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# EFFECTS ON SERUM COMPLEMENT OF NORMAL AND PRE-ECLAMPTIC PREGNANCY AND OF ORAL CONTRACEPTIVES

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**Summary.—The complement system was studied in normal gestation and puerperium, in pre-eclampsia and in women taking oral contraceptives. In 12 normal pregnancies, CH50 titres, C4, C3, C6 and C7 were increased throughout pregnancy; the serum concentration of C1 inhibitor was decreased in the last 2 quarters but greatly raised after delivery. Oral oestrogen–progestogen contraceptives also raised C3 but in contrast to pregnancy lowered CH50, C6 and C7. The different effects of pregnancy and of oral contraceptives may be due to materno-foetal immune reactions. In 9 patients with pre-eclampsia, complement levels showed a wider scatter and a significantly higher C3 level than in normal pregnancy. Conversion of C3 to C3i was found in 2 of these patients.**

SERUM complement may be activated *in vivo* by the classic pathway or by the alternative pathway. In the classic pathway, C1 is activated mainly by the Fc portion of bound IgG1, IgG3 and IgM antibodies; the sequence continues by activation of C4 and C2, which then convert C3 to active C3b. In the alternative pathway (Nicol and Lachmann, 1973) endotoxins and polysaccharides of bacterial or other origin initiate conversion of C3 to C3b by sequential activation of properdin, then factor D, and finally factor B. An active complex BC3b, or even DBC3b, is formed which potently converts more C3 to C3b and also promotes activation of D and B. Subsequent activation by C3b of the later components C5, C6, C7, C8 and C9 is identical in both pathways. The turnover of complement components is, like that of other proteins, probably influenced by changes in the blood levels of oestrogens and possibly also progestogens. When applied as oral contraceptives, these hormones raise the levels of thyroxine binding globulin (Robbins and Rall, 1960; Hollander *et al.*, 1970), of aldosterone and cortisol binding proteins (Layne *et al.*, 1962), of angiotensinogen (Weir, Tree and Fraser, 1970), of plasminogen and antiplasmin (Howie *et al.*, 1970) and of clotting factors VII and X (Poller, 1969); antithrombin factors are reduced (Howie *et al.*, 1970). The changes in pregnancy are similar and usually more extensive. The concentrations of complement components may in pregnancy also be affected by the "acute phase" phenomenon and by higher proteolytic activities in body fluids and tissues. Finally, complement may be influenced by maternal immune reactions against foetal antigens derived from the father.

Evidence for such reactions has been found in normal pregnancies (McCormick *et al.*, 1971) and their participation in several abnormalities of gestation has been claimed. They may contribute to the occurrence of spontaneous abortions in the first quarter of pregnancy and of pre-eclampsia at later stages. The involvement of complement in materno–foetal reactions is suggested by the deposition of components C3 and C4 in placentae (McCormick *et al.*, 1971); serum complement which is raised at term in normal

pregnancies (Siedentopf, Kabath and Kube, 1969) is even higher in pregnancies with ABO and Rhesus incompatibility (André, 1969) in which the incidence of pre-eclampsia is increased. In overt pre-eclampsia, however, Pietruska and colleagues (1973) found reduced serum complement, and Kitzmillar and Benirschke (1973) unchanged levels.

To clarify the role of complement, total haemolytic complement ($CH_{50}$) and the following complement constituents were measured in non-pregnant controls, in women taking oral contraceptives, in normal primigravidae, and in pre-eclampsia: (1) component C4 which is activated in the classic, but not in the alternative pathway; (2) components common to both pathways: C3 ($\beta$1 C) and its conversion product C3i ($\beta$1 A); components C6 and C7; (3) the alternative pathway was specifically investigated by observing the conversion of Factor B to active B; (4) in addition, the serum levels of C1 inhibitor (C1 INH) were measured antigenically and enzymatically. Lower levels in the second half of pregnancy have been found previously by an esterolytic method (Donaldson, 1966; Amir, Pensky and Ratnoff, 1972).

## PATIENTS AND METHODS

Blood was collected by clean venepuncture; separated serum or edetate plasma were stored at $-70°$ within 2 h of collection. Under these conditions haemolytic complement titres did not alter detectably for at least 6 months. All samples were unfrozen at 37° and then kept on ice. Pooled healthy serum (NHS) or plasma (NHP) was used for controls.

*Buffers and diluents.*—Complement fixation diluent (CFD), 0·1 mol/l barbitone buffer, pH 7·2 was prepared from tablets (Oxoid Ltd). Phosphate buffered saline (PBS) was 0·04 mol/l phosphate buffer, pH 7·2, in 0·85% NaCl. PBS with 0·01 mol/l edetate, pH 7·2 (PBS-EDTA) was used in certain assays.

