TRANSPLACENTAL BLEEDING FROM THE FOETUS*

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The suggestion that the foetus can become severely anaemic from haemorrhage into the maternal circulation was first made by Wiener (1948). A case reported by Wickster (1952) was almost certainly due to such a haemorrhage. However, neither Wiener nor Wickster made confirmatory laboratory tests, and it was Chown (1954, 1955) who first proved, both serologically and by showing excess of alkali-resistant haemoglobin, the presence of foetal cells in the blood of a woman just delivered of an anaemic child. A case in which anaemia was slight, but serological evidence strong, was reported by Dunsford (1957). The presence in the maternal blood of an excess of alkali-resistant haemoglobin without serological tests has been interpreted as an indication of transplacental bleeding by Colebatch, Pitt, and Maddison (1956) and by O'Connor, Shields, Kohl, and Sussman (1957).

Two cases are described here of neonatal anaemia in which foetal cells were demonstrated in the maternal blood by differential agglutination and other tests. The first infant had severe haemolytic disease; the second severe anaemia which appeared to be due entirely to transplacental bleeding.

Case Reports

Case 1.—Mrs. C. M., aged 30, was delivered at 4.50 p.m. on February 28, 1955, in the 37th week of her pregnancy, of a pale, shocked, hydropic female infant. Shortly thereafter she had a rigor. Her first child, born in 1947, was normal; the second (1952) died at the age of 2 days from haemolytic disease; the third (1953) was stillborn and hydropic. She had felt well after delivery of the first child, but with the second and third, as with the present fourth, she had a rigor after delivery. The present pregnancy had been normal apart from the continued presence in the

serum of rhesus antibodies (anti-D). At the 36th week the anti-D titre was 1 in 8 in saline and 1 in 256 in albumin. She was group B, Rh negative (C—, D—, E—, c+). The labour was spontaneous and there was no significant external bleeding (total loss 4 oz.). The cord was ligated immediately after delivery of the child. The placenta was large and oedematous with ragged membranes.

Laboratory Investigations.—The cord blood was group B, Rh positive (D positive). A direct Coombs test was positive. Haemoglobin was 24% (3.6 g.), R.B.C.s 1.18 m., P.C.V. 15%, M.C.V. 127 cu.µ., M.C.H.C. 24%, reticulocytes 23%. Plasma showed severe icterus.

Blood films showed severe erythroblastemia (70% of all nucleated cells) and much polychromasia, and myelocytes and a few monocytic erythrophages among the white cells. Platelets were scanty. The diagnosis was severe haemolytic disease.

The mother's blood on the day after delivery showed Hb 85% (12.6 g. per 100 ml.), P.C.V. 38%, and M.C.H.C. 33%. A film was within normal limits.

The fact of the rigor after delivery suggested that blood from the foetus had leaked into the maternal circulation and caused a minor transfusion reaction (Goodall, 1955, 1957). Therefore the buffy coat was examined for evidence of ingested red cells, films being made from a freshly spun sample and from one incubated at 37° C. for one hour. Erythrophagocytosis was found in both, especially in the latter where there were 87 erythrophages per 10,000 potential phagocytes, an erythrophagocytic ratio of 0.87% (normal up to 0.10%, Zinkham and Diamond, 1952). Most ingested red cells were in neutrophil polymorphs (see Fig. 1), the rest in monocytes. One ingested normoblast was seen.

Erythrophagocytosis is known to occur in haemolytic anaemias (Zinkham and Diamond, 1952) and in megaloblastic anaemia of pregnancy (Goodall, 1957), but this mother was not anaemic. Thus, in view of the possibility that the ingested cells might be foetal, the following tests were made in the maternal blood.

A direct Coombs test showed, in a general background of unagglutinated cells, clumps (6–8 cells) that were accepted as sensitized cells.

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FIG. 1.—This smear from the maternal buffy coat in Case 1 shows two neutrophil polymorphs with ingested red cells; the large vacuole in another cell is possibly the end-result of similar phagocytosis. Leishman, $\times 800$.

On incubation with strong "saline" anti-D, small scattered clumps were present with two different anti-D sera after two hours' incubation at 37°C .

Alkali-resistant haemoglobin was estimated by the method of Singer, Chernoff, and Singer (1951). There was no increase of alkali resistance as compared with normal controls.

