Clinical Hemorheology and Microcirculation 22 (2000) 99–106
IOS Press

100 mg acetylsalicylic acid acutely decreases red cell aggregation in women taking oral contraceptives

A. El Bouhmadi [a], F. Laffargue [a], N. Raspal [a] and J.F. Brun [b,*]
[a] *Service de Gynécologie Obstétrique Hôpital Arnaud de Villeneuve, France*
[b] *Service Central de Physiologie Clinique, Centre d'Exploration et de Réadaptation des Anomalies du Métabolisme Musculaire (CERAMM), CHU Lapeyronie 34295, Montpellier-cédex 5, France*

Received 19 April 1999
Revised 4 October 1999

Abstract. Since oral contraceptives (OC) are known to impair blood fluidity and to increase the risk of venous and arterial thrombosis, while acetylsalicylic acid (ASA) decreases the thrombotic risk and modifies some rheologic parameters, we compared the hemorheologic effects of ASA on blood rheology between women treated by OC and women who never received this medication. 25 women under OC were compared to 25 matched women who had never used OC. Blood viscosity (MT90 viscometer) and RBC aggregation (Myrenne aggregometer and AFFIBIO erythroaggregometer) were measured before and 1 hr after women received per os 100 mg ASA, after an overnight fast. The only significant difference between women under OC and controls was an increased RBC aggregation ('M' index +28%, $p < 0.04$; Affibio aggregation time −21%, $p < 0.03$). On the whole sample of 50 women as well as in the subgroup of women under OC, ASA decreased RBC partial disaggregation threshold (−1.7%, $p < 0.01$). These results confirm that RBC aggregation is increased under OC and suggest that 100 mg ASA acutely induces a partial reversal of this RBC hyperaggregation.

Keywords: Erythrocyte deformability, blood rheology, contraceptives, aspirin, erythrocyte aggregation

1. Introduction

Oral contraceptives, which are among the most widely taken medications in the healthy population, may induce clinically important side-effects. The most important of these side-effects is probably venous and arterial thrombosis [1]. Pharmacological attempts to further decrease venous thrombotic side-effects by the use of third-generation oral contraceptives have failed [1]. This places a greater emphasis on the selection of patients to help avoid giving medication to those patients with underlying thrombotic risk factors. An example of this approach has been the clear confirmation of the adverse effects of cigarette smoking leading to arterial thrombosis in oral contraceptive users. At the biochemical level, hypercoagulability testing (i.e., screening for high-frequency prothrombotic abnormalities, such as the Factor V Leiden genotype) may be useful [1]. However, the pathophysiology of these events remains uncompletely understood and a potential involvement of some hemorheological disturbances has been suggested, given the fact that blood rheology is impaired by OC treatments [2,3].

Recent literature that emphasizes the previously underestimated importance of blood rheology in the risk of cardiovascular events [4–11] gives more interest to studies on blood rheology in OC users.

*Correspondence to: Dr J.F. Brun, MD, Ph.D. Tel.: +33 467 338284; Fax: +33 467 335923; E-mail: drjfbrun.@aol.com.

A possible involvement of rheologic disturbances in thrombotic side-effects of OC deserves some attention [12]. Alternatively, it could be interesting to evaluate therapeutic approaches designed to protect against these cardiovascular side-effects of OC. In this respect, acetylsalicylic acid (ASA) is a well-known antithrombotic drug which is widely used in persons at risk for preventing thrombotic events [13]. However, its effects on blood rheology, as reported in the literature, are not likely to be so beneficial, since they include a decrease in RBC deformability [14].

Given the frequency of use of both OC and ASA, the simultaneous intake of these two medications is surely a usual situation. However, nothing is known about the combined effects of ASA and OC on the rheologic properties of blood. Thus, in this study, we aimed at comparing the hemorheologic effects of ASA on blood rheology between women treated by OC and women who never got this medication.

2. Subjects and methods

2.1. Patients

Twenty-five women under OC i.e., 25 μg ethinyl estradiol plus norgestrel, cyprotherone acetate or norethisterone acetate, were compared to twenty-five matched women who never used OC. All women were nulliparous. There was the same percentage of smokers (one third) in each group. OC taken in the first group were representative of the average French contraceptive prescriptions: ethynyl-estradiol (20–50 μg) associated with either cyprotherone acetate (2 mg), desogestrel (0.15 mg) levonorgestrel (0.15 mg), gestodene (75 μg), norgestimate (0.25 mg). Characteristics of the patients are given in Table 1. Hemorheological parameters were measured before and 1 h after they received per os 100 mg ASA, after an overnight fast. Subjects were recumbent without physical activity during this hour.