*Antisera.*—Antisera against human C3 and C4 were obtained from Hyland Laboratories Ltd and against C1 INH and Factor B from Behringwerke Ltd; all gave a single line of precipitation in immunoelectrophoresis against normal human serum.

*Agar and agarose gels.*—Gels for immunodiffusion and electrophoresis were prepared with 2% agarose (Indubiose A 37, Micro Bio Labs Ltd) or with 2% purified agar-agar (Oxoid Ltd) in 0·04 mol/l barbitone buffer, pH 8·6, with 0·01 mol/l edetate.

*Complement reagents.*—Coating of red cells with C4 and C3 (EAC43): The bulk of C3 was removed from fresh human serum by incubating with zymosan, 1 mg/ml for 45 min at 37° (R3). Sheep red cells (E) optimally coated with horse haemolysin (EA) were incubated with R3 and then treated with suramin (Lachmann, Hobart and Aston, 1973). The resulting cells coated with C1, C4, C2 and C3 (EAC1423) were converted to EAC43 by storage overnight at 4° in CFD.

Preparation of C56 rich euglobulin: Reactive serum (Thompson and Lachmann, 1970) from women 3 days after childbirth was activated with zymosan. The euglobulin precipitate which contains active C56 was redissolved in half the original serum volume of PBS-EDTA. Testing on Ouchterlony double diffusion plates showed that guinea-pig red cells (GPE) suspended in the agar were lysed at the site where 1 : 256 dilutions of this euglobulin diffused against normal sera.

Total haemolytic complement ($CH_{50}$): $CH_{50}$ titres in plasma were measured by the method of Osler, Strauss and Mayer (1952). The volume of the incubate was reduced to 1·5 ml.

Radial immunodiffusion assays of C4, C3 or C1 INH were carried out in agarose containing the appropriate antiserum (Mancini, Carbonara and Heremans, 1965). C3 and C4 were assayed in plasma-EDTA using reference sera as standards. The C1 INH content of serum samples was expressed as a percentage of the value in pooled NHS. 8% Dextran (Pharmacia Ltd) was incorporated in the gel for the C1 INH assay to obtain better definition of the precipitin line.

C1 INH was also measured by a modification (Eisen and Loveday, 1972) of the enzymatic method of Levy and Lepow (1959). The normal range was 8·5–16 inhibitor u/ml.

Haemolytic plate assays (Lachmann *et al.*, 1973) of serum C6 and plasma C7: For the C6 assay, plates were poured of 1% agarose containing 1% EAC43 and 10% v/v of C6 deficient rabbit serum. C7 was assayed in 1% agarose containing 1% GPE and 10% v/v of C56 preparation. The zone of haemolysis was measured after 12 h at room temperature and expressed as a percentage of NHS activity.

The conversion in plasma-EDTA of C3 ($\beta$1C) to the electrophoretically faster C3i ($\beta$1A) was assessed using 2-dimensional crossed immunoelectrophoresis (Laurell, 1965). Best separation of C3 from C3i was obtained in gels consisting of 2% agar (1 part) and of 2% agarose (3 parts). Conversion of Factor B to active B

was examined by immunoelectrophoresis in agarose gel (Nicol and Lachmann, 1973). On completion of radial immunodiffusion or electrophoresis, plates were washed overnight in 0·9% NaCl, dried and then stained with 0·25% Coomassie brilliant blue RB (BDH Ltd).

*Patients.*—Informed consent was obtained at their first ante-natal visit from 20 clinically healthy, normotensive primigravidae who had normal plasma levels of urea and electrolytes and no abnormal findings in MSU specimens. Abnormal pregnancies, multiple conceptions and primigravidae in whom no post-natal samples were available were excluded. Eventually, 12 women with a spontaneous onset of labour and vaginal delivery of a living, mature-for-dates infant were included. Blood samples were taken during pregnancy, at 2–7 days after delivery and at the end of the puerperium. In a few patients the final post-natal samples were taken later.

Nine abnormal pregnancies were studied in which pre-eclampsia had been diagnosed on the basis of: (a) blood pressure of 140/90 mm Hg or higher on 2 or more occasions; and (b) persistent and significant proteinuria (>0·5 gr/24 h). One patient was being treated for pre-existent hypertension, a second suffered an abruptio placentae during labour. Seven pre-eclampsias had no further concurrent complications. All blood samples were taken in the last quarter of pregnancy.