The first two tests indicate that sensitized Rh-positive cells were present in the blood of the Rh-negative mother. It is difficult to assess the number of these presumed foetal cells. The clumps were about the size (6 to 8 cells) which Mollison (1956) observed with agglutinable cells in a concentration of 1 in 200. Accepting Mollison's figure, it can be said (on six agglutination counts in a Neubauer chamber) that the proportion of foetal cells in the maternal blood was probably no higher than about 1 in 200. This is supported by the virtual absence of foetal haemoglobin (alkali resistance). It should be remembered, however, that this proportion was found on the day after the delivery and rigor, by which time many of the incompatible cells could have been destroyed. As for indirect methods of assessing the magnitude

of the "transfusion reaction," the plasma on the day after delivery was neither jaundiced nor tinged with haemoglobin and the urine did not contain abnormal pigments.

Progress of the Mother.—She remained well throughout the puerperium, with no fever or sign of infection, toxæmia, or jaundice. The urine was normal. On the third, fifth, and ninth days after delivery blood samples showed no significant erythrophagocytosis, negative differential agglutination tests, and virtual absence of alkali-resistant haemoglobin.

Progress of the Child.—Despite exchange transfusion jaundice increased and the child died the day after birth.

Necropsy (Dr. K. Rhaney).—This showed severe haemolytic disease with haemorrhagic manifestations, but no evidence of kernicterus. The plasma bilirubin level was 10.7 mg. per 100 ml.

Histology.—Findings were in keeping with the clinical and anatomical diagnosis. Siderosis of the spleen was largely concentrated in the capsule and trabeculae.

The appearance of the placenta was compatible with haemolytic disease. A few small groups of normoblasts (presumably foetal) were found in intervillous spaces.

Case 2.—Mrs. N. M., aged 24, gave birth to her second child, a mature female, on April 19, 1956. The first pregnancy and delivery were normal and the child was healthy. She was well during the second pregnancy, apart from an easily corrected retroversion of the uterus at 10 weeks' gestation. Labour was normal with a blood loss of 8 oz. The cord was tied after pulsation had stopped with the child slightly lower than the uterus. The infant was pale and shocked with peculiar sighing, rapid respirations suggestive of air hunger, yet no obvious haemorrhage was found. There was no evidence of jaundice, petechiae, ascites, splenomegaly, hepatomegaly, or skeletal deformity. The placenta showed no abnormality on routine examination, in particular, neither retroplacental clots nor vascular anomalies were seen. It was not kept for microscopy. The mother's blood group was O, Rh positive, and her Wassermann reaction negative.

Laboratory Investigations.—Preliminary investigation of the infant's blood (heel stab) on April 19 showed Hb 39% (5.8 g.), total nucleated cells 43,600 per c.mm. A differential count of 500 nucleated cells gave: neutrophil polymorphs, 63.8% (27,817 per

FIG. 2.—This film of the infant's blood in Case 2, taken shortly after birth, shows numerous small erythrocytes with excessive central pallor. Two tiny normoblasts are seen. Leishman, $\times 800$.

FIG. 3.—The maternal buffy coat in Case 2 contains many small, ring-stained erythrocytes similar to those seen in the infant's blood (Fig. 2). Tiny normoblasts are also present here. Leishman, $\times 800$.

c.mm.), eosinophil polymorphs 0.0%, basophil polymorphs 0.0%, neutrophil metamyelocytes 3.2% (1,395), neutrophil myelocytes 1.4% (610), monocytes 4% (1,740), lymphocytes 18.6% (8,110), normoblasts 7.8% (3,400), smears 1.2% (523).

On a film the red cells were generally smaller and more hypochromic than normal, and the normoblasts small with pyknotic nuclei and scanty, hypochromic cytoplasm (Fig. 2). Polychromasia was more obvious than in a normal infant. There was a neutrophil polymorphonuclear leucocytosis. Platelets appeared normal. A direct Coombs test was negative. Blood was group O, Rh positive. The plasma bilirubin level was 2.1 mg. per 100 ml.

