Metabolism of Norethynodrel in Thrombophlebitic-Thromboembolic Subjects

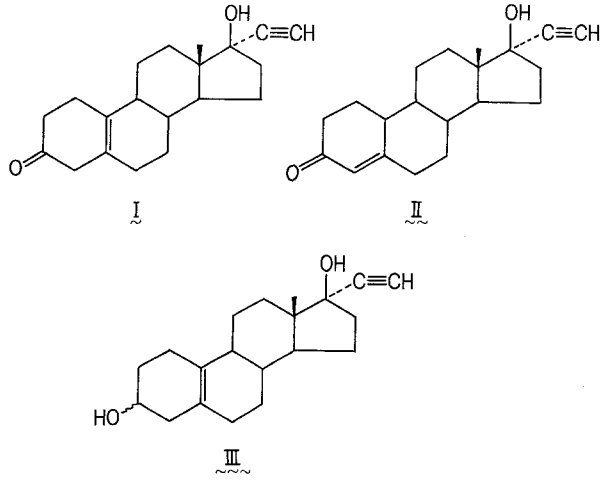
With the increasing use of oral contraceptives there has been considerable concern over the higher incidence of thrombophlebitic-thromboembolic episodes among women taking these agents[1]. It is a matter of speculation whether these vascular events are associated with a predisposition toward this condition or whether the active components of the contraceptive formulation initiate a chain of biological events culminating in the vascular accident. The latter alternative suggests the possibility that there might be a correlation between the embolic episode and some abnormal metabolic behavior of the estrogen and/or progestin contraceptive component.

Material and methods. A study was initiated to determine the manner in which a typical progestin was metabolized in women who had previously experienced a thrombophlebitic-thromboembolic episode while on some form of oral contraceptive. Norethynodrel[2] (I) is the progestin component in the currently used oral contraceptive Enovid® and was chosen for this study because its major metabolites have been identified[3] and its metabolism in humans has been investigated[4,5]. The basis for data comparison was with a segment of the general population which had never experienced a thrombophlebitic-thromboembolic event. For this purpose, we selected as our control the group of women previously used in an oral norethynodrel study by COOK et al.[4].

6,7-[^{3}H]-Norethynodrel (11 mg, 50–75 μCi), prepared according to KEPLER and TAYLOR[6], was administered orally to 7 women and plasma and urine collections were taken periodically. The samples were evaluated following the analytical methodology described by COOK et al.[4]. The enzyme preparation used for conjugated metabolite hydrolysis was type H-2 glucuronidase-sulfatase from *Helix pomatia* (Sigma Chemical Company, St. Louis, Mo.) containing approximately 120,000 Fishman units/ml (glucuronidase) and approximately 51,000 Whitehead/ml (arylsulfatase).

Results and Discussion. An average of 27.1% of the administered dose was excreted in the urine over 24 h. This compared with an average of 21.4% in the control subjects. In both groups, the urinary metabolites were almost exclusively in the conjugated form (94–97%). Approximately 40% of the conjugated metabolites were hydrolyzed by *H. pomatia*. The hydrolysate had the following composition: 5.4–6.3% norethindrone (II), 7.5–8.0% 3α-hydroxy metabolite (IIIa), 4.9–8.1% 3β-hydroxy metabolite (IIIb) and 73.5–77.3% polyhydroxy metabolites (substances more polar than 3β-metabolite on silica gel TLC). These values were similar to those found for the control group. The initially high 3β-hydroxy metabolite excretion rate observed in the control subjects was also noted in this study. The total amount of 3α-hydroxy and 3β-hydroxy metabolite excreted over 24 h was 0.82 and 0.54% of the administered dose, respectively. The same overall 3α-hydroxy/3β-hydroxy excretion ratio (3/2) was also noted in the control subjects.

The plasma data showed considerable intragroup variation. This behavior was noted in both the control and present study. The time course of total plasma metabolites present in the unconjugated form was the same in both groups (Table IA). However, plasma metabolite concentration, based on total radioactivity



Structure for steroids discussed (see footnote[2]). I. Norethynodrel. II. Norethindrone. IIIa. 3α-Hydroxy Metabolite. IIIb. 3β-Hydroxy Metabolite.