Blood samples from 2 groups of age-matched non-pregnant women were taken randomly throughout the menstrual cycle: Group 1 (22 clinically normal women who were not receiving any drugs) and Group 2 (14 women who were taking oral low dose oestrogen–progestogen contraceptives).

## RESULTS

### *Serum total haemolytic complement*

The mean $CH_{50}$ (Fig. 1a) of Group I (untreated non-pregnant women) was 110 $CH_{50}$ u/ml and of Group II (non-pregnant women taking oral contraceptives) 92·7 u/ml ($P < 0·001$). During pregnancy the titres rose gradually and were significantly increased to 124 u/ml by 21–25 weeks of gestation. At term the mean titre was further increased to 137 u/ml and reached a maximum of 162 u/ml in the first week after delivery. By the end of the puerperium the $CH_{50}$ titre had returned to normal levels.

### *Early complement components*

In contrast to $CH_{50}$ titres, levels of plasma C3 were raised in women taking oral contraceptives (Fig. 1b). C3 levels rose throughout pregnancy and reached 157 mg/100 ml at term ($P < 0·001$; a maximum of 184 mg/ml was attained in the first week of the puerperium. The plasma C4 levels (Fig. 1c) in Group I (m = 34 mg/100 ml) were similar to those of Group II (m = 33 mg/100 ml). C4 was raised throughout pregnancy. By the end of the puerperium both C3 and C4

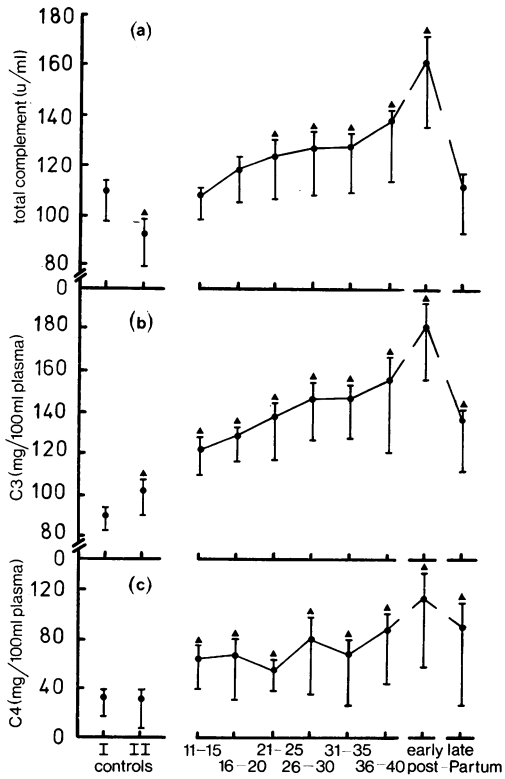


FIG. 1.—Complement levels during and after pregnancy in 12 normal primigravidae. Age-matched controls were 22 non-pregnant women not taking drugs (Group I) and 14 women taking oral oestrogen–progestogen contraceptives (Group II). During pregnancy, levels were measured sequentially at 5 week intervals (abscissa), and post partum within 7 days (early) and 6–8 weeks after delivery (late). S.e. mean are plotted above the means, and S.D. below. ▲ = Mean significantly different ($P < 0·05$) from mean of Group I. (a) Total haemolytic complement ($CH_{50}$ titres); (b) antigenically determined C3 levels; (c) antigenically determined C4 levels.

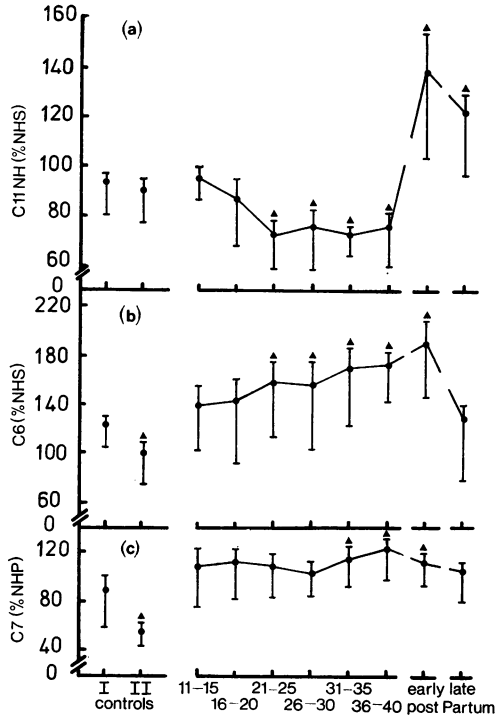


FIG. 2.—As Fig. 1. (a) C1 INH levels measured antigenically; (b) C6 levels measured by haemolytic plate method; (c) C7 levels measured by haemolytic plate method.

levels had fallen but were still higher than in Group I non-pregnant women. Normal levels were found in post-natal samples taken 3–4 months after delivery.

