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EFFECTS OF SOME CONTRACEPTIVE STEROIDS ON SERUM PROTEINS OF WOMEN

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Abstract—Daily oral doses of mestranol, from 25 to 120 μg, were given to healthy adult women for 21 days. Concentrations of serum proteins, sensitive to exogenous estrogen, were measured before and after treatment. Dose-dependent increases in ceruloplasmin and decreases in albumin, haptoglobin and orosomucoid, were observed. The effects of mestranol were significantly less than those of oral ethinylestradiol at any particular dose. Addition of 1·0 mg norethisterone to either estrogen had no effect in serum protein concentrations. When given alone, 1·0 mg norethisterone daily for 21 days had no effect on serum proteins, but 10·0 mg daily induced changes unlike those produced by estrogens. Similar results were seen with megestrol acetate.

IN A PREVIOUS report[1] significant dose-related changes in various serum proteins of healthy adult women were found after 21-day courses of oral ethinylestradiol [17α-ethinyl-1,3,5(10)-estratriene-3,17β-diol] given alone. We have now conducted a similar study of the second estrogen commonly used in oral contraceptive preparations: mestranol [3-methoxy-17α-ethinyl-1,3,5(10)-estratriene-17β-ol], given alone, or in combination with a progestogen.

METHODS AND MATERIALS

Micronized steroids were mixed with lactose and packed in gelatine capsules. The subjects were healthy women in the age range 19–36 years. None had any history of disease thought likely to complicate the study, and none had taken steroid pharmaceuticals for at least 3 months prior to the test period.

Each woman received 21 consecutive oral doses of mestranol at various concentrations. Each mestranol level investigated was represented by a group of 10–12 women. Blood was collected by venepuncture from fasting subjects at the beginning of the trial before any estrogen had been taken, then again after the 21 daily doses. Some women took a second 21-day course of mestranol at the same dose level as their first, but to which norethisterone (NE: 17α-ethinyl-17β-hydroxy-4-estren-3-one) had been added at 1·0 mg daily. A similar investigation was made using norethisterone combined with 50 μg daily of ethinylestradiol. Other groups received a second course of estrogen alone. Blood was collected from these subjects at the end of the second course of treatment.

In a second study, norethisterone or megestrol acetate (MGA: 17α-acetoxy-6-methyl-4,6-pregnadiene-3,20-dione) was given alone at either 1·0 mg or 10·0 mg daily for 21 consecutive days to each of five women. Blood was collected at the beginning and end of the trial periods.

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Finally, a similar study was conducted on women taking "sequential" oral contraceptives. Two products were examined: "Serial 28" (Glaxo) and "Ortho-Novin SQ" (Ortho). The first of these products consists of 16 daily oral doses of 100 μg ethinyl estradiol, followed by 5 daily oral doses of 100 μg EE + 1·0 mg megestrol acetate, while the second consists of 14 daily oral doses of 100 μg mestranol, followed by 7 daily oral doses of 100 μgM$^+$ 2·0 mg norethisterone. Blood specimens were taken from women before starting the first monthly course of tablets, again on days 14–16 and again on day 21. More blood specimens were collected on the same cycle days during the third monthly course of tablets. Serum was separated and analysed for albumin, haptoglobin and orosomucoid by a combination of standard methods of immunoelectrophoresis,[2] and for ceruloplasmin by *p*-phenylenediamine oxidation.[3] Mean values and standard deviations were calculated for each serum protein for each group of women. Statistical significance of any differences was assessed by a Student *t*-test.

RESULTS

Mean values and standard deviations of each group of women are shown in Tables 1–3. For comparison, we have also included the results of our previous study[1] with ethinylestradiol. Examination of these results shows that both estrogens cause a dose-dependent increase in ceruloplasmin concentration, but decrease in albumin, haptoglobin and orosomucoid concentration.