[1] A. P. FLETCHER and N. ALKJAERSIG in *Metabolic Effects of Gonadal Hormones and Contraceptive Steroids* (Eds. H. A. SALHANICK, D. M. KIPNIS and R. L. VAN DE WIELE; Plenum Press, New York-London 1969), p. 539.

[2] Structures are shown in Figure 1. Systematic names for compounds are as follows: Norethynodrel (I): 17β-hydroxy-17α-ethynylestr-5(10)-ene-3-one; Norethindrone (II): 17β-hydroxy-17α-ethynylestr-4-en-3-one; 3α-hydroxy metabolite (IIIa); 17α-ethynylestr-5(10)-ene-3α,17β-diol; 3β-hydroxy metabolite (IIIb): 17α-ethynylestr-5(10)-ene-3β,17β-diol.

[3] K. H. PALMER, F. T. ROSS, L. S. RHODES, B. BAGGETT and M. E. WALL, J. Pharmac. exp. Ther. *167*, 207 (1969).

[4] C. E. COOK, M. E. TWINE, C. R. TALLENT, M. E. WALL and R. C. BRESSLER, J. Pharmac. exp. Ther. *183*, 197 (1972).

[5] D. S. LAYNE, T. GOLAB, K. ARAI, G. PINCUS, Biochem. Pharmac. *12*, 905 (1963).

[6] J. A. KEPLER and G. F. TAYLOR, J. labell. Comp. *7*, 545 (1972).

Table I. Plasma metabolite concentrations. Study group (7 subjects); control group (4 subjects)

A) Unconjugated metabolites

Time (h)	Study (ng/ml ± SD)[a]	Control (ng/ml ± SD)
1	76.7 ± 46.8	54.5 ± 50.6
2	85.2 ± 22.0	58.5 ± 49.0
4	89.3 ± 25.1	80.3 ± 31.3
8	36.6 ± 15.3	45.0 ± 8.7
12	29.7 ± 18.7	42.3 ± 3.0
24	9.1 ± 5.5	17.0 ± 10.4

B) Total metabolites

Time (h)	Study (ng/ml ± SD)	Control (ng/ml ± SD)
1	275.1 ± 238.9	129.5 ± 91.5
2	297.2 ± 110.8	186.3 ± 102.3
4	427.7 ± 27.0	405.0 ± 140.7
8	226.6 ± 92.7[b]	389.5 ± 98.2
12	210.2 ± 143.7	329.3 ± 72.3
24	80.1 ± 35.8[b]	175.3 ± 24.6

C) Conjugated metabolites

Time (h)	Study (ng/ml ± SD)	Control (ng/ml ± SD)
1	197.0 ± 196.0	75.0 ± 49.1
2	212.0 ± 98.2	127.8 ± 57.8
4	338.3 ± 23.9	324.8 ± 114.3
8	190.0 ± 85.9[b]	344.5 ± 95.9
12	180.5 ± 127.3	287.0 ± 71.1
24	71.0 ± 27.6[b]	158.3 ± 27.6

[a] ± SD, standard deviation of the mean. [b] Significantly different from corresponding control group ($p < 0.05$) using unpaired Student *t*-test.

measurements, showed significant differences at the 8 and 24 h sampling period, the control group exhibiting higher total metabolite plasma levels at these times (Table IB). Workup of the plasma samples indicated that this increase was associated with a significantly larger conjugated metabolite fraction (Table IC). Evaluation of the plasma unconjugated fractions showed the following significant differences compared to the control data (Tables 2A–D): A) larger amounts of the polyhydroxy (polar) metabolites at 2 and 4 h, B) lower amounts of the 3β-hydroxy metabolite at 8 and 12 h and C) lower amounts of norethindrone at 8, 12 and 24 h. The causal relationship of these changes is not clear. The absolute magnitude of the concentration differences discussed above was largest for the increase in unconjugated fraction polar metabolites and the decreases in total and conjugated metabolite levels.

