

RESULTS



1. The Antibody Titre

Experiments were performed as described under 5.2 page 27. Various concentration at final dilution of 1:7,000, 1:28,000, 1:56,000 and 1:112,000 of antibody were mixed with standard progesterone in concentration of 0, 50 and 500 pg/0.5 ml and 0.1 ml of ^3H -progesterone ($\approx 10,000$ cpm). The mixtures were incubated overnight at 4°C and proceeded as described on page 28. Figure 6 (page 34) showed that among the four antibody dilutions tried, the appropriated dilution required to bind 40 to 50% of approximately 10,000 cpm of ^3H -progesterone was 1:28,000. This concentration was, therefore, used in subsequent experiments.

2. Effect of Time and Temperature of Incubation

Standard curves of progesterone were performed. The range of the standards was 2.5-1,000 pg/tube. Antibody (0.1 ml) at final dilution of 1:28,000 and ^3H -progesterone ($\approx 10,000$ cpm in 0.1 ml) were used. Four different temperatures for incubation 4°C , 20°C , 37°C and 50°C were tested. Incubation time was varried from 2, 4, 18 and 24 hours. The results are shown in table 1 (pages 35 and 36). It can be seen that at a usable working range of the standard between 50-500 pg, a reasonable percentage change

Figure 7

PROGESTERONE ANTIBODY TITRE STUDY

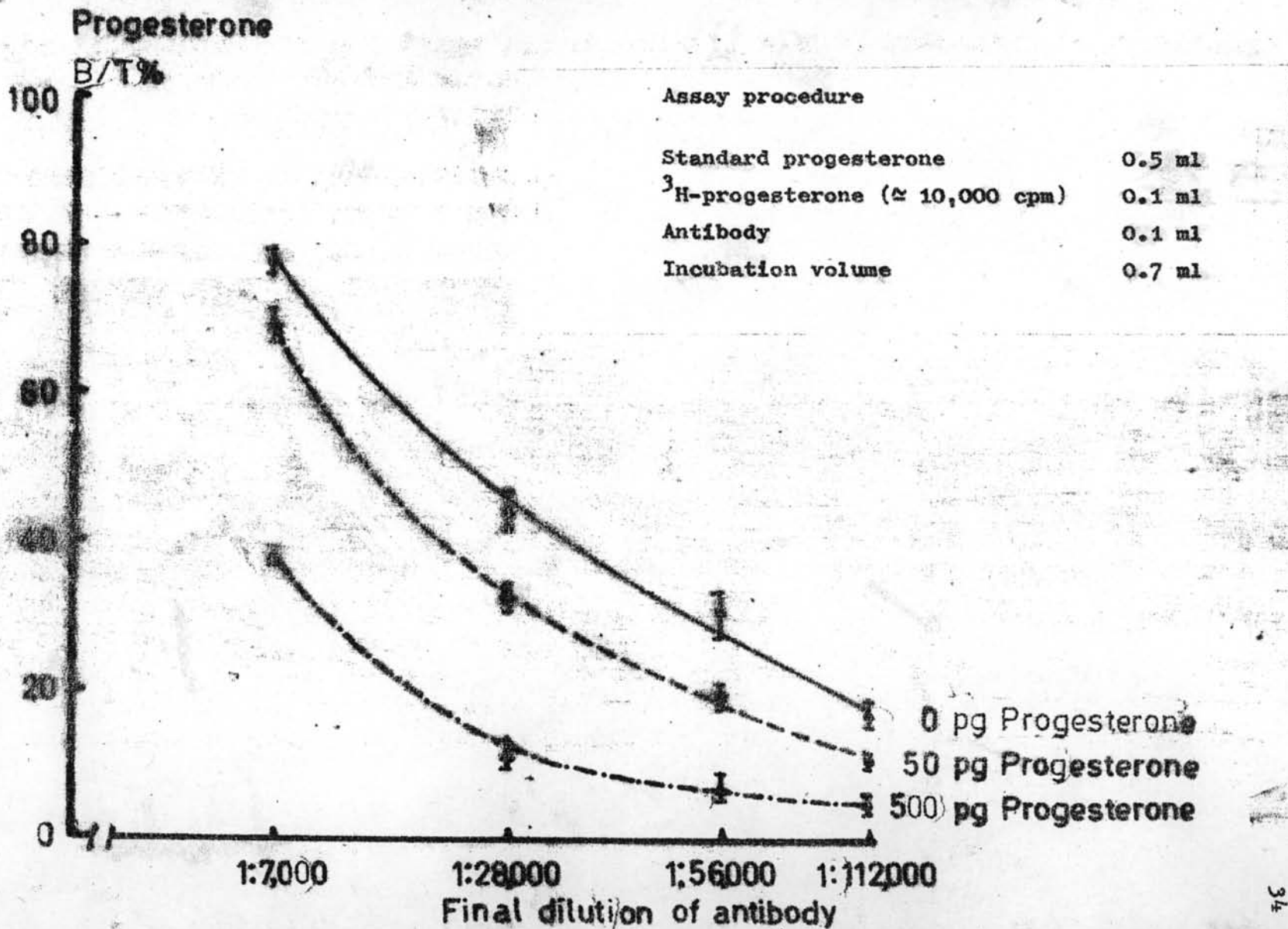


Table 1 The effect of temperature and time of incubation.

