used in the early diagnosis of invasive candida infections. The same type of procedure is also extremely promising for detecting other pathogens such as viruses.^{13 14} Enzyme immunoassay will probably find a place in the early diagnosis of invasive aspergillosis, which is currently being investigated.

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Continuous-flow plasmapheresis in management of severe rhesus disease

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Summary

Eight patients with severe rhesus disease and expected fetal loss were treated by intensive plasmapheresis using a continuous-flow cell separator. Plasmapheresis was started at 16-27 weeks' gestation, and continued until planned intrauterine transfusion or until the infant was delivered or the rhesus disease became uncontrolled again. Altogether 24 to 237 l of plasma was exchanged over periods ranging from seven to 16 weeks. In seven of the eight patients the anti-D concentration fell during the period of plasmapheresis. Amniotic fluid spectrophotometry values remained below those recorded in the preceding pregnancy in six out of seven women. In five patients an attempt was made to control the rhesus disease by plasmapheresis alone, and two of these women delivered infants who survived. In the other three cases the infants died, one from the idiopathic respiratory distress syndrome and the other two in utero.

These preliminary findings suggest that intensive plasmapheresis with a cell separator may reduce fetal haemolysis and in some cases can sustain this effect until the infant is delivered. Nevertheless, plasmapheresis may best be used to reduce haemolysis until intrauterine transfusions may be given more safely after 30 weeks' gestation.

Introduction

Continuous-flow cell separators permit plasmapheresis of up to 7 l two or three times a week and offer a possible means of

controlling severe rhesus disease. We have used this approach in eight patients with exceptionally severe rhesus isoimmunisation and have tried to answer the following questions: (1) Can circulating maternal anti-D be removed faster than it re-accumulates? (2) Is such removal accompanied by a decrease in fetal haemolysis, as judged by amniotic fluid spectrophotometry? (3) Can intensive plasmapheresis without intrauterine transfusion save affected infants when the mother has had previous rhesus stillbirths?

Patients and methods

Obstetric and serological details of the patients are shown in tables I and II. The decision to start plasmapheresis was made when the combination of these factors, usually supported by the findings on early amniocentesis spectrophotometry, suggested that the fetus was unlikely to survive with conventional management. Only highly motivated mothers were accepted, and the procedure was fully explained.

Maternal antibody level—To estimate rhesus antibodies we measured both the indirect antiglobulin (Coombs) titre assay and the anti-D immunoglobulin concentration.¹ The latter is more reliable than antibody titration² and generally correlates with the severity of haemolysis.³ All but one of our mothers had levels of IgG higher than 4.0 mg/l, mostly much higher, which by itself suggested the presence of severe rhesus disease.⁴

Amniocentesis findings—All except the first patient (case 1) underwent amniotic fluid spectrophotometry performed by the Liley method⁵ before treatment. Though no definite predictions can be made before 25 weeks' gestation, optical density (OD) measured at a wavelength (Δ) of 450 nm is probably the best index of severity and is essential to monitor progress. All eight had levels above 0.1 OD unit, five having levels of at least 0.2 OD unit when first measured, which is equivalent to the IUT zone at 34 weeks' gestation.

Plasmapheresis procedure—Plasmapheresis was carried out using an Aminco Cell Separator (American Instrument Company, Maryland).⁶ Volumes ranging from 2.5 to 7.0 l of plasma were exchanged for reconstituted freeze-dried plasma or plasma protein fraction. Fresh frozen plasma (500 ml) was given after the latter to replace clotting factors. Heparin to a total dose of about 10 000 units was used as anticoagulant, and calcium gluconate (10 ml 10%) was always given at the start and often during the procedure.⁷ Blood samples were taken before and after the procedure. A full blood count, 12 microanalyses (using the SMA/12 Microanalyser⁸), and bacteriological culture were performed,

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TABLE I—Obstetric and blood group details of rhesus-affected families