The serum levels of C1 INH (Fig. 2a) were not affected by oral contraceptives. (Group I m = 94%, Group II m = 91%). Unlike all the other complement components studied, the serum concentration of this $\alpha_2$-neuraminoglycoprotein fell during pregnancy. By the beginning of the second half of pregnancy, the mean level of C1 INH was 75% of the value in NHS ($P < 0.005$), and remained at this level until term. In the first week after delivery the level rose rapidly to 141% ($P < 0.001$), and was still elevated at the end of 6 weeks after delivery. This increase was no longer apparent in the few postnatal samples taken 3–4 months after delivery. Low levels of C1 INH at term (m = 9·3 u/ml) and high levels after delivery (m = 17·5 u/ml; $P < 0.005$) were also found by an esterolytic method.

*Late complement components*

The haemolytic activity of serum C6 was lower in women taking oral contraceptives (Group I, m = 124%, Group II, m = 100%; Fig. 2b). During pregnancy C6 rose gradually attaining at 21–25 weeks 160% of the value in NHS ($P < 0.05$) and at term 174% ($P < 0.005$). The highest level, 192%, was found in the first week post partum.

The haemolytic activity of plasma C7 (Fig. 2c) was reduced by oral contraceptives (Group I, m = 92%, Group II, m = 56%). There was only a slight increase during pregnancy. At term the mean was 126% of the value in NHP. It fell to 116% in the first week after delivery, C7 being the only complement component to be lower after delivery than at term. The haemolytic levels of C6 and C7 returned to normal by the end of the puerperium.

*C3 and factor B conversion*

At no stage of the normal pregnancy could intravascular activation of C3 or factor B be demonstrated.

*Pregnancies complicated by pre-eclampsia*

When compared with normal pregnancies of the same stage, pre-eclamptic patients showed a wider scatter of C6 and C7 activities and of $CH_{50}$ titres (Fig. 3). Although their mean values were lower ($CH_{50}$, C4, C6, C1 INH) or higher (C3, C7) than in normal pregnancies, only the change in C3 was significant. Two patients showed slight C3 conversion; no conversion of factor B to active B was found.

## DISCUSSION

It is very likely that the lower $CH_{50}$, C6 and C7 values and the higher C3 levels found in women taking oral contraceptives are due to actions of oestrogens and

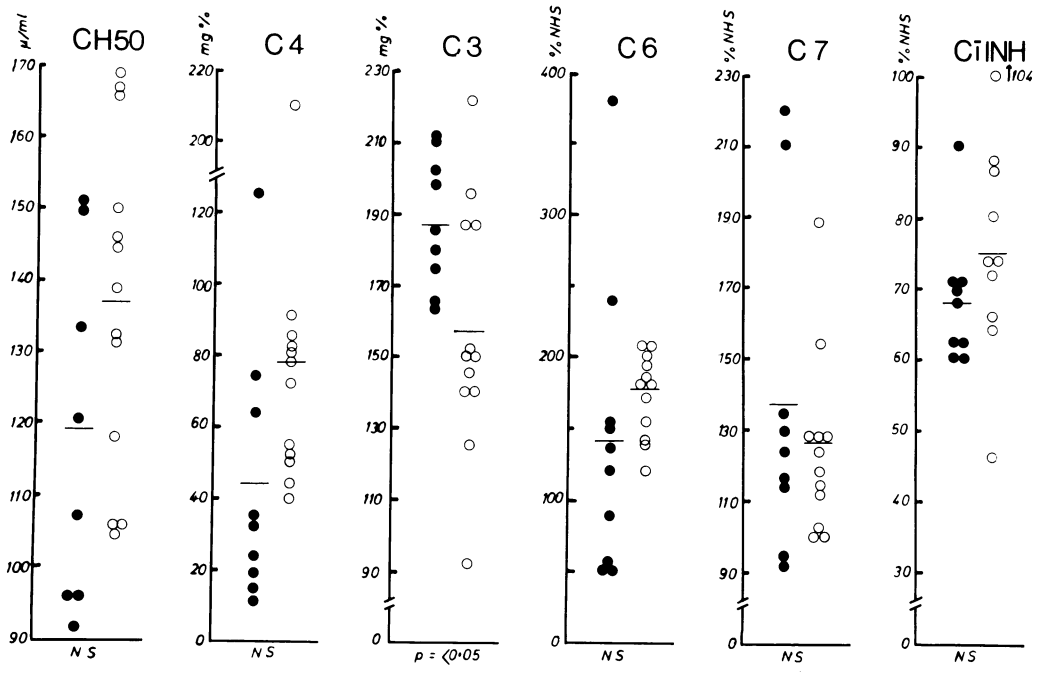


FIG. 3.—Complement levels in pre-eclampsia (●) compared with levels in normal pregnancies (○) at the same stage of gestation. Horizontal bars show mean values.

