PLASMA Xa INHIBITORY ACTIVITIES AND PLASMA CONCENTRATIONS OF NORGESTREL AND ETHINYLLOESTRADIOL IN WOMEN ON ORAL CONTRACEPTIVE STEROIDS

It has been known for a number of years that women taking oral contraceptive steroids are at relatively high risk of venous thrombosis. The degree of risk appears to be related to the dose of ethinylloestradiol in the contraceptive preparation and with the reduction in the dose of ethinylloestradiol from 100 µg to 50 µg the number of women developing venous thrombosis has been reduced (Oral Contraceptives and Health, 1974; Vessey, Doll, Fairbairn & Glover, 1970). Oral contraceptive steroids affect the levels of a number of clotting factors in the blood and the incidence of deep vein thrombosis has been closely linked to the concentration of antithrombin III in the plasma. Antithrombin III concentrations are lower in women on oral contraceptives than controls, (Von Kaulla & Von Kaulla, 1970) and Sagar, Stamatakis, Thomas & Kakkar (1976) found that those women with the lowest concentrations of antithrombin III were those who were most likely to develop deep vein thrombosis following emergency surgery.

Antithrombin III is the naturally occurring inhibitor of activated factor X and is probably the same protein as factor Xa inhibitor and heparin co-factor (Yin, 1974). Wessler, Gitel, Wan & Pasternak (1976) showed that women taking oral contraceptive steroids had significantly reduced factor Xa inhibitory activity compared to control women ($74 \pm 14\%$ compared to $85 \pm 13\%$). We have measured the factor Xa inhibitory activity in a group of women on oral contraceptive steroids to see if the reduction in activity correlates with the plasma concentration of ethinylloestradiol.

Forty-nine women between the ages of 16 and 33 years have been studied. All were attending the Local Authority family planning clinic and had been taking

combined oral contraceptive steroids for at least 6 months. None were receiving any other medication. Sixteen of the women were regular cigarette smokers. Twenty-five were taking Eugynon 30 (30 µg ethinylloestradiol (EE) and 250 µg levonorgestrel (Ng), eleven were receiving Ovranette (30 µg EE and 150 µg Ng), nine were receiving Microgynon (30 µg EE and 150 µg Ng) and four were taking Ovrin (50 µg EE and 250 µg Ng). All were seen on two successive days between 10 and 12 h after their regular dose of contraceptive steroid. After giving consent a blood sample was taken by venepuncture on each occasion. Blood (10 ml) was placed in a heparinized container and the plasma was analysed for norgestrel (Back, Bates, Breckenridge, Crawford, MacIver, Orme & Rowe, unpublished observations) and ethinylloestradiol (Back, Breckenridge, Crawford, MacIver, Orme, Rowe & Watts, 1979) using recently developed radioimmunoassays. The remaining sample of blood (2.25 ml) was placed into a tube containing 0.25 ml of 3.8% sodium citrate and after centrifugation the plasma was separated and stored in plastic tubes at -40°C .

The assay of anti Xa was as described by Biggs, Denson, Akman, Barrett & Hudden (1970) with minor modifications. Bovine factor Xa was purchased from Diagnostic Reagents (Thame, Oxon) and this was diluted 1 in 40 in an aqueous solution of 0.85% sodium chloride. Ball and Alton platelet substitute was purchased from Diagnostic Reagents and diluted 1 in 5 in an aqueous solution of 0.85% sodium chloride.

A normal range of anti Xa was established using plasma samples from 19 male and 14 female volunteers (19–40 years) all of whom were in good

Table 1 Plasma norgestrel and ethinylloestradiol concentrations (mean $\pm$ s.e. mean) and anti Xa activities (mean $\pm$ s.d.) in 49 patients

Preparation	Plasma norgestrel concentration (ng/ml)	Plasma ethinylloestradiol concentration (pg/ml)	Plasma Xa inhibitory activity (%)
Eugynon 30 <i>n</i> = 25	2.26 ± 0.23	27.8 ± 2.7	77.2 ± 9.0
Ovranette <i>n</i> = 11	1.74 ± 0.20	35.4 ± 5.7	69.5 ± 15.5
Microgynon <i>n</i> = 9	1.62 ± 0.16	20.0 ± 2.2	78.2 ± 12.6
Ovrin <i>n</i> = 4	3.75 ± 0.35	28.8 ± 5.6	73.4 ± 5.2

health and none of whom were on drug therapy. Each plasma was assayed against a plasma pool of 16 normals with an arbitrary value of 100%.

The normal $\pm$ s.d. range of anti Xa activity in the 33 volunteers was $90.4\% \pm 8.7$. The mean $\pm$ s.d. anti Xa activity in the 49 women taking oral contraceptive steroids was $77.0 \pm 9.1\%$, significantly less than the control values ($P < 0.01$). Forty-one per cent of the women taking contraceptive steroids had anti Xa activities below the lower limit of the normal range ($108-73\%, \pm 2$ s.d. of mean in normals). The mean $\pm$ s.e. mean plasma concentration of norgestrel in all the women taking contraceptive steroids was 2.2 ± 0.13 ng/ml with a range of 0.38 to 6.0 ng/ml. The mean $\pm$ s.e. mean plasma ethinyloestradiol concentration in the 49 women was 30.1 ± 2.5 pg/ml (range 6–190 pg/ml). Table 1 shows the values for the individual oral contraceptive preparations; there were no significant differences in the values between the various preparations. There was no significant correlation between the plasma Xa inhibitory activity and the plasma concentration of norgestrel (r between 0.109 and 0.196) or the plasma concentration of ethinyloestradiol (r between 0.127 and 0.236).

We have confirmed the previous observation (Wessler *et al.*, 1976) of a significantly reduced anti Xa activity in women taking long term oral contraceptive steroids. Our figures of a reduction from 90.4% to 77.0% are very similar to their figures of 85% in controls and 74% in treated women. The association of thrombotic complications with the higher dose preparations of ethinyloestradiol is well established (Vessey *et al.*, 1970) and it seemed possible that there might be a direct correlation between the plasma concentration of ethinyloestradiol and the anti Xa activity but in this study no such correlation has been found. The plasma concentration of EE varied 30 fold in the 47 women, while there was a 15 fold variation in plasma norgestrel concentrations. It is possible that a reactive metabolite of EE rather than EE itself is responsible

for the vascular complications and this is being investigated further.

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PLASMA RIFAMPICIN ASSAY BY AN IMPROVED SOLVENT EXTRACTION TECHNIQUE

Rifampicin is now one of the first-line drugs recommended in antituberculous treatment regimes. Measurement of plasma rifampicin concentrations may be required to determine the optimum drug dose for individuals, especially in patients with impaired metabolism of the drug, such as those with liver disease. At present a microbiological assay utilizing

Sarcina lutea as the test organism (Furesz, Scotti, Pallanza & Marpell, 1967) is commonly used for this purpose, but a simpler method of measuring the drug, amenable to use in routine laboratory, would be an improvement. The chemical extraction method using isoamyl alcohol to measure spectrophotometrically rifampicin in plasma (Sunahara & Nakagawa, 1972)