Incubation temperature and time	B/T	$\Delta B/B_0$				
		$\Delta 0-25$	$\Delta 0-50$	$\Delta 25-50$	$\Delta 50-500$	$\Delta 500-1000$
		pg	pg	pg	pg	pg
4°C						
2 hr.	45.52	8.73	14.70	5.93	43.38	9.04
4 hr.	51.93	10.22	21.48	11.26	50.42	5.51
18 hr.	51.04	8.54	22.28	13.74	63.73	12.52
24 hr.	50.01	14.76	14.55	0.21	51.98	12.71
20°C						
2 hr.	24.75	16.38	20.47	4.09	43.75	7.53
4 hr.	21.52	11.50	20.55	9.05	48.48	5.46
18 hr.	29.47	10.11	16.55	6.44	48.23	12.20
24 hr.	29.48	13.68	22.47	8.79	38.07	12.65
37°C						
2 hr.	21.44	4.24	18.16	13.92	44.04	9.47
4 hr.	20.91	16.13	21.44	5.31	40.19	11.51
18 hr.	29.80	3.80	19.22	15.42	46.20	8.57
24 hr.	28.53	5.69	16.13	10.44	36.45	22.55

Table 1 The effect of temperature and time of incubation. (Cont.)

Incubation temperature and time	B/T	$\Delta B/B_0$				
		$\Delta 0-25$	$\Delta 0-50$	$\Delta 25-50$	$\Delta 50-500$	$\Delta 500-1000$
		pg	pg	pg	pg	pg
50°C						
2 hr.	27.99	29.59	44.51	14.92	13.93	14.81
4 hr.	26.35	24.76	46.56	21.78	14.65	14.53
18 hr.	20.08	22.55	38.79	16.24	18.13	8.19
24 hr.	20.03	26.95	42.35	15.40	11.63	11.44

B = Labelled progesterone bound to antibody expressed as cpm/tube

B_0 = Amount of labelled progesterone bound to antibody in absence of unlabelled progesterone.

T = Total bound count

Δ = Percentage change.

in binding was obtained when the incubation was carried out at either 4°C or 20°C for 2-18 hours. The 4°C and overnight incubation was chosen for further performed experiments as it was considered to be the most practical condition.

3. Effect of Charcoal Suspension

Experimental condition for this study was shown on figure 8 page 38. Four different concentration (0.3%, 0.6%, 1.5%, 3%) were used to separate free and bound hormones.

The 0.6% charcoal was found to be the most suitable concentration since it was the lowest concentration to give reasonable blank values and steepest slope of the standard curve as well as the initial binding.

The following assay procedure was, therefore, applied for subsequent studies.

0.5 ml standard solution

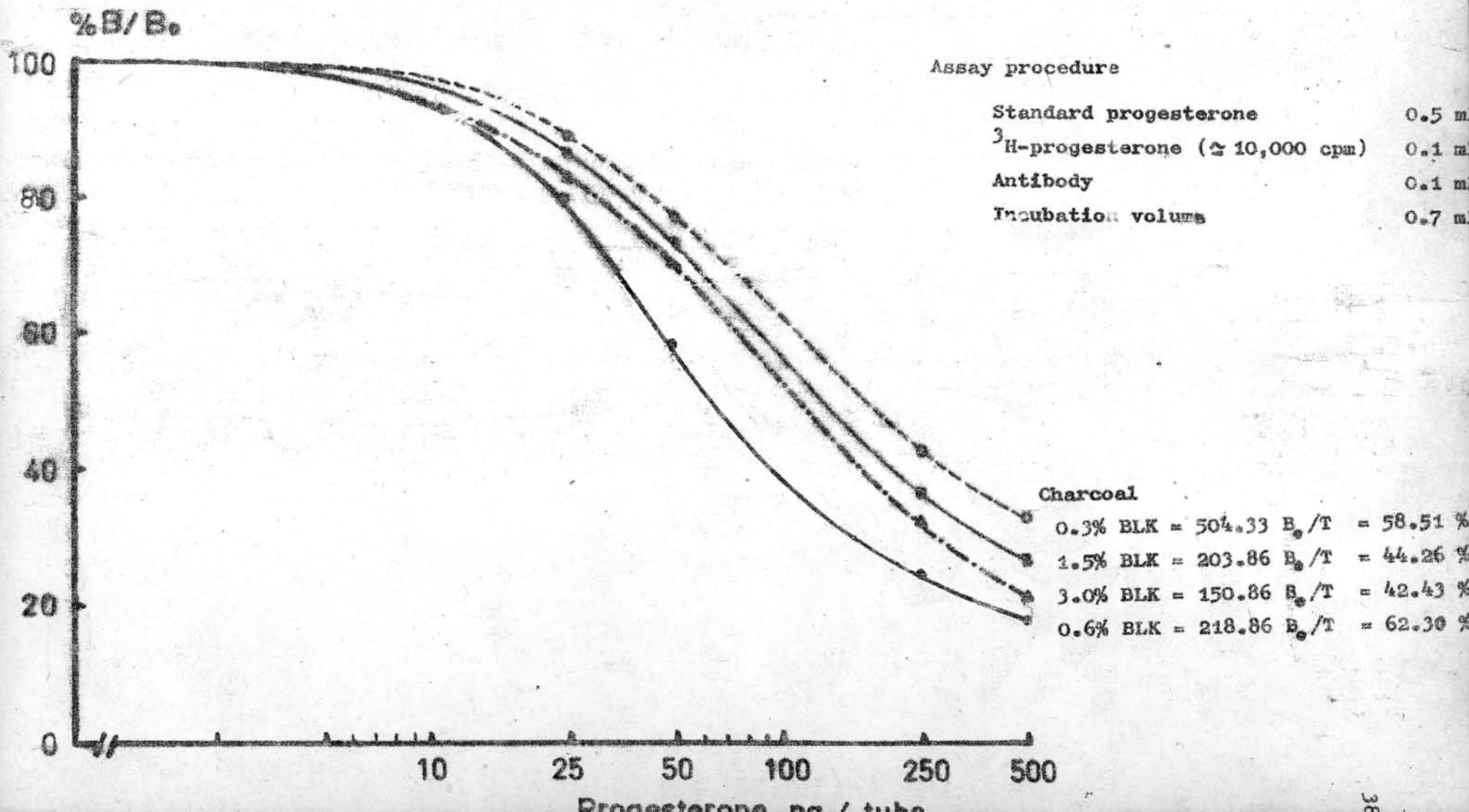
0.1 ml of antibody at a final dilution of 1:28,000

