulated leucocytes from 46 primiparae immediately after delivery of a boy (Schröder *et al*, 1974). Fetal lymphocytes and granulocytes could be found in 30% of the mothers, lymphocytes only in 37%, granulocytes only in 7%, and no fetal WBC at all in 26% (Table IX). The proportion of fetal cells varied between 0.01 and 0.2%.

TABLE VIII
PREVALENCE OF Y-BODY CONTAINING CELLS IN
MATERNAL BLOOD AT DELIVERY
(Schröder *et al*, 1974)

Code No. of Mother	Sex of Child	No. of Y-body Containing Cells per 5000 Cells Examined	
		Lymphocytes	Granulocytes
1	Male	4	3
2	Male	4	1
3	Male	2	1
4	Male	0	2
5	Male	0	1
6	Male	3	0
7	Male	4	0
8	Male	3	0
9	Male	1	0
10	Male	1	0
11	Male	1	0
12	Male	1	0
13	Male	1	0
14	Female	1	0
15–19 (five cases)	Male	0	0
20–24 (five cases)	Female	0	0

10 000 leucocytes were studied from 24 mothers after delivery.
The observer was unaware of the sex of the child.

TABLE IX
FETAL LEUCOCYTES IN THE BLOOD OF 46 WOMEN
AFTER THE BIRTH OF A MALE CHILD
(Schröder *et al*, 1974)

Y-bodies in Interphase Cells	No. of Women (n = 46)
Lymphocytes and granulocytes	14
Lymphocytes only	17
Granulocytes only	3
No Y-bodies	12

Fetal cells capable of responding to PHA and undergoing mitotic division seem, in at least some cases, to be present in maternal blood after delivery, since in three cases studied one unequivocal XY mitosis was found (Schröder *et al*, 1974). However, this study was very preliminary and does not give a true picture of the prevalence of the phenomenon.

Twenty mothers were studied at different intervals after delivery and the results indicate that fetal granulocytes disappear from the maternal circulation within 1 week after delivery, while fetal lymphocytes can be found up to 1 year post partum (Fig. 9) (Schröder *et al*, 1974).

About 22% of all mothers produce HL-A antibodies against the paternally derived fetal HL-A antigens (Schröder *et al*, 1974). A correlation exists between the presence of HL-A antibodies in the maternal serum, and the absence of fetal cells in the maternal blood (Schröder *et al*, 1974). HL-A typings in the parents, and from the umbilical cords of the infants (Tiilikainen *et al*, 1974) have given the following results. The maternally derived fetal HL-A antigens could generally be typed without

FIG. 9. Prevalence of Y-body-containing cells in the circulation of women at various intervals after birth of a male infant. The figures above the circles and squares indicate the numbers of mothers tested. (Schröder *et al* 1974)

difficulty from cord blood lymphocytes, while these cells often failed to demonstrate paternally derived HL-A antigens. These could generally be demonstrated on a later occasion after delivery, or after incubation of the cells under tissue culture conditions (Tiilikainen *et al*, 1974). It is possible that this 'masking' of the paternally derived fetal HL-A antigens is of importance in maintaining the immunological tolerance between mother and fetus.

It can be concluded that passage of leucocytes from fetus to mother is a common phenomenon, occurring in most pregnancies. The results obtained by independent methods are in good accord with each other (Walknowska *et al*, 1969; de Grouchy and Trébuchet, 1971; Schröder and de la Chapelle, 1972; Schröder *et al*, 1974). A correlation exists between the absence of fetal cells in the maternal blood, and the presence of HL-A antibodies in the maternal serum. A 'masking' of paternally derived fetal HL-A antigens may be of importance in the maintenance of tolerance between fetus and mother.

Mother to fetus. The question of whether maternal leucocytes reach the circulation of the fetus is still controversial. Desai and Creger (1963) were able to find fluorescent cells in the circulation of six fetuses after injecting quinacrine-labelled leucocytes into the circulation of nine pregnant women immediately before delivery. The maternal cell types found were platelets, granulocytes, and lymphocytes. Lymphocytes could be demonstrated in three of the cases. Olding (1972) studied mitoses

in the cord blood of 14 male infants after PHA stimulation, and found no maternal cells. XX/XY chimaerism in the lymphopoietic tissues of newborn boys has been reported by others (Kadowaki *et al*, 1965; Taylor and Polani, 1965; Turner *et al*, 1966; Benirschke and Sullivan, 1969), but generally in connection with severe immunological disease.