Case No	Age (years)	Blood group	Husband's group and Rh genotype	Previous pregnancies		
				No of survivors unaffected by rhesus disease	No of survivors affected by rhesus disease	No of deaths due to rhesus disease and gestational ages
1	31	O rr	O R ₂ r	1	1	3, including intrauterine deaths at 26 and 28 weeks
2	30	AB rr	O R ₁ R ₂	1	1	3, including intrauterine deaths at 20 and 30 weeks
3	31	O rr	O R ₁ r	0†	1	0, but survivor needed 4 intrauterine transfusions
4*	30	A rr	O R ₁ R ₁	1	0	2, including a neonatal death after intrauterine transfusion at 28 weeks
5	32	O rr	O R ₁ R ₂	1	2	1 intrauterine death after intrauterine transfusion at 32 weeks
6*	27	O rr	O R ₁ r	1	0	2 intrauterine deaths, both at 28 weeks
7	33	B rr	O R ₁ R ₂	1	1	2 intrauterine deaths at 27 and 33 weeks
8	38	A rr	A R ₁ R ₁	1	1	4, including intrauterine deaths at 29, 31, and 32 weeks
				7	7	17

*Fetus survived after plasmapheresis alone.

†Immunised by blood transfusion in childhood.

TABLE II—Plasmapheresis details, antibody levels, and amniotic fluid findings

Case No	Gestation at start of plasmapheresis (weeks)	Total cumulative plasma exchanged (l)	Average amount of plasma exchanged (l/week)	Circulating anti-D (mg/l) before and after plasmapheresis		Amniotic fluid spectrophotometry (OD units)	
				Before	After	Maximum in preceding pregnancy	Maximum in present pregnancy
1	20	29	4	5.5	1.5	0.735	0.344
2	19	24	3	28.3	13.5	0.285	0.384
3	20	73	10	55.7	4.2	0.367	0.330
4*	20	164	11	10.0	7.0	0.380	0.169
5	27	80	9	1.9	2.5	0.294	0.221
6*	20	237	15	12.2	1.5	0.660	0.254
7	21	152	19	15.5	3.8	(unavailable)	0.547
8	16	230	18	4.8	2.5	0.510	0.270
Mean	20	124	11	16.7	7.6	0.462	0.197
Significance				$t = 2.05†$; $P < 0.05‡$		$t = 2.89†§$; $P < 0.025$	

*Fetus survived after plasmapheresis alone.

†Paired *t* test.

‡One-tailed test.

§Only seven pairs were tested.

and rhesus antibody and titre and immunoglobulins (using radial immunodiffusion (Mancini)) were measured.

The amount of plasma exchanged varied with each patient but increased steadily throughout the study period. The range was from 4 l a week (2×2 l exchanges) to over 20 l a week (4.5×6 l exchanges). In general the maximum possible plasmapheresis was performed from 20 weeks' gestation onwards until or unless serial amniocentesis showed fetal haemolysis to be clearly lessening in severity.

Results

Figs 1, 2, and 3 show the general pattern of anti-D levels during repeated plasmapheresis. The level at the end of each run was about half that of the pretreatment level, but rose again before the next course of plasmapheresis; the longer the interval the greater the rise. Fig 1 shows the striking falls (from 55 to 9 mg/l) obtained with an intensive five-day course of plasmapheresis. Fig 2 shows a temporary period of "escape" with rises in antibody between 27 and 29 weeks' gestation, subsiding later during continuing plasmapheresis.

Table II shows changes in anti-D concentrations and amniotic fluid spectrophotometry values that were related to intensity of plasmapheresis. Most patients had high initial antibody levels (mean 16.7 mg/l), which were prevented from rising; the value after the final plasmapheresis many weeks later was lower in seven out of eight women (final mean 7.6 mg/l). In five it was less than 4.0 mg/l (about 15 IU/ml), below which level fetal death is unlikely in the natural disease.⁹ The fall in individual levels was statistically significant (paired *t* test: $t = 2.05$; $P < 0.05$, one-tailed). Serial amniotic fluid optical density readings ($\Delta 450$ nm) almost always remained below those in the preceding pregnancy. The highest reading in the pregnancy studied was significantly lower than that in the previous pregnancy in six of the seven women for whom previous readings are available (paired *t* test: $t = 2.89$; $P < 0.025$, one-tailed).