0.1 ml of ³H-progesterone (≈10,000 cpm)

The mixture was incubated overnight (18-24 hours) at 4°C and the free and bound hormones were separated by the addition of 0.2 ml of 0.625% charcoal suspension.

Figure 8

Effect of Different Concentrations of Charcoal on The Progesterone Standard Curve



4. The Specificity of the Assay Method

Ten steroids-pregnolone, corticosterone, cortisol, 17-hydroxyprogesterone, 20-dihydroprogesterone, testosterone, androstenedione, estradiol, estrone, estriol and the contraceptive steroidal pills "Norgestrel" were used in the concentration of 25, 100, 250, 500, 1,000 and 5,000-pg/0.5 ml to test their cross-reaction with the progesterone antibody. Assay procedure was similar to the previous experiment. The results are shown in figures 9 , 10 and 11 (pages 40 , 41 and 42). All the steroids tested showed no detectable cross-reaction with the antiserum used.

5. The Sensitivity of the Assay Method

The sensitivity of the method was determined as described under 5.5 (page 29) and found to be varied from 10-25 pg/tube (figure 12, page 43).

6. The Accuracy of the Assay System

Recovery experiments were performed by measuring the known amounts of progesterone added to 1 ml serum samples.

Standard solutions of progesterone (50 pg, 100 pg, 300 pg, 500 pg and 5,000 pg) were added into 1 ml of

Figure 9

Specificity Studies of Progesterone Antiserum

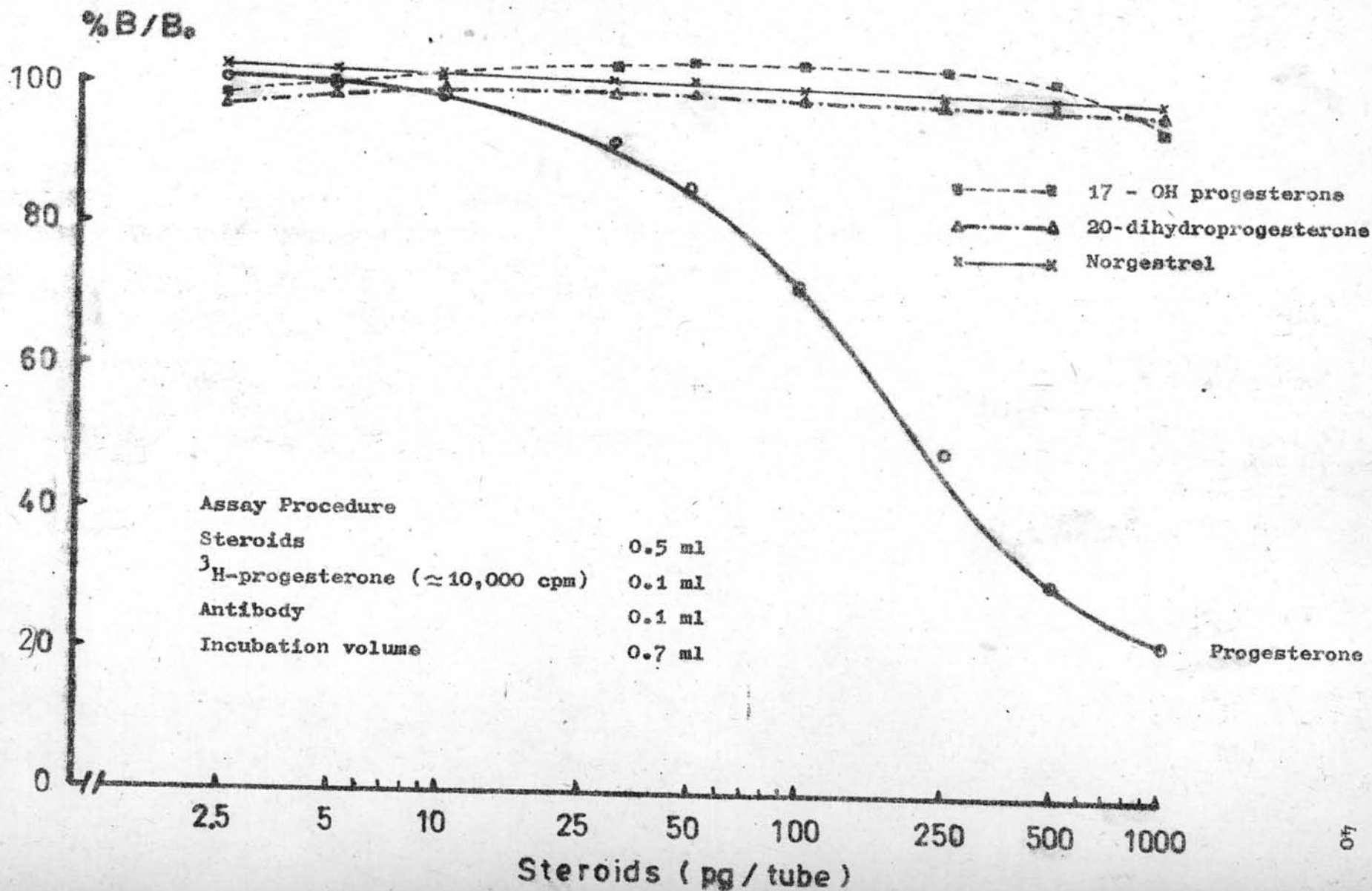


Figure 10

Specificity Studies of Progesterone Antiserum (continue)