These data suggest that no maternal mitoses are present in PHA cultures from cord blood in normal pregnancies, their presence being an exceptional occurrence connected with developmental malformations of the fetus. As chromosome studies are very time-consuming, however, rather few cells have been examined.

By using quinacrine mustard fluorescence, and mother-and-son combinations where one or more of the mother's chromosomes had a brilliantly fluorescent region not present in the child's, Schröder (1974) studied large numbers of mitoses from PHA cultures of cord blood of nine newborn boys and one girl. In all cases the infants were delivered after normal uncomplicated deliveries, and in one case 0.07% of maternal mitoses were found, four out of 5853 mitoses (Table X).

TABLE X
CORD BLOOD MITOSES FROM 10 INFANTS AFTER
PHA STIMULATION
(Schröder, 1974)

Code No.	No. of Mitoses Studied	No. of Maternal Cells
1-9	21 845	0
10	5853	4
Total	27 698	4 (0.01%)

Mitotic cells were studied after QM staining from the umbilical cords of 10 infants, to ascertain whether maternal cells had passed through the placenta. Nine of the infants were boys, and the Y chromosome and fluorescent autosomal markers were used to differentiate between fetal and maternal cells.

Maternal WBC have frequently been observed in the blood of fetuses in normal pregnancies (Desai and Creger, 1963). These cells seldom seem able to undergo mitosis in PHA cultures (Kadowaki *et al*, 1965; Taylor and Polani, 1965; Turner *et al*, 1966; Benirschke and Sullivan, 1969; Olding, 1972). If, however, a large number of cells are examined by adequate methods maternal mitoses can be found in at least some cases (Schröder, 1974).

Trophoblasts. In light of the above data it is evident that a transplacental passage of blood cells is a common, or even normal physiological event in man. What is still unanswered is why these cells so seldom cause an immune response. Actually, it is even more surprising that trophoblasts—fetal

cells in the placental villi which are in immediate contact with the maternal circulation—are tolerated. The explanation was advanced that trophoblastic villi are deficient in blood group antigens, and this has been verified (Thiede *et al*, 1965; Gross, 1966). After enzyme digestion, however, transplantation antigens have been possible to demonstrate in trophoblasts (Currie *et al*, 1968), so the 'absence' of antigens is probably a consequence of suppression by the mucoprotein layer coating these cells.

Trophoblasts have been found in the arteries of the uterus during pregnancy and after delivery (Beer and Billingham, 1971). In normal pregnancies there seems to be a chronic shedding of multicellular fragments or sprouts of syncytiotrophoblasts from placental villi into the maternal blood stream at a rate of 100 000 cells per day from the second month of pregnancy onward (Iklé, 1961). The majority of these cells are probably destroyed by enzymes in the blood stream, but some survivors are filtered out in the capillary bed of the lungs, where they gradually disappear.

Passage of trophoblasts in the reverse direction has also been reported. Iklé (1961) demonstrated trophoblastic cells in the umbilical veins of fetuses at various stages of pregnancy, and Salvaggio *et al* (1960) found them in cord blood after delivery.

Despite speculation the question of what significance such passage could have is still unanswered.

Mouse

Fetus to mother. No reports exist on the occurrence of fetal erythrocytes in the maternal circulation in the mouse. This is probably due to lack of suitable markers by which the phenomenon can be studied.

Whether fetal lymphocytes enter the maternal circulation of the mouse is totally unknown. However, a study has been initiated to answer this question. In matings between the laboratory mouse (*Mus musculus*), which has 40 acrocentric chromosomes, and the tobacco mouse (*Mus posciavinus*), which has 26 chromosomes, many of them are metacentric (Gropp *et al*, 1970) fetal mitoses are identifiable in lymphocyte cultures of maternal blood. The results, which are still tentative, indicate that such a passage of lymphocytes is very unusual, or does not occur at all (Schröder and Andersson, 1975). However, the method has the limitation that only fetal cells that have responded to PHA-stimulation by mitosis can be identified.

