day or two before the blood samples were taken had falsely high levels of serum iron and total iron-binding capacity.

Discussion

Patients on maintenance haemodialysis become iron-depleted and may require iron therapy. The exact amount of iron needed and the best route of administration are, however, uncertain It has been suggested that oral iron therapy is inconsistent in its effects and that parenteral iron therapy should be given (Carter *et al.*, 1969). Others have found that the absorption of inorganic iron is normal in chronic renal failure (Eschbach *et al.*, 1970) and that oral iron is as effective as parenteral iron (Brozovich *et al.*, 1971). The amount of blood loss and hence the amount of iron needed varies with individual patients. Because of these uncertainties and because anaemia in these patients may also be due to other causes, estimation of body iron stores is necessary at regular intervals to assess the need for iron therapy.

Our results show that serum ferritin correlates well with bone marrow iron stores in patients with chronic renal failure on maintenance haemodialysis. This estimation is more convenient than bone marrow biopsy, the amount of blood required being less than 1 ml, so that repeated estimations at frequent intervals are feasible.

The regular maintenance iron injections which these patients received provided more iron than most of them needed. A few were iron deficient despite this therapy. Whether this was because these patients omitted iron injections or because they had excessive iron loss is uncertain. With regular serum ferritin assays, however, the iron requirements of each patient can be properly assessed and maintained and those patients with excessive haemorrhage or failing to take iron therapy are easily identified.

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Pre-eclampsia in Pregnancies by Different Fathers: Immunological Studies

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Summary

Immunological studies were performed on a woman with severe pre-eclamptic toxaemia in a second pregnancy. This pregnancy followed a normal twin pregnancy by a different father four years earlier. Both fathers were also studied.

In the mixed lymphocyte culture the patient's lymphocytes reacted eight times as strongly against father 2's cells as against those from father 1. If studies along these lines are performed when a woman has toxaemic and non-toxaemic pregnancies by different fathers information may be obtained on immunogenetic aetiological factors which may be of more value than that derived from the study of large unselected populations.

Introduction

It has long been postulated that immunogenetic incompatibility may underlie at least some cases of pre-eclampsia (Dienst, 1905; Penrose, 1946, 1947; Platt *et al.*, 1958). Scott (1958) indicated how immunological and non-immunological factors could operate through a common mechanism. Stevenson *et al.* (1971) studied monozygous and dizygous twin pregnancies in inbred communities in the Middle East and provided evidence to suggest that pre-eclampsia was commoner when mother/conceptus immunogenetic disparity was greatest.

A rare opportunity arose to assess the possible role of immunogenetic factors in a complex "family unit." The mother had had two pregnancies by separate fathers. The first, a multiple gestation, was uncomplicated, while the second, a singleton gestation, was associated with severe pre-eclampsia. Both fathers were available and willing to co-operate in the study.

Case Report

The mother had an uneventful twin pregnancy by father 1 when aged 20. No hypertension was recorded during the course of regular antenatal care, and she delivered twin boys weighing 2250 g and 2500 g at 37 weeks gestation. The placenta and membranes were recorded as monochorionic by an obstetric pathologist; both twins were blood group A rhesus negative and on this evidence they were regarded as monozygotic. She was subsequently divorced. In her second pregnancy by father 2 when she was 24 her initial blood pressure was 120/80 mm Hg at eight weeks gestation, and it remained normal until 32 weeks, when she was admitted to hospital with a blood pressure of 150/100 mm Hg. Progressive albuminuria to 5 g/l developed and her blood pressure rose to 170/120 mm Hg followed by the onset of severe headaches and epigastric pain at 33 weeks gestation. Protoveratrine A and B (Puroverine, Sandoz) was infused intravenously by a log-increment pump to control the hypertension, and delivery was instituted by amniotomy and oxytocin infusion because of imminent eclampsia. A girl (1570 g) was delivered with an Apgar score of 1 who survived less than one hour as spontaneous respiration never became established. The mother's blood pressure settled to 120/80 mm Hg

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within 48 hours of delivery and the proteinuria disappeared. She was discharged on the sixth day. Six weeks post partum her blood pressure was 120/80 mm Hg and there was no proteinuria.