2.2. Laboratory measurements

Blood samples for hemorheological measurements (7 ml) were drawn with potassium EDTA as the anticoagulant in a vacuum tube (Vacutainer). Sampling was performed without tourniquet. Viscometric measurements were done at very high shear rate (1000 s^{-1}) with a falling ball viscometer (MT 90 Medicatest, F-86280 Saint Benoit) [15,16]. Accuracy of the measurements was regularly controlled with the Carrimed Rheometer 'CS' (purchased from Rhéo, 91120 Palaiseau, France) [17]. The coefficient of variation of this method ranged between 0.6 and 0.8% [18]. With this device we measured apparent viscosity of whole blood at native hematocrit, plasma viscosity, and blood viscosity at corrected hematocrit (0.45) according to the equation of Quemada [19]. Dintenfass' 'Tk' index of erythrocyte rigidity was calculated [20]. RBC aggregation was assessed with the Myrenne aggregometer [21] which gives two indices of RBC aggregation: 'M' (aggregation during stasis after shearing at

Table 1
Clinical characteristics of study subjects (mean ± SEM)

	Subgroup 1 under OC (n = 25)	Subgroup 2 without OC (n = 15)
Age (years)	23 ± 4.3	24 ± 4.7
Weight (kg)	55.4 ± 3.6	55.5 ± 6.6
Height (m)	1.63 ± 0.1	1.62 ± 0.6

600 s^{-1}) and 'M1' (facilitated aggregation at low shear rate after shearing at 600 s^{-1}). The hematocrit/viscosity (h/η) ratio, an index of oxygen supply to tissues, was calculated according to Chien [22] and Stoltz [23], with hematocrit (as percentage) divided by viscosity at high shear rate determined as described above.

The AFFIBIO-SEFAM aggregometer was used for a more precise assessment of RBC aggregation. This device is based upon the experiments of Mills [24,25] on cell disaggregation behavior in shear flow. This device measures the changes in backscattered light which are observed when sheared RBC suspensions are abruptly brought to a full stop. The decrease in the optical signal reflects the formation of RBC aggregates [26–28]. Some parameters are derived from the curve of light intensity as a function of time. The aggregation time is the reciprocal of the initial slope (calculated between 0.5 and 2 s after the shear has stopped). The aggregation index at 10 s is a measurement of the extent of erythrocyte aggregation and is the relative surface area above the curve calculated over the first 10 s. This device measures also disaggregation thresholds, by submitting blood to a succession of shear rates from 600 s^{-1} to 7 s^{-1}. The total disaggregation threshold is the shear rate below which the backscattered light intensity starts to decrease, indicating that the shear stress applied to aggregates is no longer sufficient for allowing complete dispersion of RBC aggregates. The partial disaggregation shear rate is defined as the shear rate corresponding to the intersection point of the two asymptotes drawn from the extremes (maximum and minimum shear rate).

2.3. *Statistics*

Results are presented as mean ± the SE of the mean. A value of $p < 0.05$ was considered as significant. Comparisons were made with Mann–Whitney and and Wilcoxon nonparametric tests.

3. Results

As shown in Table 1 and indicated in Section 2, the two groups of women appeared to be well matched for age, weight, height, and smoking habits. Table 2 shows the comparison of hemorheologic parameters. Hematocrit was not significantly different between the two groups, although there was a nonsignificant tendency towards a lower value in OC users ($p < 0.1$). Similarly, viscosity parameters (whole blood viscosity at either native or corrected Hct, plasma viscosity, hematocrit/viscosity ratio) were similar in the two groups and were unchanged after 100 mg ASA. The only significant difference between women under OC and controls was an increased RBC aggregability, either measured with the 'M' index (which was higher by +28%, $p < 0.04$) or with the time of aggregation TA (which was lower by −21%, $p < 0.03$). The partial disaggregation threshold exhibited only a nonsignificant tendency to be higher in women under OC ($p < 0.06$). When subgroups are considered separately, an effect of ASA on blood rheology could be found only in OC users, while there was no significant effect in non-OC users. In OC users, ASA moderately but significantly decreased both hematocrit (−1%, $p < 0.01$) and the partial RBC disaggregation threshold (−1%, $p < 0.05$).