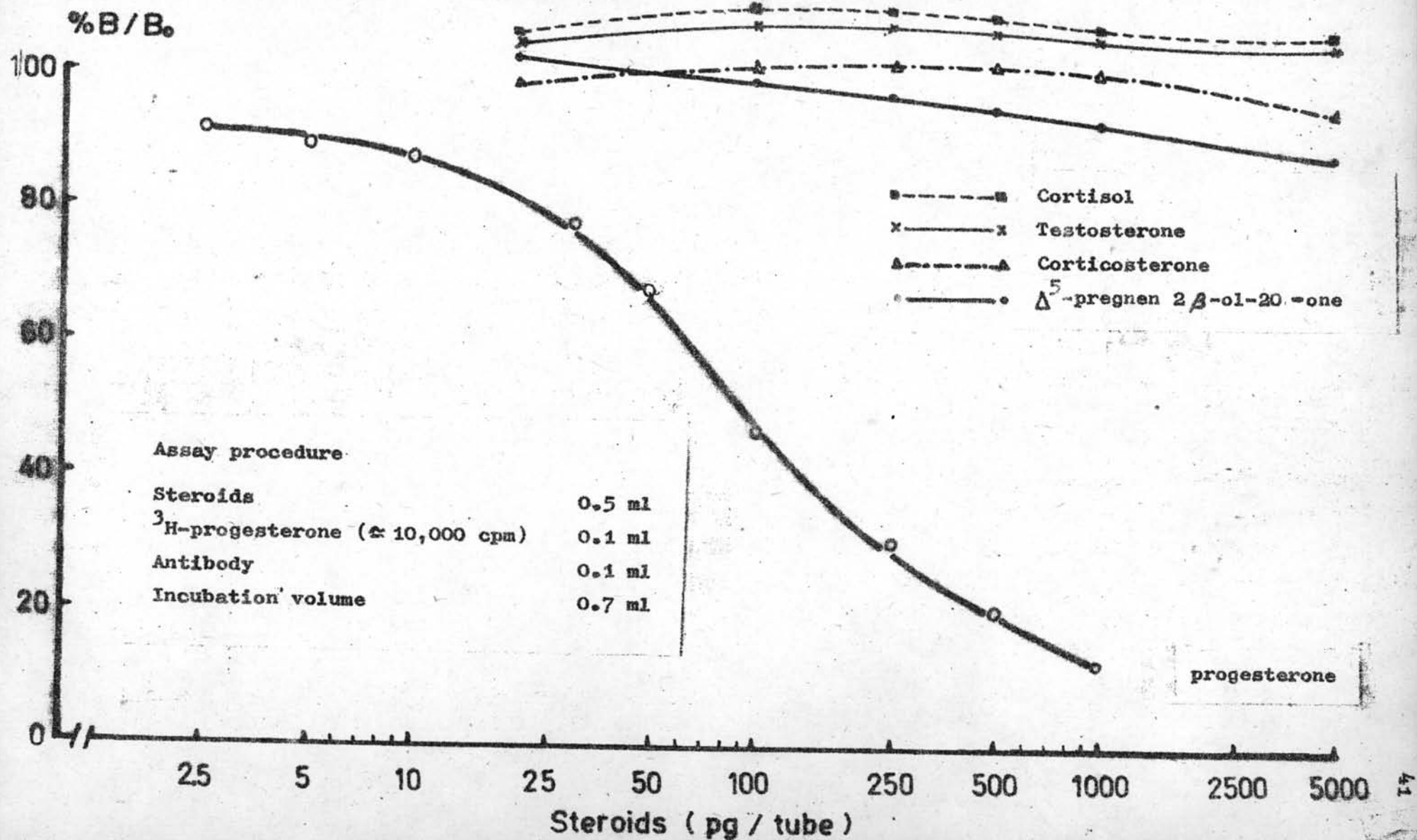


Figure 11

Specificity Studies of Progesterone Antiserum (continue)