Haemolytic disease due to rhesus incompatibility could be excluded by the negative direct Coombs test, and there was no ABO incompatibility; thus a haemolytic basis was unlikely. There was nothing to suggest any primary disease of the haemopoietic system. The presence of tiny, hypochromic, pyknotic normoblasts in a generally microcytic, hypochromic red cell population recalled a chronic post-haemorrhagic anaemia, while the very low haemoglobin level just after birth also suggested that bleeding had taken place some time before delivery. Nevertheless the clinical evidence of acute post-haemorrhagic shock and the neutrophil leucocytosis were in keeping with more rapid, recent haemorrhage. In sum, the evidence

pointed to a post-haemorrhagic anaemia of considerable duration with a final exsanguination at the time of delivery. Thus, since there was no sign of local bleeding or haemorrhagic tendency in the infant, no retroplacental clot and no antepartum, intrapartum, or postpartum haemorrhage from the mother, we thought that the mother's blood might be shown to contain a "transfusion" of foetal cells.

The mother's blood was examined on April 20, when Hb was 83% (12.3 g.), P.C.V. 37%, M.C.H.C. 33%, and a film of the red cells showed them to be generally normochromic, normocytic, with a small proportion of microcytes, some hypochromic, and an occasional pyknotic normoblast. The leucocytes were normal and the platelets adequate.

On buffy coat films, hypochromic normoblasts were present in large number (Fig. 3), but no erythrophagocytosis. The presence of tiny, hypochromic, pyknotic normoblasts in the blood of a woman who did not herself have hypochromic microcytic anaemia was certainly unusual. Its occurrence in a woman who had just given birth to a child with severe and as yet unexplained post-haemorrhagic anaemia and with the same type of normoblasts in its blood gave further support to the idea that these nucleated red cells must be derived from the foetus.

Serological Investigation.—Both mother and infant were group O, Rh positive (D+), and there was no evidence of iso-immunization. Nevertheless more extensive rhesus typing was undertaken in the hope of finding some difference in group that would allow differential agglutination tests on the mother's blood. Table I shows the results.

TABLE I
BLOOD GROUP DATA IN CASE 2

ABO Group	Rhesus Group				Probable Genotype
	(Anti)C	(Anti)D	(Anti)E	(Anti)c	
Baby M, O	+	+	—	+	CDe. cde (R ₁ r)
Mr. M, O	—	+	—	+	cDE. cde (R ₂ r)
Mrs. M, O	+	+	—	±*	CDe. CDe. (R ₁ R ₁)

* On August 10, 1956, and subsequently Mrs. M. was completely negative with anti-c.

The result with our anti-c serum on the mother's blood is the important one. This particular serum was known to be very avid when used against red cells suspended in albumin and in the indirect Coombs test. With both techniques the mother's blood showed scattered clumps in a background of unagglutinated cells. A sample field from the anti-c albumin test is shown in Fig. 4. The indirect Coombs test showed slightly larger agglutinates. Fig. 5 shows a simultaneous control using known CDe/CDc cells showing complete absence of agglutination. The infant's blood obtained before transfusion was similarly tested, and, as shown in Fig. 6, was almost completely agglutinated, incidentally showing that it contained no appreciable number of maternal cells. Thus, though the maternal blood was generally c-negative, it contained c-positive cells. As the foetal cells were

FIG. 4.—The maternal blood in Case 2 has been treated with anti-c (albumin method) and shows the small agglutinates which are presumably foetal cells. $\times 340$.

known to be c-positive, and the mother was not a twin and had never been transfused, it was considered that this partial agglutination of the mother's blood with anti-c was a confirmation of the presence of foetal cells in her circulation. It was shown on August 10, 1956, and subsequently that the mother was indeed c-negative and that the foetal cells had disappeared from her blood.

An attempt was made to assess the proportion of c-positive cells in the blood of the mother by comparing the results of differential agglutination on her blood and on prepared proportionate mixtures of CDe/cde and CDe/CDc cells. While it was almost certain that at least 5% of c-positive cells must have been present in the mother's blood, it was not possible to give an exact estimate by this method.

There was not at this time any evidence of iso-immunization in the mother's serum (saline, albumin, and indirect Coombs test).

Results in other blood group systems are not given here as the groups of the mother and child were so similar that they were useless for practical differentiation.