4. Discussion

These results confirm that RBC aggregation is moderately increased under OC and suggest that 100 mg ASA acutely lowers RBC aggregation in OC-treated women, without affecting this parameter in non-OC users.

Table 2

Hemorheological parameters in the 50 women of the study before and 1 h after 100 mg ASA

	Subgroup 1 under OC ($n = 25$)		Subgroup 2 without OC ($n = 25$)	
	before ASA	after ASA	before ASA	after ASA
Hct	39.72 ± 1.86	39.32 ± 1.74***	40.56 ± 1.52	40.48 ± 1.96
TA	2.49 ± 0.76*	2.46 ± 0.76	3.02 ± 0.92	2.90 ± 0.85
γ_D	47.07 ± 15.60	46.44 ± 15.02***	40.30 ± 7.23	38.55 ± 5.06
M	5.56 ± 1.47**	5.85 ± 2.39	4.59 ± 1.63	4.71 ± 2.3
M1	8.77 ± 2.78	8.66 ± 2.54	5.97 ± 2.80	7.44 ± 3.50
η_s	2.59 ± 0.39	2.55 ± 0.34	2.64 ± 0.37	2.63 ± 0.40
η_p	1.35 ± 0.14	1.33 ± 0.14	1.37 ± 0.14	1.35 ± 1.14
Tk	0.57 ± 0.081	0.58 ± 0.078	0.58 ± 0.070	0.58 ± 0.070
k	1.41 ± 0.18	1.42 ± 0.18	1.41 ± 0.16	1.42 ± 0.17
η45%	2.90 ± 0.41	2.91 ± 0.35	2.96 ± 0.35	2.97 ± 0.4
hct/η	0.153 ± 0.018	0.152 ± 0.016	0.148 ± 0.016	0.150 ± 0.020

Means ± SEM. Abbreviations: Hct, hematocrit (%); TA, time of RBC aggregation measured by laser backscattering; γ_D (s^{-1}), RBC partial disaggregation threshold measured by laser backscattering; M, RBC aggregation index measured by light transmission in stasis after total disaggregation at high shear; M1, RBC aggregation index at low shear rate, measured by light transmission after total disaggregation at high shear; η_s, whole blood viscosity at native hematocrit at a high shear rate (mPa.s); η_p, plasma viscosity (mPa.s); Tk, Dintenfass' index of RBC rigidity calculated from viscometry at high shear rate; k, Quemada's index of RBC rigidity calculated from viscometry at high shear rate; η45%, viscosity of whole blood corrected for Hct with Quemada's equation (mPa.s); hct/η, hematocrit/viscosity ratio ($mPa^{-1}.s^{-1}$).

*$p < 0.04$ vs non-OC users; **$p < 0.03$ vs non-OC users; ***$p < 0.01$ vs values before ASA.

Alterations of blood rheology in OC users were first reported in 1971 by Aronson et al. [29] and thus most precisely described in the early eighties by Lowe et al. [2,30] and Buchan and MacDonald [3]. An elevation of fibrinogen levels [30–32] has been repeatedly observed and is likely to explain most of the rise in aggregability we observe here. However, it is interesting to compare the hemorheologic profile of the women of our studies, taking contraceptives of the late nineties, with data reported two decades ago by the first authors who investigated this question. As reviewed in 1981 by Buchan and MacDonald [33], OC users in the late seventies were mostly characterized by a lower red cell deformability and a slightly higher whole blood viscosity despite normal values of plasma viscosity. Hematocrit was also reported to be increased in some studies [30] but not all [33]. The progestin component of the pill was assumed to be responsible for a rise in fibrinogen which explained most of this pattern. Actually, this finding on old progestin compounds of OC pills (mostly 19-nortestosterone derivatives), contrasts to some extent with the more recent physiological investigations [12] which evidenced in normally cycling women that estradiol levels were positively correlated to whole blood viscosity, plasma viscosity and fibrinogen, and negatively correlated to red cell deformability. In physiological conditions, therefore, estrogens were likely to impair blood fluidity. On the opposite, progesterone in physiological conditions had the opposite effect, decreasing both fibrinogen and blood viscosity, and increasing red cell deformability [12]. These physiological findings are in disagreement with a study performed ten years ago by Derham and Buchan, which demonstrated that pharmacologic intake of estrogens results in increased hematocrit and fibrinogen, while synthetic progestogens increase hematocrit and decrease red cell deformability [34].