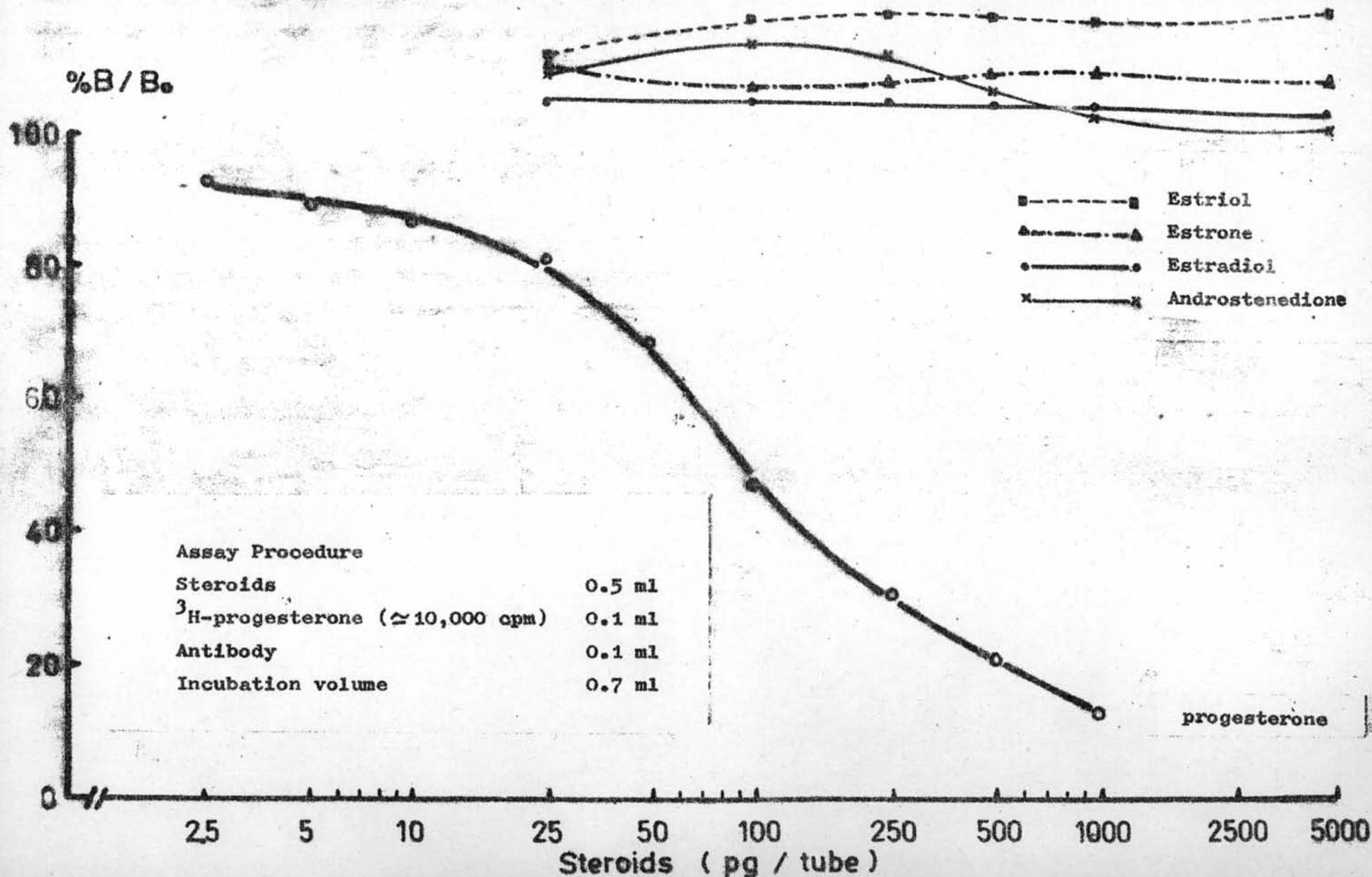
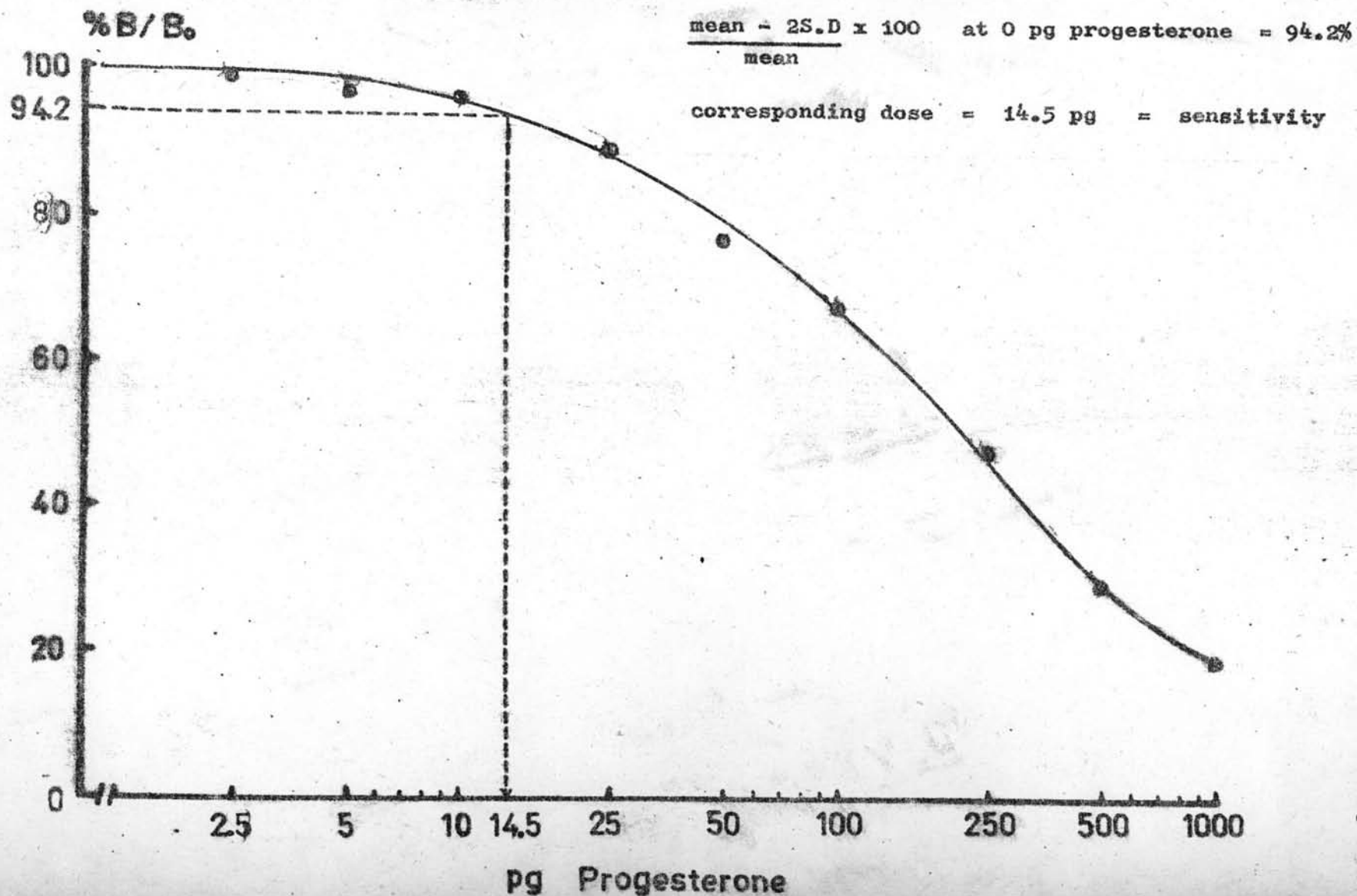