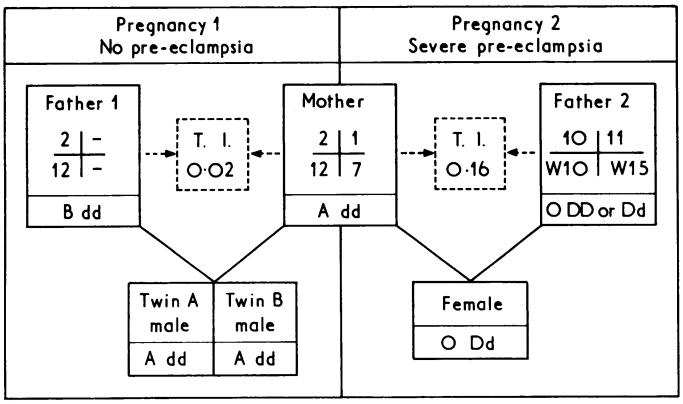
INVESTIGATIONS

Mixed Lymphocyte Reactivity.—The patient's lymphocyte response to both fathers' blood in the one-way mixed lymphocyte culture (M.L.C.) was assessed. The stimulating cells (fathers') were incubated with 60 mg/l of mitomycin C for 30 minutes and washed three times before resuspending in culture medium to the original whole blood volume. Aliquots of this treated blood (3 ml at a 1/10 dilution with culture medium) and the responding (mother's) whole blood were transferred to sterile plastic tubes (allogeneic cell mixture) and incubated at 37°C for six days. Five μCi of tritiated thymidine (specific activity = 27 Ci/mmol, Radiochemical Centre, Amersham) were added for the last 16 hours. The cultures were set up in triplicate. Autologous cell mixtures were set up as control cultures with responding cells treated with mitomycin C and untreated responding cells. For harvesting the erythrocytes were lysed with acetic acid in normal saline at 4°C, the cell protein precipitated with two washes of 5% acetic acid, and the acid-insoluble protein button dehydrated with methanol. The remaining precipitate was digested with hyamine hydroxide and transferred to 10 ml of scintillator fluid for counting in an automatic scintillation counter. Results were expressed as a transformation index —that is, the ratio of degradations per minute of the allogeneic mixture:the degradations per minute of the autologous mixture.

Other Investigations.—ABO and rhesus blood grouping, typing for human leucocyte HL-A antigens, and screening for anti-HL-A antibodies were also performed. (The HL-A panel consisted of antisera to antigens 1, 2, 3, 9, 10, 11, and 28 in the first series and 5, 7, 8, 12, 13, 14, 17, 27, W 5, W 10, W 15, W 18, and W 22 in the second series.) Serum seromucoid was measured by the orcinol method of Winzler (1955).

RESULTS

The results are summarized in the diagram. In the one-way M.L.C. the response of the maternal lymphocytes against father 2 was eight times greater than against father 1. Only two HL-A antigens were shown in father 1 and he may have been homozygous for these antigens. Both these antigens were also carried by the mother. No maternal anti-HL-A antibodies were detected during or after the second pregnancy. The seromucoid level in the mother's serum was measured at delivery (16.1 mg/l) and after delivery (214 mg/l) (normal value (± S.D.) 11·7 ± 240 mg/l, Good *et al.*, 1973).



Results of immunological studies in mother and two fathers. In each patient's box HL-A grouping is given (+). Figures above horizontal line are first series antigens, those below are second series. No figures to right of vertical line indicates that no second antigen was detected—possibly a homozygous state. Letters at base of box show ABO and rhesus (D) groupings. T.I.—Transformation index.

Discussion

Pre-eclampsia most often complicates first pregnancies and it has an even greater prevalence in first twin pregnancies. In our hospital the prevalence of pre-eclampsia in nulliparous mothers carrying twins is 27% (A. McFarlane, personal communication), but in second pregnancies in young women who showed no signs of pre-eclampsia in the first pregnancy the incidence is extremely low—about 1%. The risk of pre-eclampsia would be expected to be even lower in a second singleton pregnancy when a first twin pregnancy had not shown any pre-eclampsia. It was, therefore, very much against the probabilities that this mother's second pregnancy was complicated by severe pre-eclampsia.

The results of both HL-A typing and the M.L.C. point to a greater degree of histoincompatibility between mother and father 2 than between mother and father 1. Father 1 may have been completely HL-A compatible with the mother. As no anti-HL-A antibodies were detected in the mother's serum it seems that no humoral sensitization by the second husband's antigens occurred during the second pregnancy, but the increased degree of maternal reactivity in the M.L.C. with father 2 compared with father 1 suggests a considerable difference in antigenicity. The M.L.C. reactivity is probably not controlled by the HL-A antigens but by a closely linked locus (Cepellini 1971; Yuins and Amos 1971). The raised seromucoid levels are consistent with the findings of Good *et al.* (1973) that clinically severe toxaemic pregnancies are associated with seromucoid levels above the normal range, which might represent a non-specific immunological response of the mother to her fetal antigenic challenge.

No reports have been found of group studies in this unusual situation in which two fathers are responsible for consecutive pregnancies, one normal followed by one with pre-eclampsia. The information regarding the immunogenetic relationships in pre-eclamptic toxaemia gained from studying such situations, however, is potentially of more value than that derived from the study of large unselected obstetric populations.

The results of these studies support the possibility that immunogenetic factors operate in pre-eclampsia. More information is needed, however, and the plan adopted here seems suitable for application in similar situations which may occur in the future. A series of such reports would greatly increase knowledge of the immunogenetic relationships in pre-eclampsia. If the circumstances permitted it, of course, even more specific information could be obtained from studies of the mother's cells in relation to those of the children of the respective pregnancies.

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