Figs 2 and 3 show the serial amniotic fluid optical density readings

in the two patients (cases 4 and 6) whose babies survived. An initial upward trend was reversed by plasmapheresis, notably in case 6 when the intensity was increased.

Table III shows the obstetric and fetal outcome in the eight cases.

FIG 1—Case 3. Antibody and other plasma protein levels. This diagram first appeared in the *Lancet*¹⁵ and is reproduced by permission of the editor of the *Lancet*.

In the first three (cases 1-3) our objective was to control the rhesus disease until intrauterine transfusion could safely be performed, since the obstetric history in each case led us to fear intrauterine death before transfusion was feasible. In all three patients this objective was achieved but the fetus died as a direct result of intrauterine transfusion.

FIG 2—Case 6. Antibody levels and amniotic fluid spectrophotometry values compared with those in previous pregnancy. IUT = intrauterine transfusion.

We therefore changed our policy in the remaining five patients and tried to control the rhesus disease by plasmapheresis alone, using transfusions only when serial amniocentesis suggested failure and impending fetal death. Two infants survived after delivery at 36 weeks' gestation and one died of idiopathic respiratory distress syndrome after delivery at 37 weeks (cord blood haemoglobin = 11 g/dl, bilirubin 7.5 mg/100 ml (128 μ mol/l)). In the other two cases (cases 7 and 8) the rhesus disease worsened at 27 and 33 weeks respectively, and neither infant survived; one died at the time of a second intrauterine transfusion (case 7).

The only side effects suffered by the mothers were hypocalcaemic symptoms due to the citrate content of freeze-dried plasma. These were controlled with intravenous calcium gluconate, which was later withdrawn when we started using plasma protein fraction. Immunoglobulin levels remained normal throughout intensive plasmapheresis (fig 1).

Discussion

Despite current preventive measures, a few patients still have severe rhesus isoimmunisation. This situation will continue because anti-D immunoglobulin prophylaxis will fail in those who have unusually large fetomaternal haemorrhages. There will always be highly motivated women with a history of repeated intrauterine or neonatal deaths due to rhesus disease who will be willing to undergo considerable inconvenience and even slight hazard in the hope of having a normal live infant.

Intrauterine transfusion has been widely used, but the success rate in saving the fetus has been less than 50% in most series,^{10 11} and it is particularly hazardous before 28 weeks' gestation. As the severity of haemolysis generally correlates with absolute antibody concentrations,⁹ it is logical to try removing antibody by plasmapheresis, particularly as this is harmless to the fetus.

Conventional plasmapheresis using plastic bags and exchanging 1-4 l a week¹²⁻¹⁴ has given promising results when used either alone between 28 and 34 weeks or together with intrauterine transfusions for more severe disease from an earlier stage of pregnancy (12-14 weeks). In the largest reported series¹² plasmapheresis alone was successful in 29 out of 40 (72.5%)

FIG 3—Case 4. Antibody levels and amniotic fluid spectrophotometry values compared with those in previous pregnancy. IUT = intrauterine transfusion.

TABLE III—Outcome in patients treated by plasmapheresis

Case No	No of weeks treated	Intrauterine transfusion (weeks' gestation)	Fetal outcome	Cord blood findings		
				Haemoglobin (g/dl)	Bilirubin (μmol/l)	Direct Coombs test
Patients treated by intrauterine transfusion						
1	8	27, 29, 31	Intrauterine death due to 3rd transfusion	10.0 (= 50% fetal)	164	+
2	8	27	Premature delivery at 28 weeks, after transfusion			
3	9	28	Intrauterine death due to transfusion			
Cases in which intrauterine transfusion was avoided unless plasmapheresis failed to control Rh disease						
4	17	0	Live male infant; normal development at 1 year	10.0	108	+
5	9	0	Neonatal death at 37 weeks due to idiopathic respiratory distress syndrome	11.0	128	+
6	16	0	Live female infant; normal development at 1 year	15.4	99	+
7	8	27, 29	Intrauterine death due to 2nd transfusion (Rh disease not controlled by plasmapheresis)			
8	13	0	Neonatal death due to severe Rh disease (Rh disease not controlled by plasmapheresis)	2.0	51	+