Figure 12

THE SENSITIVITY OF STANDARD CURVE



"hormone-free serum". These sera were then extracted and assayed as described previously. The results (table 2, page 45) indicated that if the progesterone concentration in the serum samples was more than 300 pg/ml, the percentage recovery was between 88.2-104.2% and the value of measured progesterone did not differ by more than 13% from the expected values. Thus, any values in the present study of less than 300 pg/ml was considered to be inaccurate and unreliable.

7. The Precision of the Method

Three pooled serum A, B, C containing 0, 500 and 5,000 pg/ml serum respectively of added progesterone were used in the determination of "within-assay precision". The results are shown in table 3, page 46.

The "between-assay precision" was tested in the same manner except that 2,500 pg instead of 5,000 pg, of standard progesterone was added to pooled C. Experiments were performed for 3 consecutive days. Table 4, page 47 shows the results obtained.

The within-assay coefficient of variation of progesterone in pooled serum containing 0, 500 and 5,000 pg/ml were 0, 8.6 and 7.2% respectively (table 3, page 46). For the between-assay precision, the coefficient of variation of progesterone in pooled serum containing 0, 500 and 2,500 pg/ml were 19.5, 7.1 and 3.4 respectively (table 4, pages 47 and 48).



Table 2 Percentage recovery of progesterone added into 1 ml of serum.

Amount of progesterone added	Number of determinations	Mean \pm S.D. (pg/ml)	Percentage recovery (%)
0 pg	8	6.2 \pm 3.1	-
50 pg	10	222.1 \pm 35.5	445.3
100 pg	10	180.1 \pm 35.1	180.1
300 pg	10	312.6 \pm 24.9	104.2
500 pg	10	441.0 \pm 25.1	88.2
5,000 pg	10	4979.0 \pm 51.0	99.6

Table 3 Precision of measuring progesterone levels
in pooled serum within-assay.

Pooled Serum	Progesterone added (pg/ml)	Progesterone assay obtained (pg/ml)	Mean \pm S.D. (pg/ml)	cv
1	0	0	-	-
2		11.4		
3		0		
4		8.2		
5		0		
6		0		
1	500	535.4	553.2 \pm 47.6	8.6
2		488.8		
3		628.6		
4		556.1		
5		531.1		
6		579.4		
1	5000	5546.1	5065.4 \pm 363.5	7.2
2		4607.5		
3		4992.3		
4		4800.3		
5		5005.4		
6		5440.7		

Table 4 Precision of measuring progesterone levels in pooled serum between-assay.

Number of samples	Amount of progesterone added in pooled serum (pg/ml)	1st day of experiment		2nd day of experiment		3rd day of experiment	
		progesterone (pg/ml)	mean	progesterone (pg/ml)	mean	progesterone (pg/ml)	mean
1	0	57.9	47.5	85.1	79.4	71.0	87.3
2		58.6		82.5		71.0	
3		57.4		85.1		84.6	
4		43.7		56.7		86.4	
5		40.1		88.6		105.7	
6		47.5		78.7		104.8	
1	500	513.2	577.9	527.9	507.7	525.4	516.3
2		555.1		522.6		423.7	
3		578.1		469.9		418.9	
4		591.5		470.6		424.2	
5		613.9		546.5		643.3	
6		615.3		508.5		622.2	
1	2500	2335.0	2550.7	2574.3	2581.7	2289.5	2404.1
2		2384.6		2485.5		2215.5	
3		2601.1		2529.1		2400.0	
4		2502.4		2597.0		2431.3	
5		2656.1		2652.0		2800.0	
6		2824.8		2652.0		2288.3	

Table 4 Precision of measuring progesterone levels in pooled serum between assay. (Cont.)

Pooled serum (pg/ml)	mean (pg/ml)	S.D. (pg/ml)	cv
0	71.4	21.0	19.5
500	533.9	38.2	7.1
2500	2512.1	94.8	3.7

8. Studies on Menstrual Cycle

Two groups of subjects were studied.

Group 1. Normal menstrual cycle. Four normally menstruating subjects were studied for three consecutive cycles. Blood samples were drawn on days 2, 8, 14, 15, 16, 17, 18, 19, 22, 23, 24, 26 and 29 of the cycles.

Group 2. Norgestrel-treated menstrual cycle. Blood samples were obtained from 10 women taking the contraceptive pills "Norgestrel". The samples were taken during the second, sixth and twelfth cycles of the contraceptive treatment in four subjects; during the second and sixth in four subjects and during only the second cycle in two subjects.