The albumin concentration was significantly different ($P < 0·05$) from the untreated control for ethinyl-estradiol at the 20 μg daily dose level, but not until the 85 μg daily dose level for mestranol. The minimum daily oral dose of estrogen required to induce

TABLE 1. EFFECTS OF ESTROGENS ON SERUM PROTEIN CONCENTRATIONS*

Steroid	Daily dose (μg)	No. of women	Serum protein concn (mean value ± S.D.) Albumin (g/100 ml)	Ceruloplasmin (mg/100 ml)	Haptoglobin (mgHbBC/100 ml)	Orosomucoid (mg/100 ml)
None (pretreatment)	—	45	4·1 ± 0·4	39 ± 8	115 ± 9	77 ± 14
MEE*	25	12	4·0 ± 0·3	41 ± 11	105 ± 12	78 ± 12
	50	12	3·9 ± 0·4	45 ± 13	95 ± 14	72 ± 15
	60	10	3·9 ± 0·3	50 ± 12	90 ± 16	74 ± 16
	75	10	3·7 ± 0·5	53 ± 10	89 ± 18	66 ± 15
	85	10	3·5 ± 0·4	59 ± 11	90 ± 15	68 ± 11
	100	12	3·6 ± 0·3	65 ± 13	86 ± 21	60 ± 17
	120	10	3·5 ± 0·5	71 ± 15	82 ± 20	61 ± 18
EE†	10	10	3·8 ± 0·3	48 ± 5	101 ± 20	65 ± 15
	20	12	3·5 ± 0·3	54 ± 15	92 ± 21	59 ± 14
	50	11	3·3 ± 0·2	76 ± 8	88 ± 28	54 ± 18
	75	10	3·2 ± 0·3	78 ± 10	80 ± 31	53 ± 15

* MEE = mestranol.
† EE = ethinylestradiol.

TABLE 2. EFFECTS OF ESTROGENS COMBINED WITH PROGESTOGEN ON SERUM PROTEIN CONCENTRATIONS*

Treatment	T group	Daily dose (μg)	No. of days	Cycle no.	No. of women	Serum protein concn (mean values ± S.D.) Albumin (g/100 ml)	Cerulo-plasmin (mg/100 ml)	Hapto-globin (mgHb BC/100 ml)	Oroso-mucoid (mg/100 ml)
None	all	—	—	—	51	4·1 ± 0·4	40 ± 9	114 ± 10	76 ± 12
MEE	A	50	21	1	5	3·9 ± 0·4	44 ± 12	97 ± 13	74 ± 13
	A	50	21	2	5	3·9 ± 0·4	46 ± 14	94 ± 15	71 ± 16
MEE	B	100	21	1	5	3·5 ± 0·5	68 ± 14	88 ± 18	62 ± 15
	B	100	21	2	5	3·6 ± 0·4	65 ± 12	85 ± 20	60 ± 17
EE	C	50	21	1	5	3·4 ± 0·5	77 ± 10	84 ± 21	55 ± 19
	C	50	21	2	5	3·3 ± 0·5	74 ± 12	87 ± 23	53 ± 21
MEE	D	50	21	1	12	3·9 ± 0·4	46 ± 13	95 ± 22	58 ± 16
MEE + NE	D	50+1000	21	2	12	3·8 ± 0·4	48 ± 12	98 ± 19	56 ± 14
MEE	E	100	21	1	12	3·5 ± 0·3	65 ± 13	80 ± 26	55 ± 14
MEE + NE	E	100+1000	21	2	12	3·6 ± 0·2	62 ± 11	82 ± 25	58 ± 17
EE	F	50	21	1	12	3·4 ± 0·4	78 ± 14	87 ± 18	56 ± 19
EE + NE	F	50+1000	21	2	12	3·5 ± 0·5	82 ± 11	90 ± 17	59 ± 21

* Abbreviations as for Table 1.

TABLE 3. EFFECTS OF PROGESTOGENS ALONE ON SERUM PROTEIN CONCENTRATIONS*

Treatment	Daily dose (μg)	No. of days	No. of women	Serum protein concn (mean value ± S.D.) Albumin (g/100 ml)	Cerulo-plasmin (mg/100 ml)	Hapto-globin (mgHbBC/100 ml)	Oroso-mucoid (mg/100 ml)
None (pre-treatment)	—	—	20	4·2 ± 0·3	37 ± 9	112 ± 10	79 ± 11
NE	1000	21	5	4·1 ± 0·5	49 ± 18	109 ± 27	64 ± 18
	10,000	21	5	3·8 ± 0·6	54 ± 15	139 ± 23	66 ± 13
MGA	1000	21	5	4·0 ± 0·5	39 ± 12	110 ± 23	68 ± 12
	10,000	21	5	3·7 ± 0·6	41 ± 14	142 ± 28	65 ± 15

* Abbreviations as for Table 1.

a statistically significant change ($P < 0.05$) in concentration of the other three serum proteins was as follows:

Ceruloplasmin—ethinylestradiol 10 μg, mestranol 60 μg
Haptoglobin —ethinylestradiol 50 μg, mestranol 75 μg
Orosomucoid —ethinylestradiol 20 μg, mestranol 100 μg

Addition of 1·0 mg norethisterone to either 50 μg or 100 μg daily mestranol, or to 50 μg daily ethinylestradiol had no significant effect on the estrogen-induced changes in any of the four serum proteins (Table 2).