Conversion: SI to traditional units—Bilirubin: 1 μ mol/l $\approx$ 0.06 mg/100 ml.

patients treated after 28 weeks' gestation and three out of 12 patients treated before 28 weeks' gestation. This highlights the problem of severe disease early in pregnancy, when intrauterine transfusion remains extremely hazardous, yet plasmapheresis alone even using a cell separator to exchange over 20 l a week may fail to control the rate of fetal haemolysis.

Several points have emerged from our study of patients with exceptionally severe rhesus disease treated by intensive plasmapheresis with a cell separator. Firstly, the expected rise in anti-D level as pregnancy progresses can be prevented. Concentrations in seven of our eight patients actually fell ($P < 0.05$), and five had a final level below 4 mg/l (table II). Secondly, amniotic fluid spectrophotometry readings ($\Delta 450$ nm) can be maintained at levels below those recorded in preceding pregnancies. This was achieved in six out of seven patients ($P < 0.025$). The apparent reversal of a rising trend in some patients (figs 2 and 3) presumably reflected reduced haemolysis. Thirdly, the fetus can be saved through plasmapheresis alone, even in cases severe enough to warrant treatment from 20 weeks' gestation. Two of the five mothers treated by this policy delivered live babies with cord blood haemoglobin concentrations of 10.0 and 15.4 g/dl. Both mothers had previously lost two babies as early as 28 weeks' gestation (table I). Lastly, plasmapheresis must be intensive. We were successful only when up to 7 l two or three times a week was exchanged. In cases in which we exchanged about 20 l weekly plasmapheresis eventually failed to control the rhesus disease (table III).

These preliminary observations suggest that fetal haemolysis may be reduced by intensive plasmapheresis using a cell separator and that in some cases this effect can be sustained by plasmapheresis alone until a viable fetus is delivered at 36-37 weeks. Unfortunately babies still die from prematurity and the idiopathic respiratory distress syndrome, in spite of lessening of haemolysis (table III), and in others haemolysis cannot remain controlled by plasmapheresis alone. These factors must be taken into account when considering a patient for this form of manage-

ment. Only highly motivated patients should be selected, as fetal loss after prolonged treatment is particularly distressing.

If the effect of intensive plasmapheresis in reducing fetal haemolysis is accepted its optimum contribution to managing severe rhesus disease may still be to tide the baby over from about 24 weeks until 30 weeks' gestation or later, when intrauterine transfusion is less hazardous.

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Changes in breast sensitivity at puberty, during the menstrual cycle, and at parturition

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Summary

Sensitivity to pain and touch was measured in the nipple, areola, and cutaneous breast tissue of prepubertal boys and girls, postpubertal men and nulliparous women, and pregnant women before and after delivery. Before puberty there were no differences between the sexes, but after puberty the tactile sensitivity of all areas of the women's breasts was significantly greater than the men's. Tactile sensitivity of all areas also varied during the menstrual cycle, with maximal sensitivity at mid-

cycle and at menstruation; the mid-cycle peak was absent when the women were taking oral contraceptives. But the most dramatic changes occurred within 24 hours of parturition, when there was a great increase in breast sensitivity. This may be the key event for activating the suckling-induced discharge of oxytocin and prolactin and inhibiting ovulation during lactation.

Introduction

The breasts undergo volume changes in response to changing hormone concentrations at puberty,¹ during the menstrual cycle,² and during pregnancy.³ Although the human breast serves a subsidiary function as an organ of sexual attraction,⁴ its primary purpose is to nourish the newborn infant. Breast-feeding also establishes an intimate tactile bond between mother and child, which may be important in establishing and maintaining the mother-infant relationship.^{5, 6}

Afferent suckling stimuli from the breast have important endocrine consequences for the mother, as they initiate a

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