Presumably, the modern evolution towards more physiological progestogens at lower doses tends to suppress these deleterious effects and to fit more closely with the physiological picture: this assumption is further supported by more recent studies on blood rheology in OC-users [35,36]. These modern studies suggest that the recent, low dose, compounds that represent most of our sample of women are almost devoid of hemorheological side-effects [35,36], in contrast to older preparations with higher doses [33].

Consistent with this literature, we fail to observe any differences in blood viscosity and red cell deformability between OC-users and non users, suggesting that this evolution toward better-tolerated molecules and lower concentrations has been beneficial from a hemorheologist's viewpoint. However, there is still a moderately higher red cell aggregability as indicated by the value of the M1 index which is 21% higher in OC users than in non-users (see Table 2). Such a difference may theoretically be assumed to induce some microcirculatory [37] or prothrombotic effects [38]. Presumably, changes in fibrinogen [30–32], although this protein was not measured in this study, may explain this high value of aggregability. This finding suggests that the question of the hemorheologic effects of OC, although it has been investigated since 1971, still remains a potentially important field. Some recent findings on estrogen circulatory effects, such as their vasodilatory action which is mediated by NO synthesis and release by endothelial cells [39], may offer new areas of research for hemorheologists. Estrogen-response elements have been identified in the promoting region of the gene coding for the endothelial nitric oxide synthase [39]. Moreover, estrogens have been suggested to act more rapidly via membrane receptors, resulting in an increase in cytosolic Ca^{++} in some cells [39]. Estrogens are also likely to exhibit antioxidant properties which may delay NO clearing in blood [39].

A second finding in our study is that at the low dose of 100 mg daily, ASA lowers the RBC partial disaggregation threshold in OC users by 1.7%. Data on blood rheology and ASA remain scarce and are mostly related to higher doses. On the whole, two hemorheological effects of aspirin have been described. Some authors but not all [14] have observed that ASA decreases RBC deformability, and may exert some inhibiting effect on red cell aggregability [42]. Clearly, ancient techniques (mostly RBC filtration) have been employed in these works. Thus, the picture remains confusing due to the strong dependence of RBC filterability upon shape and volume alterations. There is very little recent literature on this topic. A recent report by Bozzo et al. [40] evaluated the influence of treatment with aspirin *in vitro*, alone or combined with dipyridamole, on red cell aggregability. Aggregability was estimated through digital analysis of light microscopy images. With this technique, red cells that had been exposed to aspirin showed a high aggregation rate, while dipyridamole alone or combined with aspirin provoked echinocytosis, disturbing the rouleaux arrangement. Washing red cells after treatment restored about 90% of echinocytes to their biconcave shape, but aggregation rate did not recover in parallel. Not surprisingly, those *in vitro* data are not fully in agreement with our *in vivo* findings. *In vivo* rheologic effects of aspirin are likely to be more complex than *in vitro*, due to an involvement of leukocyte and endothelium. However, this paper by Bozzo et al. [40] confirms that ASA has a direct effect on both erythrocyte shape and erythrocyte rheology. Another recent paper by Korbut and Gryglewski [41] investigated the effect of ASA (compared with prostacyclin and nitric oxide) on deformability of red blood cells in septic shock in rats. That study showed that administration of NG-nitro-L-arginine (L-NNA, 30 mg/kg, i.p.), as that of aspirin (50 mg/kg, i.p.), did not affect red blood cell deformability in non-septicaemic rats. However, L-NNA in contrast with aspirin, significantly improved deformability of red blood cells in LPS-treated animals. Thus prostacyclin, camonagrel and L-NNA can act as protective agents against LPS-induced loss of red blood cell deformability, due to specific effects of these agents on biochemical function of leukocytes present in RBC suspension. By contrast, aspirin did not improve RBC deformability.