On April 20 a preliminary test by the method of Singer *et al.* (1951) showed the presence in the mother's blood of approximately 5% alkali resistance in excess of the maximum alkali resistance found in 10 normal blood donors. On May 4 the mean of four such estimations showed 5.9% excess of alkali resistance above the maximum found in 30 normal blood donors and 15 normal pregnant women. These findings gave further evidence of foetal cells in the mother's blood.

FIG. 5.—This is the control for Fig. 4, these erythrocytes being c-negative (CDe/CDe; R_1R_1). The mother was later shown to be of this very group. $\times 340$.

Progress of Child.—An initial small transfusion relieved shock, but did not materially affect the blood picture. A venous sample showed Hb 37% (5.5 g.), reticulocytes 5.8%. In order to raise the haemoglobin without causing too great an increase in blood volume an exchange transfusion was carried out on April 20 (Dr. J. Doyle). The amount withdrawn was 450 ml., the amount transfused 360 ml., the deficit being necessitated by overloading of the right heart, as shown by tachycardia and venous engorgement. On April 22 the Hb (heel stab) was 65% (9.6 g.). The Hb level remained almost the same for two months, but rose thereafter (see Table II). No iron was given to the child, which was breast-fed, with later supplements, but the mother took iron (ferrous gluconate,

TABLE II
PROGRESS OF CASE 2 MEASURED BY HAEMOGLOBIN LEVELS

Date	Haemoglobin (g. per 100 ml.)
19/4/56	5.8
20/4/56 (small transfusion)	5.5*
22/4/56 (exchange transfusion)	9.6
26/4/56	10.1
15/6/56	9.4
22/6/56	9.6
13/7/56	12.0
10/8/56	12.9

* Venous sample, all others heel stab.

gr. 5, once daily). The child gained weight normally, and, when last seen on July 2, 1957, was in good health.

Follow-up of Changes in Maternal Blood.—The detection of transfused cells by differential agglutination is only of limited sensitivity. Thus Mollison (1956) states that this reaction is of use only if the concentration of readily agglutinable cells is of the order of at least 1 in 200. Wiener (1942) is not so optimistic. The alkali resistance test is an even less sensitive index of the presence of foetal cells, as up to nearly 2% of alkali-resistant haemoglobin may be present in normal blood (Singer *et al.*, 1951). It was thought that spinning the mother's blood might concentrate the foetal erythrocytes in a particular layer of the packed cells. This was done in Wintrobe tubes at 3,000 r.p.m. for 30 minutes and the packed cells divided into eight approximately equal portions. The fractions were tested with anti-c in albumin and by the indirect Coombs test after incubation with anti-c. The results are shown in Table III and Figs. 7, 8, and

FIG. 6.—The infant's blood in Case 2 obtained before transfusion gave practically complete agglutination with anti-c. $\times 340$.

TABLE III
RATE OF DISAPPEARANCE OF FOETAL CELLS FROM
MATERNAL CIRCULATION IN CASE 2

Date	Method	Ordinary Sample	Centrifuged Fractions (from Above Down)			
			1 to 5	6	7	8
4.5.56	D.A.	+	0	(+)	+	++++
	A.R.	5.9	Not done			
22.6.56	D.A.	(+)	0	(+)	+	++
	A.R.	0	0		0.5	4.3
13.7.56	D.A.	? trace	0	0	(+)	(+)
	A.R.	0	0	0	0	0
10.8.56	D.A.	0	0	0	0	0
	A.R.	0	0	0	0	0

D.A. = Differential agglutination tests using anti-c.
A.R. = Alkali-resistant haemoglobin in excess of normal maximum expressed as a percentage of total haemoglobin (see text).

9. The symbol + is used to denote the degree of agglutination in the general sample tested on May 4. The symbol (+) means definite agglutination, but of the order of not more than half of the original general sample. The results with the Coombs test were substantially the same as with the albumin suspension. As the fractions of packed cells were small, it was not possible to estimate their alkali resistance by the method of Singer *et al.* The technique is shown in the Appendix. The mean results are given in Table III with the results of differential agglutination.

From these data it is clear that differential agglutination was a more sensitive index of the presence of foetal cells than was the estimation of alkali-resistant haemoglobin, though it is obvious that the two tests run in parallel and thus confirm each other.

FIG. 7.—The mother's blood in Case 2 two months after delivery now shows only tiny clumps with anti-c. $\times 375$.

