Effects of oestrogen and progestogen on serum levels of α_2 -macroglobulin, transferrin, albumin, and IgG

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SYNOPSIS The levels of four serum proteins, assayed by a radial immunodiffusion technique, have been measured in healthy women who had been given either the oestrogen or progestogen component of a combined oral contraceptive preparation for three weeks. Raised α_2 -macroglobulin and transferrin levels were found after oestrogen treatment but albumin and IgG did not significantly alter. In the progestogen-treated group all four proteins remained unchanged. The four proteins have also been assayed at frequent intervals during the normal menstrual cycle. No evidence of cyclical variation was found.

In a previous study using a radial immunodiffusion technique (Horne, Howie, Weir, and Goudie, 1970) we demonstrated significant increases in the levels of serum α_2 -macroglobulin, transferrin, and IgG in normal females who had received combined oestrogen/progestogen oral contraceptives. It therefore seemed of interest to determine whether these changes could be produced by the action of oestrogen or progestogen components administered alone and whether endogenous production of steroid hormones during the normal menstrual cycle caused physiological variation in the serum levels of these proteins.

Subjects and Methods

Two groups of 14 healthy females (mean ages 20.6 and 20.9 years, ranges 17-28 and 17-30) were given respectively either 50 μ g of the oestrogen mestranol or 1 mg of the progestogen ethynodiol diacetate daily for a period of three weeks beginning on day 5 of a menstrual cycle. Before administration of these preparations serum was obtained on day 3 of the cycle; a further six samples were taken at weekly intervals during treatment and the subsequent three weeks.

To determine the effects of endogenous hormones sera were also obtained from a third group of 14 healthy females (mean age 19.9 years, range 19-23) at three- to four-day intervals throughout one complete menstrual cycle.

The sera were stored for up to 16 weeks at -20°C before the serum protein levels were measured by a

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radial immunodiffusion technique (Fahey and McKelvey, 1965; Mancini, Carbonara, and Heremans, 1965). The specific antisera were prepared as described in a previous communication (Horne *et al.*, 1970). To minimize the effects of interplate variation (Thompson, Horne, Steele, and Goudie, 1969) sera from any one subject were always tested in duplicate in a single assay plate in randomized wells. 'Absolute' values for each protein were calculated from a calibration curve prepared with solutions of a freeze-dried, reconstituted human serum containing 3, 6, 12, and 18 g protein per 100 ml and standardized with reference to a Behringwerke serum containing specified concentrations of the proteins.

Total serum iron-binding capacity (TIBC) was measured by the method of Young and Hicks (1965) on sera obtained before and after three weeks' treatment with oestrogen and progestogen.

Results

Table I shows that mestranol (M) leads to significant maximum increases in the serum levels of α_2 -macroglobulin and transferrin of 23% and 28% above the pretreatment levels ($P < 0.01$). The increases were maximal one week after completion of treatment and even two weeks later the levels had not returned to the pretreatment levels. There was no significant change in α_2 -macroglobulin or transferrin following administration of ethynodiol diacetate; albumin and IgG did not significantly alter with either mestranol or ethynodiol diacetate.

No significant change in the serum levels of any

Protein (mg/100 ml)	Preparation	Treatment				After Treatment		
		Day 0	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
α_2 -Macroglobulin	M	291 $\pm$ 57 ¹	331 $\pm$ 51	348 $\pm$ 57	352 $\pm$ 57	358 $\pm$ 63 ¹	343 $\pm$ 35	334 $\pm$ 61
	ED	287 $\pm$ 67	291 $\pm$ 67	299 $\pm$ 63	305 $\pm$ 60	296 $\pm$ 67	297 $\pm$ 63	293 $\pm$ 74
Transferrin	M	257 $\pm$ 56 ¹	275 $\pm$ 63	297 $\pm$ 61	306 $\pm$ 69	330 $\pm$ 70 ¹	278 $\pm$ 62	288 $\pm$ 54
	ED	269 $\pm$ 54	288 $\pm$ 62	289 $\pm$ 44	276 $\pm$ 41	289 $\pm$ 55	286 $\pm$ 34	292 $\pm$ 66
Albumin	M	4318 $\pm$ 402	4454 $\pm$ 271	4371 $\pm$ 285	4304 $\pm$ 368	4415 $\pm$ 398	4311 $\pm$ 225	4300 $\pm$ 402
	ED	4293 $\pm$ 385	4461 $\pm$ 396	4361 $\pm$ 268	4346 $\pm$ 340	4413 $\pm$ 503	4317 $\pm$ 356	4221 $\pm$ 353
IgG	M	1183 $\pm$ 307	1122 $\pm$ 318	1200 $\pm$ 359	1092 $\pm$ 285	1242 $\pm$ 349	1121 $\pm$ 373	1138 $\pm$ 359
	ED	1130 $\pm$ 351	1112 $\pm$ 277	1115 $\pm$ 290	1041 $\pm$ 65	1064 $\pm$ 240	1128 $\pm$ 224	1100 $\pm$ 314