Mother to fetus. Since the methods used to study the occurrence of maternal cells in the haemopoietic tissues of the offspring are not always

sensitive enough to distinguish between erythrocytes and leucocytes, I shall consider both under the same heading.

The results presented in the literature are somewhat confusing. Finegold and Michie (1961) hold the view that maternal cells can seldom be found in the blood of the offspring. They injected erythrocytes labelled with radioactive chromium into the circulation of pregnant female mice, and found labelled cells in only two out of 279 fetuses studied. Barnes and Tuffrey (1970) also used chromium labelled erythrocytes, and the fetal blood smears were studied by autoradiography. However, their figures differ considerably from those of Finegold and Michie (1961). Maternal erythrocytes were found in the majority of the cases after both normal deliveries and Caesarean sections.

Tuffrey *et al* (1969a) reported a high frequency of maternal cells in the haemopoietic tissues of 4- to 60-day-old mice that had developed in the uterus of foster mothers after transplantation at the blastocyst stage. A chromosome marker (T_6) was used to differentiate between maternal and fetal mitoses. Similar observations were later reported by the same authors (Tuffrey *et al*, 1969b) after normal pregnancies, in which they used matings between CFW and CBA T_6T_6 mice. However, Billington *et al* (1969) were unable to confirm these results, and suggested that the findings of Tuffrey *et al* (1969a; 1969b) might be due to misinterpretation of the T_6 marker.

In a later study, Barnes and Tuffrey (1970), using autoradiography, have found maternal lymphocytes in the circulation of newborn mice. However, Sellar (1970), working with mice with macrocytic anaemia and using the T_6 marker, was unable to find any evidence of a traffic of blood cells from mother to fetus.

As the studies mentioned above indicate, it is still uncertain whether passage of cells occurs between mother and fetus in mice. The conflicting reports are probably due to pitfalls in the methods used.

When a small number of cells has to be recognized among a large number of other cells, the T_6 marker method and radioactive labelling of cells do not seem to be sensitive enough.

Using matings between the laboratory mouse and tobacco mouse we have also looked for maternal cells in the haemopoietic tissues of the offspring (Schröder and Andersson, 1975). So far we have found no sign of large-scale passage of blood cells from mother to fetus.

DISCUSSION

Correlation between erythrocyte and leucocyte data

Fetus to mother. The data concerning fetal erythrocytes and leucocytes in the maternal circulation during pregnancy and after delivery have been considered separately in the previous sections. All the results indicate that both RBC and WBC of the fetus are present in the maternal circulation at these times. Fetal erythrocytes are supposed to pass the placenta as a result of a bleeding (Zilliacus, 1963; Clayton *et al*, 1964; Cohen *et al*, 1964; Cohen and Zuelzer, 1967; Betke and Nierhaus, 1968; Bartsch, 1972), since they are not motile. The fact that fetal erythrocytes are found more frequently in the mother's blood when the fetus or placenta has been subject to mechanical violence also supports this theory (Wimhöfer *et al*, 1962; Bartsch, 1972). On the other hand, it is well known that WBC are capable of active movement. Hence it has been suggested that fetal lymphocytes cross the placental barriers in most, if not all, pregnancies (Walknowska *et al*, 1969; de Grouchy and Trébuchet, 1971; Schröder and de la Chapelle, 1972; Grosset *et al*, 1974).

Light might be shed on these questions by counting both white and red fetal blood cells in the circulation in a large series of mothers at different stages of pregnancy. Such a study is under way at the Folkhälsan Institute of Genetics, Helsinki. Until such counts are available, however, we may try to relate the erythrocyte and lymphocyte findings from data in the literature.

