The effect of some oral contraceptive steroids on platelet electrophoretic mobility *in vitro*

A cause-effect relation between oral contraceptive steroid intake and thrombogenesis seems to have been established although the incidence of thrombosis in women taking oral contraceptive steroids over long periods of time is low (Inman & Vessey, 1968; Vessey & Doll, 1968). Recently, Dugdale & Masi (1971) in a review of the literature on hormonal contraception and thromboembolic disease concluded that oral contraceptives lead to an increase in platelet adhesiveness and aggregation. They tentatively concluded that the thrombogenic potential of oral contraceptives resided in the oestrogenic component and that this may be due to the effect of this component on platelet function.

As there is evidence that many steroid hormones are surface-active (Baret & Roux, 1968; Munck, 1957) and interact with lipids extracted from platelet suspensions spread as monolayers on water (Florence & Rahman, 1972), it was thought possible that steroids adsorb at the platelet surfaces, decrease surface charge density and therefore decrease the electrostatic repulsive forces which operate between the platelets. Such an effect would lead to an increase in the tendency of the platelets to adhere to each other, which will predispose to thrombus formation.

We have studied the effect of saturated aqueous solutions of a number of oestrogens and progestagens on the electrophoretic mobility of bovine platelets *in vitro* using a Zetameter (Zeta-Meter Inc., New York) with a glass-Teflon cell. These experiments differ from those of Bolton, Hampton & Mitchell (1968) who measured the mobility of platelets from patients on a regime of oral contraceptives. Such changes as they observed may have been due to changes in plasma lipid rather than to a direct effect of the steroids on the platelets.

In our experiments, progesterone, mestranol, norethindrone, medroxyprogesterone acetate, megestrol and norethynodrel decreased the electrophoretic mobility of the platelets (Table 1). Oestradiol and lynestrenol had no significant effect, while ethinyloestradiol increased the electrophoretic mobility.

Table 1. *Electrophoretic mobility of bovine platelets in the presence of steroids at saturation concentrations.*

Steroid	Electrophoretic Mobility* $\mu\ s^{-1}\ V^{-1}cm^{-1}$
None	$-2{\cdot}1 \pm 0{\cdot}03$
Oestrogens	
Oestradiol	$-2{\cdot}05 \pm 0{\cdot}04$
Ethinyloestradiol	$-2{\cdot}80 \pm 0{\cdot}06$
Mestranol	$-1{\cdot}70 \pm 0{\cdot}02$
Stilboestrol	$-2{\cdot}23 \pm 0{\cdot}05$
Progestagens	
Progesterone	$-1{\cdot}50 \pm 0{\cdot}01$
Chlormadinone acetate	$-1{\cdot}90 \pm 0{\cdot}07$
Lynestrenol	$-2{\cdot}07 \pm 0{\cdot}06$
Medroxyprogesterone acetate	$-1{\cdot}80 \pm 0{\cdot}02$
Megestrol acetate	$-1{\cdot}65 \pm 0{\cdot}02$
Norethynodrel	$-1{\cdot}67 \pm 0{\cdot}02$
Norethindrone	$-1{\cdot}87 \pm 0{\cdot}02$

* $\pm$ standard deviation calculated from at least four sets of determinations of mobility (total of 20 single determinations).

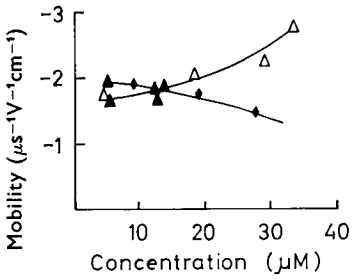

FIG. 1. Plot of platelet electrophoretic mobility against steroid concentration; oestrogen (△) progestagens (▲). ◆ Progesterone at saturation and intermediate concentrations.

The steroids were studied at their maximum concentration, but intermediate concentrations of oestradiol and stilboestrol were also used. If the electrophoretic mobility results are plotted against steroid concentration, i.e. solubility (Fig. 1), the trend for oestrogens and progestagens is different: progestagens tend to decrease electrophoretic mobility and the oestrogens either do not influence the net mobility or increase it. The latter may be due to the presence in stilboestrol, ethinyloestradiol and oestradiol of phenolic hydroxyl groups; an increase in electrophoretic mobility is not compatible with the physico-chemical explanation of increased aggregation put forward above. Progesterone, which lowers the electrophoretic mobility of the platelets, has been shown by Gershfeld & Maramatsu (1971) to decrease the surface potential of a number of lipid films, but it was suggested that the reduction is caused by an alteration in the hydration layer of the surface film. As the phenolic hydroxyl groups of the oestrogens which increase mobility are not ionized in water or at physiological pH, any effect is probably due to an increase in the hydration of the platelet surfaces. This may result from interaction of water molecules with the hydroxyl groups.