On the whole, it can be concluded from this literature that ASA may induce alterations in RBC membrane properties and shape that may in some situations impair RBC deformability and decrease RBC aggregability. In our study investigating the short-term effects of a low dose, we did not observe the former effect, but significantly evidenced the latter. Interestingly, it could be observed only in women whose RBC aggregability was increased by the use of OC, while there was no decrease of aggregation when it was normal. Furthermore this effect remained moderate and did not reverse the effect of oral contraceptive intake.

Besides, a very moderate (−0.6%) decrease in hematocrit is observed in all study subjects after aspirin, since in the whole sample Hct decreased from 40.14 to 39.9%. An artifact due to blood sampling and thus RBC withdrawal should be discussed. In fact, we removed only 7 ml (see Section 2.2) representing 0.2% of circulating blood if we assume a blood volume representing [40] in women 5.5–7% of body weight (i.e., grossly 3500 ml for the average weight of our subjects). If we assume also that plasma volume is equal to blood volume × (1 − 0.0087 × Hct) [41] it can be calculated that we removed 4.5 ml of plasma and 2.5 ml of red cells. However, the same calculation indicates that when hematocrit decreases from 40.14% to 39.9% (if blood volume is constant) there is a "loss" of 7 ml of red cells, i.e., 2.8-fold the amount of withdrawn red cells. In other terms our drawing reduces the red cell mass by 0.20% and not by 0.58% so that it is very unlikely to explain the hematocrit change. Thus, the hematocrit change is probably better explained by an effect of aspirin on body fluid distribution.

Thus, women representative of the common picture of oral contraceptive use in the late nineties still exhibit some rheologic disturbances (mostly a higher red cell aggregability). The use of 100 mg of acetylsalicylic acid slightly reduces this hyperaggregability but does not totally reverse this effect. In the light of the recent literature demonstrating an epidemiologic link between blood rheology and atherothrombotic events, a possible role of hemorheologic disturbances in the pathogenesis of the vascular risk in OC users still deserves attention.

References

[1] C. Carter, The pill and thrombosis: epidemiological considerations, *Baillieres Clin. Obstet. Gynaecol.* **11** (1997), 565–585.
[2] G.D.O. Lowe, M.M. Drummond, C.D. Forbes and J.C. Barbenel, Increased blood viscosity in young women using oral contraceptives, *Am. J. Obstet. Gynecol.* **137** (1980), 840–842.
[3] P.C. Buchan and M.N. MacDonald, Altered haemorheology in oral-contraceptive users, *Br. Med. J.* **280** (1980), 978–979.
[4] E. Ernst, K.L. Resch, A. Matrai, M. Buhl, P. Schlosser and H.F. Paulsen, Impaired blood rheology: a risk factor after stroke?, *J. Intern. Med.* **229** (1991), 457–462.
[5] W. Koenig, M. Sund, E. Ernst, U. Keil, J. Rosenthal and V. Hombach, Association between plasma viscosity and blood pressure. Results from the MONICA-project, Augsburg, *Am. J. Hypertens.* **4** (1991), 529–536.
[6] G.D.O. Lowe, W.C.S. Smith, H. Tunstall-Pedoe, I.K. Crombie, S.E. Lennie, J. Anderson and J.C. Barbenel, Cardiovascular risk and hemorheology. Results from the Scottish Heart Study and the MONICA Project, Glasgow, *Clin. Hemorheol.* **8** (1988), 517–524.
[7] G.D.O. Lowe, D.A. Wood, J.T. Douglas, R.A. Riemersma, C.C.A. McIntyre, I. Takase, E.G.D. Tuddenham, C.D. Forbes, R.A. Elton and M.F. Oliver, Relationships of plasma viscosity, coagulation and and fibrinolysis to coronary risk factors and angina, *Thromb. Haemost.* **65** (1991), 339–343.
[8] G.D.O. Lowe, F.G.R. Fowkes, J. Dawes, P.T. Donnan, S.E. Lennie and E. Housley, Blood viscosity, fibrinogen, and activation of coagulation and leukocytes in peripheral arterial disease and the normal population in the Edinburgh Artery Study, *Circulation* **87** (1993), 1915–1920.
[9] G.D.O. Lowe, M.M. Drummond, C.D. Forbes and J.C. Barbenel, Occlusive arterial disease and blood rheology, in: *Clinical Aspects of Blood Viscosity and Cell Deformability*, G.D.O. Lowe, J.C. Barbenel and C.D. Forbes, eds, Springer-Verlag, Berlin, 1981, pp. 133–148.