FIG. 8.—The result of centrifugation on the previous sample (Fig. 7) shows in the top layer no clumps with anti-c (cf. Fig. 9). $\times 375$.

It also appeared that the foetal cells at this later stage in their ectopic intramammary life were concentrated in the lower part of the haematocrit. On July 13 the results of agglutination with anti-c, recorded as " ? trace," would undoubtedly have been recorded as negative had one not looked very intently over several samples from the tube. Thus, 12 weeks after delivery, the ordinary sample was for all practical purposes negative whereas the two lowest samples from the haematocrit were definitely positive. It must be remembered, however, that, one day after delivery, there were tiny normoblasts, presumably foetal, in the buffy coat at the top of the packed cells. It is considered that the foetal cells became more dense as they matured and thus were concentrated in the bottom of the haematocrit.

Throughout the investigation serum from each sample of maternal blood was tested by the indirect Coombs method for the presence of anti-c, but results have been entirely negative.

Subsequent Pregnancy.—The mother was delivered of another daughter on July 3, 1957. The pregnancy was uneventful and the maternal blood showed no evidence of foetal cells or iso-immunization before or after delivery. The infant is not anaemic.

To summarize, an infant was born with severe anaemia which could be explained only by massive haemorrhage from the placental vasculature of the foetus into the maternal circulation. This was confirmed by the presence in the maternal blood of

FIG. 9.—The lowermost layer with anti-c (same time as Figs. 7 and 8) shows more agglutination than the ordinary sample did at the time of delivery, two months previously (see Fig. 4). $\times 375$.

nucleated red cells not associated with maternal anaemia and similar in type to those in the foetus, by differential agglutination and by the finding of a significant amount of alkali-resistant haemoglobin in the mother. The nature of the placental lesion allowing this leakage of blood was not ascertained as the placenta was not available for detailed study.

The proportion of foetal red cells in the maternal blood just after delivery was more than 5%, possibly nearly 10%. From weight and height the mother's blood volume was estimated as approximately 4.5 to 5 litres, while that of the infant, which weighed 6 lb. 4 oz. at birth, should normally have been about 250 ml. Thus the amount of blood lost from the foetus must have been at least that of its expected blood volume. Such a large amount of blood must have been lost over a considerable period of time, otherwise the foetus would have died *in utero*. Confirmation of the long duration of the bleeding arises from the facts that the child's haemoglobin was very low at birth and that the hypochromic microcytic erythrocytes and tiny erythroblasts resembled exactly those of chronic blood loss at other periods of life.

Discussion

Until the discovery of the relationship between the rhesus blood groups and haemolytic disease of the newborn, little was understood of neonatal anaemia, and the prognosis was poor. As knowledge of haemolytic disease has increased, it has also become apparent that haemorrhage from the foetus, though less common than haemolysis, is nevertheless an important cause of anaemia. Any systematic classification of the causes of such bleeding tends to be rather lengthy, as the causes are numerous (Wickster and Christian, 1954), but

it is convenient for the clinician to group them either as overt, if haemorrhage was observed at delivery, or occult, as in the two cases reported here. The site of bleeding may be the placenta, the umbilical vessels or anomalous vascular connexions, or the foetus itself.

Clinical Diagnosis of Post-haemorrhagic Anaemia of the Newborn.—The main clinical features in the infant, as summarized by Colebatch *et al.* (1956), are pallor, weakness, loss of muscle tone, tachycardia, absence of jaundice, absence of enlargement of liver and spleen, failure to revive with oxygen, but a dramatic response to transfusion. Given such signs and an obvious source of haemorrhage, the diagnosis is easy. When the bleeding is occult only particular regard to the character and rate of respiration will prevent the misdiagnosis of asphyxia pallida or intracranial injury.

Examination of the Placenta.—A thorough inspection of the placenta, cord, and membranes is essential, for, even when haemorrhage has not been obvious clinically, the naked-eye appearances may give an important clue, as in Chown's cases (1955).

Normoblasts in groups were found within the maternal intervillous lake by Kline (1948) and by Javert and Reiss (1952). These were presumed to be foetal cells, and to occur in clumps only if the number of erythroblasts in the foetus had been high, as in prematurity or haemolytic disease (Javert and Reiss).

