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Effect of Oral Contraceptives and Pregnancy on Erythrocyte Deformability and Surface Charge (39037)

JOHN R. DUROCHER,[1] M. SUSAN WEIR, EDWARD G. LUNDBLAD, WARREN E. PATOW, AND MARCEL E. CONRAD[2]

Department of Hematology Walter Reed Army Institute of Research and Department of Obstetrics-Gynecology Walter Reed Army Medical Center, Washington, D.C., 20012

The use of oral contraceptives has been associated with thrombotic episodes in seemingly healthy young women (1). Although increased activity of plasma coagulation factors and alterations in platelet function occur in users of oral contraceptives, a direct causal relationship to thromboses has not been proven (2, 3). An increase in blood viscosity and a reduction in blood flow rate have been reported and might contribute to thromboses (4, 5).

Recently, Oski *et al.* reported that erythrocytes from patients taking oral contraceptives had diminished deformability (6). Erythrocyte membrane deformability is a factor in blood viscosity. Progesterone and synthetic progestational steroids interact with the erythrocyte membrane and may be related to the decreased deformability (7, 8). In women an additional biophysical change in the erythrocyte membrane is a reported reduction in net negative surface charge during pregnancy (9). A reduction in surface charge might overcome normal electrostatic repulsion of erythrocytes and promote agglutination, especially in a situation of reduced blood flow rate (10). We therefore studied erythrocyte deformability and surface charge in normal women, oral contraceptive users, and pregnant women in an attempt to correlate membrane changes with clinical status.

Materials and methods. Blood was collected in heparin from patients in a Family Planning Clinic and an Obstetrical Clinic. Patients taking drugs other than oral contraceptives were excluded from the study. All patients taking oral contraceptive[3] had used them for more than 2 months.

Erythrocytes were washed thrice in Ringer's-12 m*M* Tris buffer (pH 7.4) containing 0.25% albumin. Filtration was performed as described by Oski *et al.* (6) and the "$T_{1/2}$" calculated from least squares analysis. In addition, polycarbonate filters were used in a manner similar to that described by Gregersen *et al.* (11). A 2% erythrocyte suspension in buffer was filtered through 3 μm pore polycarbonate filters under 10 cm water vacuum. The filters were held in a Millipore filter holder on a stainless steel screen with a filtration area of approximately 2.5 cm.^{2} The time required for 2 ml to filter was taken as a measure of erythrocyte deformability and is called "filtration time." Significance was determined using a nonpaired Student's *t* test. ATP determinations were done using an ATP STAT-PACK (Calbiochem). Mean corpuscular volumes were calculated from the microhematocrit and red blood cell counts done on a Coulter Model B.

Erythrocytes were washed in saline and cell electrophoresis performed at 23° in a low ionic strength phosphate–sorbitol buffer (12). A Zeiss cytopherometer fitted with platinum electrodes was used. In each sample, at least ten cells in the frontal plane were measured before and after the reversal of polarity.

Results and discussion. As seen in Table I there was no significant difference in erythrocyte deformability as measured by filterability between normal women and oral contraceptive users. There was a significant

[1] Present address: Pennsylvania Hospital, Philadelphia, Pennsylvania 19107.

[2] Present address: University of Alabama, Birmingham, Alabama 35294.

[3] The contraceptives taken included Ovulen (2), Ovral (7), Norinyl 1 + 80 (7), Norlestrin 1 mg (2), and Demulen (2).

TABLE I. ERYTHROCYTE DEFORMABILITY.

		T½[a]	Filtration time[b]	ATP levels[c]	Mean corpuscular volume[d]
Women (controls)	(18)	4.46 ± 0.31	10.0 ± 1.1	1.23 ± 0.04	95.2 ± 1.5
Oral contraceptive users	(20)	4.24 ± 0.20 (NS)	9.8 ± 0.8 (NS)	1.33 ± 0.07 (NS)	95.4 ± 2.3 (NS)
Pregnant women	(28)	5.59 ± 0.39 (NS)	14.2 ± 1.0 ($P < 0.01$)	1.37 ± 0.07 (NS)	95.3 ± 1.7 (NS)

[a] Min ± 1 SE.
[b] Sec ± 1 SE.
[c] μM ATP/ml packed cells ±1 SE.
[d] μm^3 ± 1 SE.
NS = Not statistically significant from controls ($P > 0.05$).