progestogens on the synthesis of complement components. The hormones may act directly on metabolic activities of macrophages in the liver, gut and other tissues, which are a major source of serum complement (Ruddy, Gigli and Austen, 1972).

Previously found effects of oral contraceptives on serum proteins were of a similar direction though less extensive than the changes occurring during pregnancy (Laurell, Kullander and Thorell, 1967; Studd, Blainey and Bailey, 1970*a*). This differs from the present findings. The increase during pregnancy in $CH_{50}$, C4 and C7 levels and the decrease in C1 INH levels are the opposite of the corresponding changes brought about by oestrogen–progestogen preparations. It may be assumed that in pregnancy the high concentrations of endogenous oestrogen and progestogen have the same effect on complement turnover as do the exogenous hormones. The different findings in the two situations could well be due to materno–foetal immune reactions which probably enhance both synthesis and consumption of complement. Enhancement of synthesis by these reactions will increase complement levels in plasma, which may mask local complement consumption at the placenta. A comparable situation has been described in rheumatoid arthritis where serum complement levels are raised in spite of increased complement catabolism in the affected joints (Hedberg, Lundh and Laurell, 1970).

C1 INH inhibits not only C1 esterase but also C1r, kallikrein, plasmin and clotting factors XI and XII (Amir *et al.*, 1972). Some of these enzymes are also inhibited by $\alpha_1$-antitrypsin which is increased during pregnancy. Reduced levels of functional C1 INH in the second half of pregnancy have been found in earlier studies (Donaldson, 1966; Amir *et al.*, 1972). The possibility was raised that this might be due to synthesis of non-functional inhibitor (Amir *et al.*, 1972), but the present findings that C1 INH is

also reduced antigenically argue against this possibility. It is not known whether the fall in C1 INH during the second half of pregnancy is a cause or a consequence of enhanced C1 activation, or of the increased fibrinolytic activity in late pregnancy (Bonnar, McNicol and Douglas, 1971).

In the early puerperium, plasma proteins show changes typical of the " acute phase " reaction, and the reported complement changes undoubtedly form part of this reaction. Some of the factors operating during " acute phase " episodes may also be active during pregnancy and alter levels of complement in the same direction as seen post partum. These factors, however, cannot explain changes during pregnancy, which are the opposite of post partum changes, as in the case of C1 inhibitor and C7.

The involvement of complement fixing immune reactions in pre-eclampsia is suggested by reports of circulating antibodies against the placenta (Levanon and Rossitini, 1968), deposits of IgG and C3 in decidual vessels (Kitzmillar and Benirschke, 1973) and decreased levels of serum complement (Pietruska *et al.*, 1973). In renal glomeruli, complement is deposited particularly in afferent and efferent arterioles; in capillary loops it occurs together with IgM and IgG (Petrucco *et al.*, 1974). Antisera against homologous placentae cause a pre-eclamptic syndrome in pregnant animals (Langford, Douglas and Arhelger, 1967). The alternative pathway is implicated in the Schwartzman reaction which has an absolute requirement for complement (Brown, 1972). Pre-eclampsia is similar to the Schwartzman reaction in that it involves a state of disseminated intravascular coagulation (Howie, Prentice and McNicol, 1971). Magara (1960) isolated a constituent from the human placenta which initiated a Schwartzman reaction.

The finding that C3 was significantly increased in pre-eclampsia does not agree with reports that C3 was unchanged (Studd, Blainey and Bailey, 1970*b*) or lowered (Pietruska *et al.*, 1973). The conversion of C3 ($\beta$1C) to C3i ($\beta$1A) found in 2 patients suggests that immune reactions activating complement occur at least in some patients with pre-eclampsia. Moreover, significant activation of complement may take place without detectable formation of C3i ($\beta$1A). More sensitive methods, such as measurement of the breakdown product C3$\alpha$2D or of the catabolism of labelled complement components (Charlesworth *et al.*, 1974) might reveal enhanced catabolism of complement in a larger proportion of pre-eclamptic patients.

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