Table 5 (page 50) and figures 13, 14, 15 and 16 (pages 51 and 52) show that the levels of progesterone in four subjects during normal menstrual cycle varied from 0.48 to 0.60 ng/ml (mean value 0.53 ± 0.04 ng/ml) in the follicular phase; and during the luteal phase, varied from 2.04 to 13.50 ng/ml (mean value 6.31 ± 2.47 ng/ml).

It can be seen that in group 2 (table 7 pages 53, 54, 55 and 56), all ten except one (SG₉) of the Norgestrel-treated patients has the reduction in progesterone production to less than 3 ng/ml and the estradiol levels were less than 150 pg/ml. Only one patient (SG₉) produced progesterone and

Table 5 Progesterone levels during different phases of normal menstrual cycle.

Physiological condition	Mean \pm S.D. (ng/ml)	Range (ng/ml)	Number of subjects
Follicular phase	0.54 \pm 0.04	0.48 - 0.60	4
Luteal phase	6.31 \pm 2.47	2.04 - 13.50	4

Table 6 Levels of serum progesterone during various stages of pregnancy.

Weeks of gestation	Mean \pm S.D. (ng/ml)	Range (ng/ml)	Number of subjects
4-8	17.20 \pm 6.24	9.01 - 22.79	7
16-28	62.00 \pm 36.10	28.92 - 116.65	7
32-38	169.48 \pm 52.71	107.66 - 225.33	9
39-40	177.76 \pm 4.36	173.39 - 182.12	4