Time of occurrence, frequency, and proportion. Fetal erythrocytes have been demonstrated in the maternal circulation even during the first trimester (Clayton *et al*, 1964; Cohen *et al*, 1964; Betke and Nierhaus, 1968; Bartsch, 1972). However, they have generally been found in less than 1 fetal cell per 50 000 maternal cells, and then only in about 10% of women. Fetal lymphocytes have been found in the mother's blood during the first trimester in almost all cases studied at this stage (Tables III, VII, Walknowska *et al*, 1969; de Grouchy and Trébuchet, 1971; Grosset *et al*, 1974). The ratio of fetal to maternal cells is about 1/500. During the second and third trimesters fetal erythrocytes are found in 30–40% of mothers, but still in a low proportion, mostly less than 1 fetal cell/50 000 maternal cells. The figures for fetal lymphocytes in the maternal circulation during the second and third trimesters are of magnitude 1 fetal cell/1000 maternal cells or higher, and fetal cells are found in more than 80% of cases (Walknowska *et al*, 1969; de Grouchy and

Trébuchet, 1971; Schröder and de la Chapelle, 1972).

If, in contrast to what is indicated by experimental data so far fetal erythrocytes and lymphocytes are assumed to pass into the maternal circulation in normal relative proportions, two theoretical explanations would be possible; (1) fetal lymphocytes are tolerated better than fetal erythrocytes in the mother's blood or (2) the frequency of fetal mitoses in cultures of the mother's blood does not reflect the frequency of fetal WBC in the maternal circulation.

(1) Fetal erythrocytes occur twice as frequently in the maternal blood in blood-group-compatible pregnancies than in blood-group-incompatible ones (see above). In blood-group-incompatible pregnancies fetal erythrocytes seem to be eliminated rapidly, but in homospecific pregnancies the survival values of the fetal red cells in the mother's blood are in good accord with the normal life span of the cells. In about 95% of all primigravidae the HL-A antigens of the fetus are different from those of the mother, and in 60% of these cases at least two different HL-A antigens are involved (Tiilikainen *et al*, 1974). Since at least 70% of the mothers have fetal leucocytes in their blood at the same time, it is surprising that only 22% of them produce HL-A antibodies (Schröder *et al*, 1974). On the other hand, a negative correlation exists between the occurrence of HL-A antibodies directed against fetal HL-A antigens in the mother's serum, and the proportion of fetal leucocytes in the maternal blood (Schröder *et al*, 1974). This correlation can be shown immediately after delivery, but is even stronger later.

Fetal lymphocytes are probably better tolerated in the maternal blood than fetal erythrocytes. This may to some extent be due to a masking of paternally derived fetal HL-A antigens during pregnancy (Tiilikainen *et al*, 1974).

A greater tolerance is not in itself enough to explain the great difference in the relative proportion of fetal erythrocytes and lymphocytes in the maternal circulation during pregnancy and after delivery.

(2) Walknowska *et al* (1969) suggested that the high rates of XY mitoses in maternal blood cultures did not reflect the proportion of fetal WBC *in vivo*. Preferential stimulation of fetal cells by PHA in cultures of maternal blood would exaggerate the proportion of fetal mitoses. This would also explain the large numbers of fetal mitoses reported by de Grouchy and Trébuchet (1971), and the high proportion of Y-body containing cells in the mother's blood in 5- to 6-day PHA cultures found by Grosset *et al* (1974). On the other hand, when testing the theory of preferential stimulation of fetal

cells, Schröder and de la Chapelle (1972) and Schröder *et al* (1974) have found cultured and uncultured interphase cells in roughly similar numbers.

Validity of mitotic findings. Walknowska *et al* (1969) and de Grouchy and Trébuchet (1971) used conventional methods of chromosome staining. The use of such methods for this purpose has been criticized (Jacobs and Smith, 1969). Although methodological difficulties may possibly lead to misinterpretations all data indicate that fetal leucocytes do occur in the maternal circulation during pregnancy, in much higher relative proportions than fetal erythrocytes.