Table I Effects of mestranol (M) and ethynodiol diacetate (ED) on serum protein levels (mean $\pm$ SD in mg/100 ml) in 14 subjects in each group¹Student's *t* = 2.9556 *P* < 0.01²Student's *t* = 3.00557 *P* < 0.01

Protein (mg/100 ml)	Day of Menstrual Cycle						
	1-4	5-8	9-12	13-16	17-20	21-24	25-28 ⁺
α_2 -Macroglobulin	420 $\pm$ 75	433 $\pm$ 139	407 $\pm$ 66	407 $\pm$ 117	385 $\pm$ 55	441 $\pm$ 139	385 $\pm$ 57
Transferrin	226 $\pm$ 39	227 $\pm$ 42	227 $\pm$ 45	233 $\pm$ 37	227 $\pm$ 38	227 $\pm$ 31	211 $\pm$ 37
Albumin	4277 $\pm$ 395	4414 $\pm$ 332	4417 $\pm$ 464	4279 $\pm$ 415	4612 $\pm$ 557	4414 $\pm$ 622	4200 $\pm$ 424
IgG	1218 $\pm$ 415	1164 $\pm$ 304	1310 $\pm$ 398	1175 $\pm$ 375	1131 $\pm$ 358	1321 $\pm$ 474	1337 $\pm$ 701

Table II Serum protein levels (mean $\pm$ SD) in 14 subjects during one menstrual cycle

of the four proteins was observed during one menstrual cycle (Table II).

Table III shows a significant rise of 20% in TIBC in the mestranol-treated group but not in the progestogen-treated group in keeping with the results obtained with the immunoassay of transferrin.

Preparation	Day 0 (Before Treatment)	Day 7 (After Treatment)
M	354 $\pm$ 42 ¹	424 $\pm$ 61 ¹
ED	360 $\pm$ 48	379 $\pm$ 63

Table III Effects of mestranol (M) and ethynodiol diacetate (ED) on total serum iron-binding capacity (mean $\pm$ SD in μ g/100 ml)¹*t* = 3.531 *P* < 0.0025

Discussion

Our results clearly show that oestrogen is responsible for significant increases in α_2 -macroglobulin and transferrin of a similar order of magnitude to those found following administration of combined oral contraceptives in our previous study (Horne *et al*, 1970).

Like Laurell, Kullander, and Thorell (1969) we do not find a significant effect of progestogen on transferrin levels whereas Briggs and Staniford (1969) have found an increase in total iron-binding capacity, presumably attributable to a rise in transferrin, following administration of progestogen. Furthermore, Musa, Doe, and Seal (1967) find no

increase in transferrin levels following oestrogen treatment in marked contrast to our findings. Unfortunately in all the above studies different synthetic steroid hormones have been used for varying periods. Since the other workers measured TIBC (Briggs and Staniford, 1969) and obtained different results, we wondered whether there might be a discrepancy between transferrin levels and TIBC following hormone therapy but in our cases this proved not to be so.

In our short-term experiments we have again found no significant change in albumin, which is known to fall by approximately 10% in patients receiving combined oral contraceptives for several months (Hønger and Rossing, 1969).

We suspect that our previous finding of a 17% rise in serum IgG levels following combined oestrogen/progestogen oral contraceptives is fortuitous. Laurell, Kullander and Thorell (1967) demonstrated no such increase nor have we in our present study with oestrogen and progestogen administered separately.

Finally our failure to demonstrate changes in the levels of these proteins during the menstrual cycle suggests that if physiological variations do occur they must be too small to be measured by our methods.

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