When norethisterone was given alone (Table 3), 1·0 mg daily had little action on serum proteins, though ceruloplasmin was slightly increased ($P < 0.05$) and orosomucoid slightly decreased ($P < 0.05$). However, 10 mg norethisterone daily induced a non-significant decrease in albumin and orosomucoid, but a significant increase in both ceruloplasmin ($P < 0.02$) and haptoglobin ($P < 0.01$). Similar results were seen with megestrol acetate given alone. This change in haptoglobin concentration is the reverse of that seen with estrogens alone, or with estrogens plus a low dose of progestogen.

Results with sequential oral contraceptives (Table 4) are generally similar to those with estrogens given alone. Changes with the mestranol product ("Ortho-Novum SQ") are significantly less than those with the ethinylestradiol product ("Serial 28"). Changes induced by the first course of estrogen were not altered by addition of progestogen during the second half of the cycle, and were similar in the first and third cycles.

TABLE 4. EFFECT OF SEQUENTIAL ORAL CONTRACEPTIVES ON SERUM PROTEIN CONCENTRATIONS*

Product	Treatment	Cycle	Daily dose (μg)	No. of days	No. of women	Serum protein concn (mean value ± S.D.) Albumin (g/100 ml)	Ceruloplasmin (mg/100 ml)	Haptoglobin (mgHbBC/100 ml)	Orosomucoid (mg/100 ml)
Ortho-Novin SQ	pre-treatment	—	—	—	10	4·1 ± 0·5	40 ± 12	108 ± 16	80 ± 16
	MEE	1	100	14	10	3·6 ± 0·4	66 ± 14	88 ± 19	59 ± 18
	MEE+	1	100+						
	NE		2000	7	10	3·7 ± 0·5	62 ± 15	83 ± 21	63 ± 21
	MEE	2	100	14	10	3·5 ± 0·5	64 ± 11	86 ± 20	61 ± 17
	MEE+	2	100+						
	NE		2000	7	10	3·6 ± 0·5	68 ± 12	85 ± 18	60 ± 18
Serial 28	pre-treatment	—	—	—	10	4·2 ± 0·4	38 ± 10	112 ± 12	76 ± 15
	EE	1	100	16	10	3·1 ± 0·6	75 ± 15	79 ± 15	51 ± 12
	EE+	1	100+						
	MGA		1000	5	10	3·0 ± 0·5	79 ± 18	83 ± 18	55 ± 15
	EE	2	100	16	10	3·2 ± 0·6	81 ± 19	81 ± 20	58 ± 18
	EE+	2	100+						
	MGA		1000	5	10	3·0 ± 0·5	76 ± 14	86 ± 19	52 ± 20

* Abbreviations as for Table 1.

DISCUSSION AND CONCLUSIONS

In animals, mestranol and ethinylestradiol have approximately the same anti-gonadotrophic activity, but ethinylestradiol is a more potent estrogen.[4] The estrogenic activity of these compounds in humans is exceedingly difficult to quantify, but mestranol is generally accepted to be somewhat weaker than ethinylestradiol, and is included in pharmaceutical products at a slightly higher level.

From effects on serum proteins sensitive to exogenous estrogens, it appears that mestranol is less active in adult women than ethinylestradiol. The dose-related effects on the four proteins investigated during this study indicate activity ratios for ethinyl-estradiol to mestranol of between 1:1·5 and 1:6.

The clinical significance of these results is uncertain, but the increased incidence of thrombo-embolic disease in women taking oral contraceptives is thought[5] to be related to effects of the estrogen component on blood biochemistry though the precise mechanism is unknown. Our findings indicate significantly less alteration in protein concentrations with mestranol than with ethinylestradiol at any dose level. Most oral contraceptive preparations of the "combined-type" now contain 50 μg ethinylestradiol, though some contain 50 μg mestranol. The latter products will be associated with less change in serum protein concentrations.

The progestogens used in this study (norethisterone and megestrol acetate) had little action on serum proteins when given alone at the dose used in oral contraceptives, though larger amounts produced significant changes. Addition to 50 μg daily of either estrogen at 1·0 mg daily had no effect on the estrogen-induced changes.

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