Mother to fetus. Very different values have been given for the occurrence of maternal erythrocytes in the blood of their newborn infants (Naeslund and Nylin, 1946; Macris *et al*, 1958; Cohen and Zuelzer, 1965). However, reports of high proportions of maternal erythrocytes have been based on counts of cells labelled with radioactive isotopes (Naeslund and Nylin, 1946; Naeslund, 1950/1951; Mengert *et al*, 1955; Zarou *et al*, 1964). These studies are open to criticism because free isotope molecules may pass through the placenta and be taken up by fetal cells. If all but the studies based on agglutination tests, immunofluorescence, or the use of marker cells (sickle cells, elliptocytes) are disregarded, maternal cells have been found in 10–80% of all newborns (Hedensted and Naeslund, 1946; Mengert *et al*, 1955; Macris *et al*, 1958; Cohen and Zuelzer, 1965; Fischer and Müller, 1967; von Mural, 1967; Eimer and Weiland, 1969). Even in normal pregnancies maternal leucocytes occur in the blood of newborns (Desai and Creger, 1963; Schröder, 1974), but they have generally been reported in connection with malformations of the fetus and placenta (Kadowaki *et al*, 1965; Taylor and Polani, 1965; Turner *et al*, 1966; Benirschke and Sullivan, 1969). This might be due to the fact that most teams have studied only cells from PHA cultures. Since only a proportion of the leucocytes (T lymphocytes) respond to PHA by entering mitosis, these results give a very limited picture of the situation *in vivo*. With Atebrin-labelled leucocytes Desai and Creger (1963) were able to show passage of maternal cells through the placenta in the majority of their cases. A further factor limiting chromosome studies is their time-consuming nature, for which reason only small numbers of cells have been counted (Turner *et al*, 1966; Benirschke and Sullivan, 1969; Olding, 1972).

If a correlation is sought between the data for erythrocytes and lymphocytes the facts mentioned

above have to be taken into consideration. In the light of these one cannot exclude the possibility that lymphocytes and erythrocytes pass from mother to fetus to the same extent, and in the same normal relative proportions.

Biological importance

What is the biological importance of this passage of blood cells between mother and fetus? Is it a pathological phenomenon followed by antibody formation and immunological disease, or is it of importance in the immunological tolerance between mother and fetus? Or is the occurrence of these cells in the mother's or infant's blood merely a secondary consequence of immunological tolerance? These questions cannot be fully answered at the moment, but if the phenomenon were of basic importance for the tolerance between mother and fetus, one would expect it to be wide-spread among other mammals.

The passage of blood cells has been studied in detail in the mouse (Finegold and Michie, 1961; Billington *et al*, 1969; Tuffrey *et al*, 1969a; 1969b; Barnes and Tuffrey, 1970; Seller, 1970), but the question is so far unanswered. However, these studies involved the use of cytological markers. In the rat Beer *et al* (1971; 1972) and Beer and Billingham (1971, 1973) have been able to induce tolerance or runt disease in the offspring by giving living cellular homografts to females 1–2 weeks before mating. This they have taken to reflect a transmission of cells from mother to fetus. Here, however, the constellation has been a pathologic one, and caution is called for in drawing parallels with normal pregnancies.

In conclusion, the passage of blood cells between mother and fetus is understandable in many pathological conditions, but the importance of the phenomenon in normal pregnancies is not yet clear.

Practical implications

Since the recognition that the passage of erythrocytes from Rh⁺ fetuses to Rh⁻ mothers is of importance in Rh immunization, programmes have been developed in several countries to avoid Rh toxæmia (Clarke, 1967; Bartsch, 1972; Eklund and Nevanlinna, 1973).

How can the occurrence of fetal lymphocytes in the maternal circulation be turned to useful purposes by the clinician? A very tempting thought would be to use fetal cells in the maternal blood for antenatal diagnosis instead of amniocentesis. So far the method has not proved sensitive enough for the purpose (Schröder and de la Chapelle, 1972).

The main difficulty lies in the low frequency of fetal lymphocytes in the mother's blood and the persistence of fetal lymphocytes from earlier pregnancies. A possible method to enrich fetal cells in blood samples from the mother would perhaps be to destroy the maternal cells by HL-A antibodies directed against maternal, but not fetal HL-A antigens (Tiilikainen *et al.*, 1970). Another method which seems possible on theoretical grounds at least, is to use a cell separator of the type described by Hulett *et al.* (1973). With this device different lymphocyte populations have been separated successfully from each other.

Although some data already exist on the passage of blood cells between mother and fetus, many of the most important questions are still unanswered. From a combination of cytological and immunological methods, it seems realistic to expect the answer to most of these questions in the near future.

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