[10] J.W.G. Yarnell, I.A. Baker, P.M. Sweetnam, D. Bainton, J.R. O'Brien, P.J. Whitehead and P.C. Elwood, Fibrinogen, viscosity and white blood cell count are major risk factors for ischemic heart disease. The Caerphilly and Speedwell Heart Disease studies, *Circulation* **83** (1991), 836–844.
[11] W. Koenig and E. Ernst, The possible role of hemorheology in atherothrombogenesis, *Atherosclerosis* **94** (1992), 93–107.
[12] S.B. Solerte, M. Fioravanti, F. Magri, A. Spinillo, G. Guaschino and E. Ferrari, Influence of sex hormones on blood rheology and plasma protein pattern during the menstrual cycle, *Rev. Fr. Gynecol. Obstet.* **86** (1991), 139–142.
[13] C. Patrono, Prevention of myocardial infarction and stroke by aspirin: different mechanisms? Different dosage?, *Thromb. Res.* **92**(Suppl. 1) (1998), S7–12.
[14] I.B. Kovacs and J. O'Grady, Impaired red blood cell deformability after oral administration of aspirin in man, *Biomed. Biochim. Acta* **43** (1984), 395–398.
[15] J. Doffin, R. Perrault and G. Garnaud, Blood viscosity measurements in both extensional and shear flow by a falling ball viscometer, *Biorheology* (Suppl. 1) (1984), 89–93.
[16] M.F. Aillaud, C. Poisson, M. Buonocore, M. Billerey, P. Lefèvre and I. Juhan-Vague, Etude du viscosimètre médical à chute de bille, *Le Pharmacien Biologiste* **159** (1985), 291–294.
[17] J. Bouton and M. Ansermin, Rheomètre Carrimed CS. Appareil à contrainte imposée pour measure de fluides viscoélastiques et de fluides à seuil. Techniques en Biorheologie, in: *Séminaire INSERM*, Vol. 143, J.F. Stoltz, M. Donner and E. Puchelle, eds, 1986, pp. 121–124.
[18] C. Fons, J.F. Brun, I. Supparo, C. Mallard, C. Bardet and A. Orsetti, Evaluation of blood viscosity at high shear rate with a falling ball viscometer, *Clin. Hemorheol.* **13** (1993), 651–659.
[19] D. Quemada, Rheology of concentrated disperse systems. A model of non-Newtonian shear viscosity in steady flows, *Rheol. Acta* **17** (1978), 632–642.
[20] L. Dintenfass, *Blood Viscosity, Hyperviscosity & Hyperviscosaemia*, MTP Press, Melbourne, 1985, 482 pp.
[21] H. Schmid-Schönbein, E. Volger and H.J. Klose, Microrheology and light transmission of blood. III: The velocity of red cell aggregate formation, *Pflugers Arch.* **254** (1975), 299–317.
[22] S. Chien and L.A. Sung, Physicochemical basis and clinical implications of red cell Newtonian shear viscosity in steady flows, *Clin. Hemorheol.* **7** (1987), 71–91.
[23] J.F. Stoltz, M. Donner and S. Muller, Introduction de la notion de profil hémorhéologique: "Hémorhéologie et Facteurs de Risque" 7e Réunion Conjointe de la Société Hémorhéologie de l'Ouest et de la Societé de Biorhéologie de Langue Française. laser, in: *Hémorhéologie et Agrégation Érythrocytaire*, Vol. 2: *Applications Cliniques*.
[24] P. Mills, D. Quemada and J. Dufaux, Etude de la cinetique d'agregation érythrocytaire dans un écoulement Couette, *Rev. Phys. Appl.* **15** (1980), 1357–1366.
[25] P. Snabre, M. Bitbol and P. Mills, Cell disaggregation behavior in shear flow, *Biophys. J.* **51** (1987), 795–807.
[26] B. Pignon, S. Muller, D. Jolly, M. Siadat, E. Petitfrère, B. Vessel, M. Donner, G. Potron and J.F. Stoltz, Validation d'une méthode d'approche de l'agrégation érythrocytaire par rétrodiffusion laser, in: *Hémorhéologie et Agrégation Érythrocytaire*, Vol. 2: *Applications Cliniques*, J.F. Stoltz, ed., Médicales Internationales, Paris, 1988, pp. 65–74.
[27] M. Donner, M. Siadat and J.F. Stoltz, Erythrocyte aggregation: approach by light scattering determination, *Biorheology* **25** (1988), 367–375.
[28] A. Chabanel and M. Samama, Evaluation of a method to assess red blood cell aggregation, *Biorheology* **26** (1989), 785–797.
[29] H.B. Aronson, F. Magora and J.G. Schenker, Effect of oral contraceptives on blood viscosity, *Am. J. Obstet. Gynecol.* **110** (1971), 997–1001.
[30] G.D.O. Lowe, M.M. Reavey, C.D. Forbes and C.R.M. Prentice, Increased blood viscosity in young women taking oral contraceptives, *Lancet* **ii** (1970), 1329–1332.
[31] L. Poller, Oral contraceptives, blood clotting and thrombosis, *Br. Med. Bull.* **34** (1978), 151–156.
[32] P.W. Howie, C.R.M. Prentice, A.C. Mallinson, C.H.W. Horne and G.P. McNicol, Effect of combined oestrogen–progestogen oral contraceptives, oestrogen, and progestogen on antiplasmin and antithrombin activity, *Lancet* **ii** (1970), 1329–1332.
[33] P.C. Buchan and H.N. MacDonald, Rheological studies in obstetrics and gynaecology, in: *Clinical Aspects of Blood Viscosity and Cell Deformability*, G.D.O. Lowe, J.C. Barbenel and C.D. Forbes, eds, Springer-Verlag, Berlin, Heidelberg, New York, 1981, pp. 175–192.
[34] R.J. Derham and P.C. Buchan, Haemorheological consequences of oestrogen and progestogen therapy, *Eur. J. Obstet. Gynecol. Reprod. Biol.* **32** (1989), 109–114.
[35] S.B. Solerte, M. Fioravanti, A. Spinillo, F. Ferrari and S. Guaschino, Influence of oral contraceptives on blood rheology and hemostatic and metabolic patterns in young women. Results of a three year study, *J. Reprod. Med.* **37** (1992), 725–732.
[36] E. Ernst, C. Schmolzl, A. Matrai and W. Schramm, Hemorheological effects of oral contraceptives, *Contraception* **40** (1989), 571–580.
[37] E. Vicaut, X. Hou, L. Decuypere, A. Taccoen and A. Duvelleroy, Red blood cell aggregation and microcirculation in rat cremaster muscle, *Int. J. Microcirc.* **14** (1994), 14–21.