Gaarder & Laland (1964) have suggested that the binding of ADP to platelet surfaces provides a mechanism for the formation of calcium bridges with neighbouring platelets. We suggest that in the light of the present findings it would be possible to reconcile the thrombogenic activity of the oestrogenic component of oral contraceptives (mainly ethinyloestradiol) with an ability to increase the (negative) surface potential of the circulating platelets. This increased negativity could increase the likelihood of interaction with the 6-amino-group and the adenino nitrogen of adenosine diphosphate (ADP), or more directly, encourage the formation of radially oriented calcium bridges linking adjacent anionic groups at the platelet surfaces (Weiss, 1970).

A preliminary study of the effect of these steroids on platelet aggregation *in vitro* (unpublished observations) supports this conclusion. Ethinyloestradiol ($3{\cdot}4 \times 10^{-5}$M) does not itself affect aggregation, but it increases the sensitivity of the platelets to the action of ADP, while stilboestrol ($2{\cdot}9 \times 10^{-5}$M) only slightly increases the effectiveness of ADP, and oestradiol ($1{\cdot}8 \times 10^{-5}$M) does not affect it. On the other hand, progesterone induces a greater degree of aggregation *in vitro* than the control in the absence of ADP, a result which might be expected from a lowering of electrical forces of repulsion.

This work has been supported, in part, by Glaxo Research Ltd.

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July, 3, 1972

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Penicillin allergy: imidazole-catalysed formation of the penicilloyl determinant

The major antigenic determinant in penicillin allergy is the penicilloyl group (Levine, 1965; de Weck & Blum, 1965), and it has been reported that most of the immunogenicity of the penicillins is due to *in vivo* formation of penicilloyl conjugates (de Weck, Schneider & Gutersohn, 1968; Schneider & de Weck, 1970). Such conjugates may be formed by reaction of penicillin with free amino-groups of protein and/or by reaction via the reactive degradation product, penicillenic acid (see e.g. Schneider, 1970). The claim (Schneider & de Weck, 1968, 1969) that the imidazole group cannot be penicilloylated by penicillin has recently been disproved in this laboratory (Bundgaard, 1971, 1972a, b). The studies have shown that imidazole at neutral pH reacts with several penicillins with the quantitative formation of corresponding penicillenic acids, the initially formed products being *N*-penicilloylimidazoles. Since the rate of this nucleophilic imidazole catalysis is much higher than the rate of aminolysis of penicillins by other amines at physiological pH and temperature, and since the penicilloylating properties of the reaction product, penicillenic acid, in some respects are different from those of penicillins [e.g. the free thiol group can be penicilloylated by penicillenic acid, but not by penicillin (Wagner, Davis & Gorman, 1969)], the imidazole reaction must be considered as a potential pathway in the formation of penicilloyl conjugates. However, the question arises whether imidazole can catalyse penicilloylation of amino- or other functional groups of proteins by penicillins which are structurally incapable of undergoing rearrangement to penicillenic acids. Such penicillins have been demonstrated to be as immunogenic as benzylpenicillin (Schneider & de Weck, 1966). The present report shows that imidazole at pH 7·4 and 37° catalyses the penicilloylation of amino- and hydroxyl groups by 6-ethoxycarbonylaminopenicillanate, a penicillin in which the alkoxy side chain prevents penicillenate formation.

Sodium 6-ethoxycarbonylaminopenicillanate (ethoxypenicillin sodium) was prepared by treatment of 6-aminopenicillanic acid with ethyl chloroformate in aqueous acetone with sodium bicarbonate as acid acceptor. The penicillin was isolated as its sodium salt by treating the free acid in ether with a 30% solution of sodium 2-ethylhexanoate in n-butanol. The infrared spectrum showed characteristic bands at 1770 (β-lactam carbonyl), 1710 (carbamate carbonyl), and 1600 cm^{-1} (carboxylate). Found: C, 40·2; H, 5·9; N, 8·3. Calc. for $C_{11}H_{19}N_2O_5S$ Na,H_2O: C, 40·2; H,5·2; N, 8·5%.

The reactivity of ethoxypenicillin toward the nucleophilic agents imidazole, glycylglycine, and hydroxide ion was found to be similar to that of benzylpenicillin (Table 1).