Examination of the Infant's Blood.—A detailed study of the morphology of the cord blood or peripheral blood from the infant may well give important timous information, as in the second case, where the presence of a hypochromic microcytic blood picture was an indication not just of haemorrhage but of prolonged haemorrhage, probably of several weeks' duration. In the second case the normoblasts numbered only 7.8% of the total nucleated cell count whereas in the predominantly haemolytic first case they were 70% ; a differential count therefore appears to be of diagnostic value.

Appropriate blood group data and the direct Coombs test provide further distinguishing information, and estimation of the plasma bilirubin may also be useful.

After even severe haemorrhage, if it occurred only at the time of delivery, the cord blood haemoglobin might well be normal, but there would be subsequent haemodilution, leucocytosis, and a normoblastic reaction ; thus in a clinically doubtful case one should look for haemodilution by serial haemoglobin estimations.

Proof of Foeto-maternal Transfusion.—The occurrence of a rigor or a febrile reaction in the mother, as in Chown's Case 3 (1955) and the present Case 1, may well be a sign that incompatible foetal blood had entered her circulation, but this is of value only if infection can be completely excluded, and it must be remembered that amniotic embolism can cause a rigor. If a relatively large amount of incompatible foetal blood were to cross the placental barrier, haemoglobinuria, anuria, and jaundice might occur, but in the absence of incompatibility it is most unlikely that any evidence of foeto-maternal transfusion would be detected clinically in the mother.

Laboratory Investigation of Maternal Blood.—Examination of the buffy coat may show a significant degree of erythrophagocytosis (as in Case 1) or the presence of otherwise unexplained normoblasts (as in Case 2). Many cases of megaloblastic anaemia show numerous erythrophages in the buffy coat, but confusion is not likely to arise since examination of the buffy coat is a direct way to diagnosing the megaloblastic anaemias of pregnancy (Goodall, 1955, 1957). The many tiny normoblasts in the buffy coat of Case 2 pointed to foeto-maternal bleeding because the mother herself was not anaemic. These diagnostic cells were in fact seen in the ordinary blood films, where they were accompanied by more numerous, non-nucleated hypochromic microcytes.

The phagocytosis of foetal red cells in cases of rhesus incompatibility may well depend on an opsonin effect produced by the antibody, as for example the anti-D in the maternal serum of Case 1. It should be mentioned, however, that anti-Rh sera differ greatly in their property of promoting erythrophagocytosis of Rh-positive cells (Conway, 1953 ; Harkink, Doorman, and van Loghem, 1953 ; Bonnin and Schwartz, 1954) ; in fact many are inactive and others relatively weak as compared with anti-A or anti-B, especially lytic anti-A. Thus the occurrence of haemorrhage into a rhesus-negative mother with antibodies might not be followed by erythrophagocytosis. On the other hand, when an ABO incompatibility exists between foetus and mother (a heterospecific pregnancy) foeto-maternal haemorrhage would almost certainly lead to rapid ingestion of the foetal cells by the maternal leucocytes.

The most valuable serological test is differential agglutination, which, of course, depends on the presence of a different antigen or antigens in the maternal and foetal cells. The possibility of carrying this out depends on the availability of sera containing the relevant antibodies. The maternal blood is incubated with the appropriate serum in saline and in albumin, and the indirect Coombs test is performed. If the mother is immunized to the foetal cells (as in Case 1) the direct Coombs test on her blood may give valuable information by revealing the clumps of affected cells. Fractionation of the maternal blood by differential centrifuging may enable one to obtain a greater concentration of foetal cells in one or more fractions as in Case 2.

Changes in maternal antibody titre during pregnancy may give a useful indication that foeto-maternal bleeding has occurred ; postpartum changes certainly can, as in the case described by Dunsford (1957). Such changes can be taken only as presumptive evidence, but suggest that other tests are worth doing, as in Dunsford's case.

Adsorption of antibody *in vitro* is another possible indirect pointer, and Chown (1954) has described the adsorption of anti-D by an Rh-negative mother's blood containing D-positive cells from the foetus.

The occurrence of anti-rhesus immunization after an episode of foeto-maternal bleeding is described by Chown (1954), but, though highly suggestive, this again is only presumptive evidence.