Table II. Composition of plasma free fraction. Study group (7 subjects); control group (3 subjects)

A) Polyhydroxylated (polar) metabolites

Time (h)	Study (ng/ml ± SD)[a]	Control (ng/ml ± SD)
1	32.6 ± 7.7	13.2 ± 4.9
2	36.4 ± 6.7[b]	16.0 ± 4.2
4	41.1 ± 10.4[b]	19.5 ± 1.6
8	17.2 ± 6.6	21.2 ± 7.5
12	15.2 ± 9.0	17.2 ± 7.6
24	4.0 ± 2.4	9.2 ± 6.4

B) β-Hydroxy metabolite

Time (h)	Study (ng/ml ± SD)	Control (ng/ml ± SD)
1	7.2 ± 5.0	6.8 ± 7.0
2	7.1 ± 2.8	4.7 ± 1.9
4	5.6 ± 2.3	5.5 ± 2.7
8	1.9 ± 1.3[b]	4.2 ± 0.90
12	1.6 ± 0.92[b]	3.4 ± 0.40
24	0.44 ± 0.34	1.4 ± 1.4

C) α-Hydroxy metabolite

Time (h)	Study (ng/ml ± SD)	Control (ng/ml ± SD)
1	25.6 ± 16.4	17.9 ± 7.0
2	29.2 ± 13.7	15.1 ± 4.9
4	30.2 ± 11.5	19.3 ± 11.3
8	8.2 ± 3.2	11.2 ± 3.2
12	7.2 ± 5.3	6.9 ± 3.8
24	1.2 ± 1.1	3.2 ± 2.2

D) Norethindrone

Time (h)	Study (ng/ml ± SD)	Control (ng/ml ± SD)
1	5.8 ± 4.1	7.1 ± 4.7
2	7.1 ± 2.5	4.8 ± 1.6
4	8.2 ± 4.2	6.7 ± 2.5
8	3.1 ± 1.5[b]	6.9 ± 2.6
12	1.9 ± 0.84[b]	4.8 ± 0.69
24	1.0 ± 0.80[b]	3.0 ± 1.7

[a] ± SD, standard deviation of the mean. [b] Significantly different from corresponding control group ($p < 0.50$) using unpaired Student t-test.

The results obtained in this study indicate that there is no basic qualitative difference in the metabolism of norethynodrel in subjects with a history of thrombophlebitic-thromboembolic episodes and in normal healthy women. Although some statistically significant differences were noted, it seems more reasonable to ascribe them to nonspecific effects (e.g., conjugation, polyhydroxy metabolite formation) and not to factors specifically affecting norethynodrel metabolism[7].

Zusammenfassung. Der Metabolismus von Norethyndrol, der progestiven Komponente verschiedener derzeitig verwendeter oraler Kontraceptiva, wurde bei Frauen mit einer thrombophlebetischen-thromboembolischen Krankengeschichte sowie bei normalen gesunden Frauen einer Kontrollgruppe untersucht. Der Norethyndrolmetabolismus beider Gruppen zeigte keine wesentlichen Unterschiede.

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Zygotic Mortality in *Ellobius lutescens* (Rodentia: Microtinae)

Matthey[1,2] reported that two species of voles, *Microtus oregoni* and *Ellobius lutescens*, had $2n = 17$ and demonstrated that males and females of *E. lutescens* had the same odd number of chromosomes. White[3] suggested that *E. lutescens* had 16 autosomes and that the heterochromosome was formed by fusion of X and Y elements in males, and two X elements in females; it followed that combinations of gametes with chromosomal numbers of $8+8$ or $9+9$ were lethal. White's hypothesis included *M. oregoni*, but it was later found that *M. oregoni* was a gonosomic mosaic[4] and this species was not characterized by 50% mortality of all zygotes[5]. Similarity in structure of the unpaired element in both sexes of *E. lutescens* was shown autoradiographically[6], and a hypothesis was developed that the sex structure was $XAAY$ (A = autosome) in males and XAA in females. This hypothesis is similar to that of White, since it admits a possibility of 50% mortality of males.

[1] R. Matthey, Arch. Julius Klaus-Stift. VererbForsch. *28*, 65 (1953).

[2] R. Matthey, Experientia *14*, 240 (1958).

[3] M. J. D. White, Proc. Zool. Soc. Calcutta, Mookerjee Mem. 1957, vol. 113.

[4] S. Ohno, J. Jainchill and C. Stenius, Cytogenetics *2*, 232 (1963).

[5] L. M. Brown and C. H. Coneway, J. Hered. *55*, 7 (1964).

[6] E. Castro-Sierra and U. Wolf, Cytogenetics *6*, 268 (1967).