[38] P. Riha, F. Liao and J.F. Stoltz, The effect of rouleaux formation on blood coagulation, *Clin. Hemorheol. Microcirc.* **17** (1997), 341–346.
[39] R.O. Cannon III, Effects of estrogen on the endothelium in postmenopausal women: implications for atherosclerosis, in: *Atherosclerosis XI. Excerpta Medica, International Congress Series*, Vol. 1155, B. Jacotot, D. Mathé and J.C. Fruchard, eds, pp. 817–823.
[40] J. Bozzo, M.R. Hernández, A. Del Giorgio and A. Ordinas, Red blood cell aggregability is increased by aspirin and flow stress, whereas dipyridamole induces cell shape alterations: measurements by digital image analysis, *Eur. J. Clin. Invest.* **26** (1996), 747–754.
[41] R. Korbut and R.J. Gryglewski, The effect of prostacyclin and nitric oxide on deformability of red blood cells in septic shock in rats, *J. Physiol. Pharmacol.* **47** (1996), 591–599.
[42] M. Staubli, W. Reinhardt and P.W. Staub, Blood viscosity and red cell deformability after aspirin in vivo, *Atherosclerosis* **41** (1982), 167–170.
[43] F. Lang, The body compartments and dynamics of water and electrolytes, in: *Comprehensive Human Physiology*, R. Greger and U. Windhorst, eds, Springer-Verlag, Berlin, Heidelberg, 1996, pp. 1545–1556.
[44] M. Greger and M. Bleich, Normal values for physiological parameters, in: *Comprehensive Human Physiology*, R. Greger and U. Windhorst, eds, Springer-Verlag, Berlin, Heidelberg, 1996, pp. 2427–2449.