Of chemical tests, the most useful is the estimation of the alkali-resistant haemoglobin of the erythrocytes in the maternal blood. The widely

advocated "one minute" test of Singer *et al.* (1951) is a relatively simple method. This had to be modified for the fractionation experiments, because of the smallness of the available blood samples. None the less with adequate controls the modified though relatively crude method gave significant results. On the other hand, the qualitative type of investigation advocated by O'Connor *et al.* (1957) is not ideal. Their results appear to indicate that foeto-maternal haemorrhage is quite common, but a quantitative method seems to be required, preferably supported by other data. Thus a quantitative alkali-resistance test on the red cells in the maternal blood is an important part of the proof of transplacental bleeding, but care must be taken to obtain clear haemoglobin solutions (Singer *et al.*, 1951) free from red cell stroma (Iversen and Larsen, 1956). Caution must be exercised in the interpretation of the results of such estimations, and, if at all possible, this chemical method should be supported by morphological and serological evidence.

The estimation of alkali-resistant haemoglobin in the plasma of the mother is certainly worth doing if a mother has a rigor that might be due to the passage of incompatible cells from the foetus. This test has been done in a case of severe pre-eclampsia with haemoglobinaemia and haemoglobinuria: the haemoglobin in the plasma in this case showed no alkali resistance, thus excluding a foetal origin.

As for other tests for foetal haemoglobin, electrophoresis is known to be of no practical value in the detection of small proportions of foetal haemoglobin, and we have no experience of the immunological method used by Rucknagel and Chernoff (1955) or of the test recently described by Beaven, Ellis, and White (1956).

Conclusions.—Though the danger to the foetus of bleeding from itself, its cord, or its placenta has long been recognized, it is only since the discovery of the Rh factor and its relationship to haemolytic disease that this very different type of anaemia, post-haemorrhagic anaemia of the newborn, has received the attention it merits. Bleeding from the placental vessels of the foetus into the maternal circulation, postulated by Wiener and proved by Chown, is now confirmed and shown to be of possibly lethal extent. The confident confirmation of a suspected case by morphological, serological, and chemical methods is not beyond the resources of the routine clinical laboratory. Finally, it should be stressed that post-haemorrhagic anaemia of the newborn is an eminently treatable condition, and a condition

which, when characterized by pallor and severe shock, requires transfusion without delay.

Summary

Two cases of transplacental bleeding from the foetus have been described; in the first the bleeding was slight and associated with severe haemolytic disease; in the second the bleeding was the cause of severe neonatal anaemia.

A description is given of the various methods of diagnosis suitable for use in a routine laboratory.

Post-haemorrhagic anaemia is an important cause of pallor in the newborn. Asphyxia pallida may be closely mimicked by post-haemorrhagic shock. Rapid diagnosis and treatment of the latter by prompt blood transfusion may save the life of an otherwise normal infant.

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APPENDIX

Method for Foetal Haemoglobin

The cells were washed three times in normal saline and lysed with distilled water. The solution was cleared by shaking with a third volume of toluene and centrifuging at 2,500 r.p.m. for 20 min. As this

did not always give a clear solution, filtration through a No. 1 Whatman paper was sometimes required, followed by further centrifuging. When only a small volume was available the solution had to be diluted with distilled water to get an adequate yield from filtration. These fractions and control haemoglobin solutions (30 normal blood donors, 15 normal pregnant women) were diluted with 0.007 N. NH_4OH until they gave a reading of 80 in the EEL photoelectric colorimeter using a 625 filter, and exactly 7 ml. quantities of the standardized solutions measured into tubes. The tubes were arranged at random, each being given only a serial number. Each solution had its reading of 80 checked. Then 0.5 ml. of

N. NaOH was added and mixed for 30 sec. by a piston-shaped glass rod. With the top of the colorimeter closed the deflection was read at exactly one minute from the time of adding the NaOH. The alkaline solution was kept and read again in two hours, this reading being taken as the equivalent of complete conversion to alkaline haematin. Any excess of resistance between the one-minute reading of the fractions and the maximum one-minute reading among controls was considered as significant alkaline resistance. By standardizing the EEL with the 625 filter and known concentrations of haemoglobin, it was thus possible to calculate the percentage of alkaline resistance in excess of normal.