TABLE II. DURATION OF PREGNANCY AND ERYTHROCYTE DEFORMABILITY

		T½[a]	Filtration time[b]
Controls	(18)	4.46 ± 0.31	10.0 ± 1.1
Trimester:			
First	(4)	4.81 ± 0.63 (NS)	11.0 ± 1.9 (NS)
Second	(11)	5.25 ± 0.41 (NS)	12.0 ± 1.2 (NS)
Third	(13)	5.50 ± 0.54 (NS)	16.9 ± 1.4 ($P < 0.001$)

[a] Min ± 1 SE.
[b] Sec ± 1 SE.
NS = Not significantly different from controls ($P > 0.05$).

difference in filtration time between these groups and pregnant women. These differences were not explained by changes in erythrocyte ATP levels. The prolongation of filtration time of red blood cells was related to the duration of pregnancy (Table II) correlating with increases of hormone levels as pregnancy proceeds (13).

No significant difference in net negative surface charge, as measured by electrophoretic mobility, was found between controls and users of oral contraceptives or pregnant women (Table III).

Unlike the studies of Oski *et al.* (6), we were unable to show filtration abnormalities in erythrocytes from women receiving oral contraceptives. In a recent publication reporting changes of filterability through paper filters of banked sickle trait blood (14), erythrocytes from users of oral contraceptives did not filter differently from controls.

TABLE III. ERYTHROCYTE ELECTROPHORETIC MOBILITY.

Subjects		Electrophoretic mobility (μm/sec/V/cm ± 1 SE)
Women (controls)	(16)	2.06 ± 0.02
Oral contraceptive users	(15)	2.07 ± 0.02 (NS)
Pregnant women	(24)	2.09 ± 0.02 (NS)

NS = Not significantly different from controls ($P > 0.025$).

Progesterone has been shown to interact with the erythrocyte membrane (7). However, synthetic progestogens (e.g., norethynodrel and norethindrone) do not share all of progesterone's properties to the same degree, e.g., solubility in lipids or protection against

hemolysis (8). Elevated "progesterone" levels as measured by urinary pregnanediol levels are not found in users of oral contraceptives (15). Therefore, our inability to find filtration changes is not surprising.

The high levels of female hormones in pregnancy, especially during the latter months of pregnancy, are correlated with decreased deformability of the erythrocytes. Murphy found an increased viscosity of erythrocytes from normal or pregnant women when suspended in serum from pregnant women, but no alterations in filterability through Millipore filters (16). Patients with Hemoglobin CC disease had decreased erythrocyte deformability, which became more abnormal during pregnancy. In our filtration system, in which a standard buffer was used to obviate serum effects, we found changes in the filterability of pregnant women's erythrocytes which could be explained by decreases in cellular deformability and not by serum factors alone.

In 1962, Angers and Rottino reported a decreased erythrocyte net negative surface charge in pregnant women (9). As seen in Table II we were unable to find any difference in erythrocyte net negative surface charge in normal women, oral contraceptive users, and pregnant women. In contrast to Angers and Rottino (17), we used a low ionic strength buffer which affords greater sensitivity and reproducibility than a physiologic ionic strength buffer. The main determinant of surface charge in the erythrocyte is sialic acid (18). A reduction in surface charge would involve masking or cleavage of superficial sialic acid moieties or introduction of cationic groups, a property not ascribed to progesterone.

The cause of thrombotic episodes in oral contraceptives users is still not clarified. A reduction in erythrocyte surface charge was not found in pregnant women or users of oral contraceptives. The finding of normal filterability of erythrocytes in women receiving progestational agents suggests that alterations in erythrocyte deformability do not play a role in the thromboses seen in this group. However, the decreased deformability of erythrocytes in pregnant women might contribute to thromboses in pregnancy (19), and would aggravate hemolysis noted in patients with certain hemoglobinopathies (16).

Summary. Erythrocyte deformability and surface charge were studied in normal premenopausal women, oral contraceptive users, and pregnant women. The increased incidence of thrombosis in women taking oral contraceptives could not be explained by decreased erythrocyte deformability or surface charge. However, the decreased erythrocyte deformability of late pregnancy may relate to thrombosis during this period and to increased hemolysis in patients with certain hemoglobinopathies.

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