Figure 13 Progesterone Levels During The Normal Menstrual Cycle

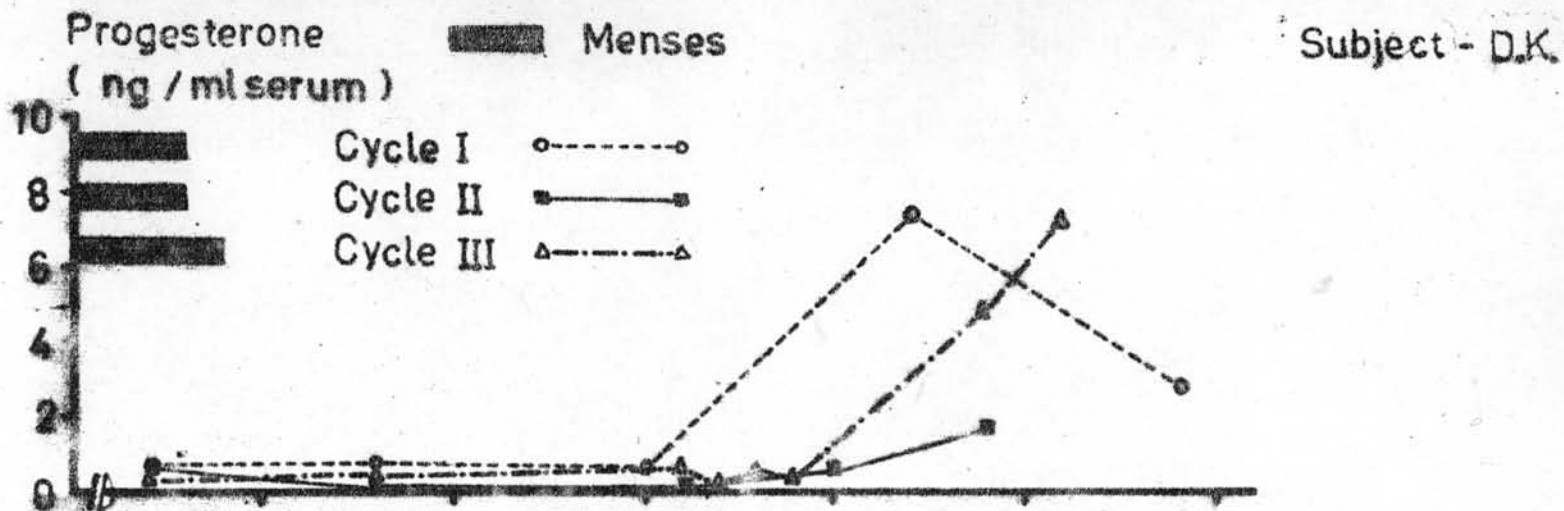


Figure 14

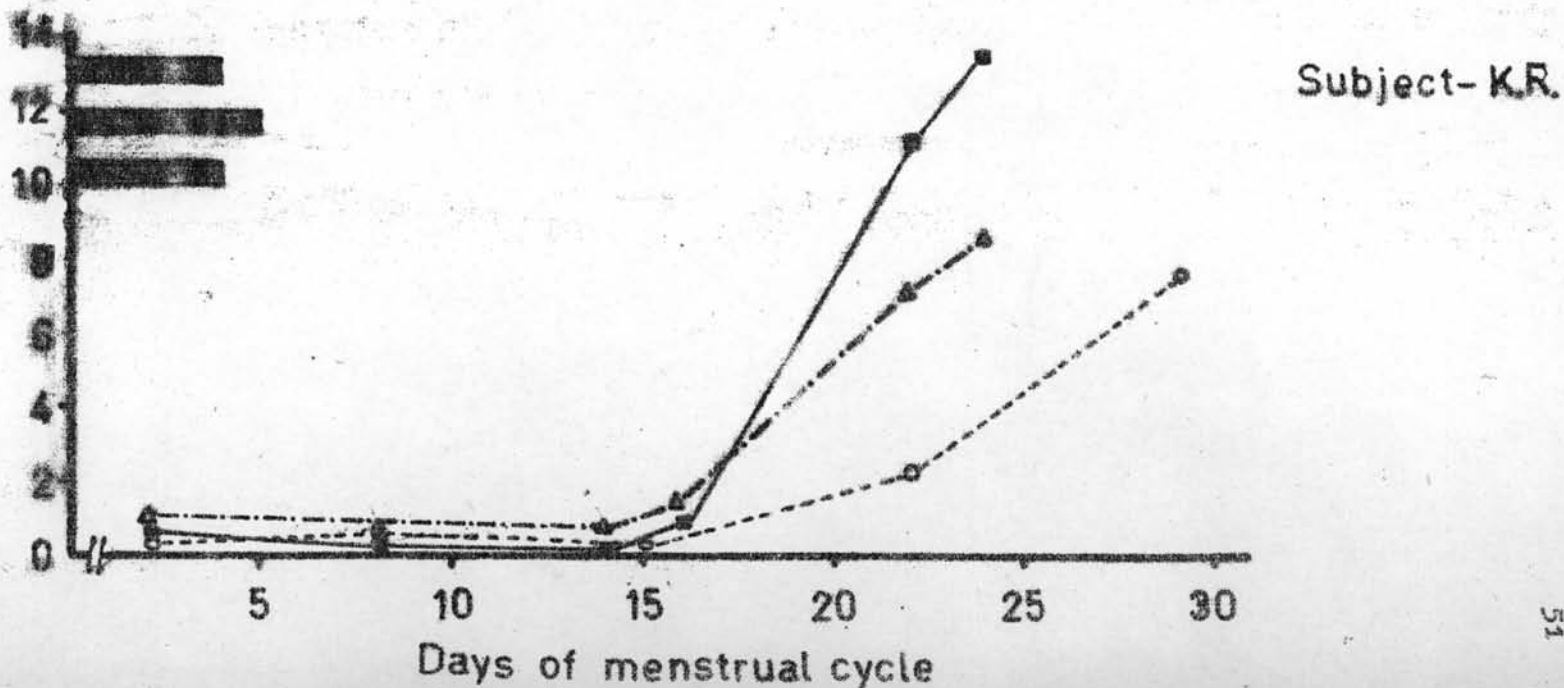


Figure 15

Progesterone Levels During The Normal Menstrual Cycle

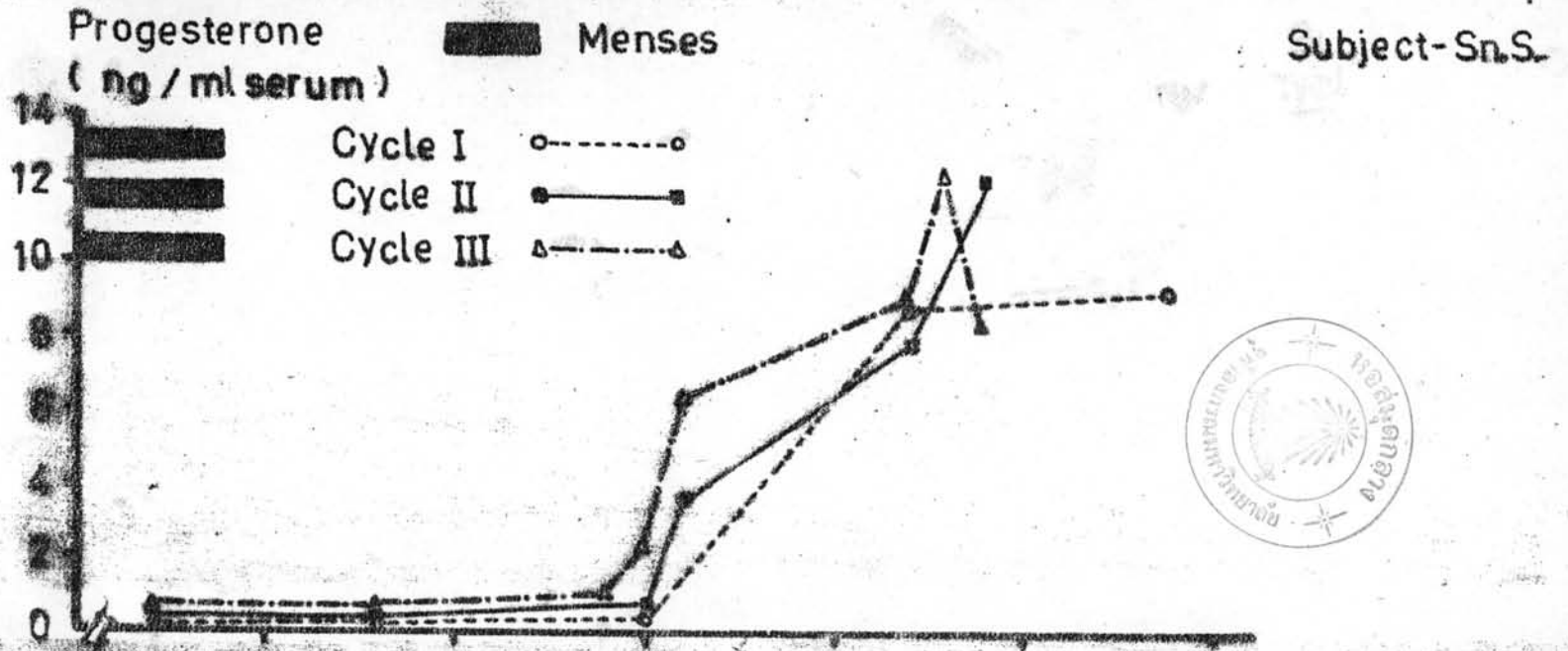


Figure 16

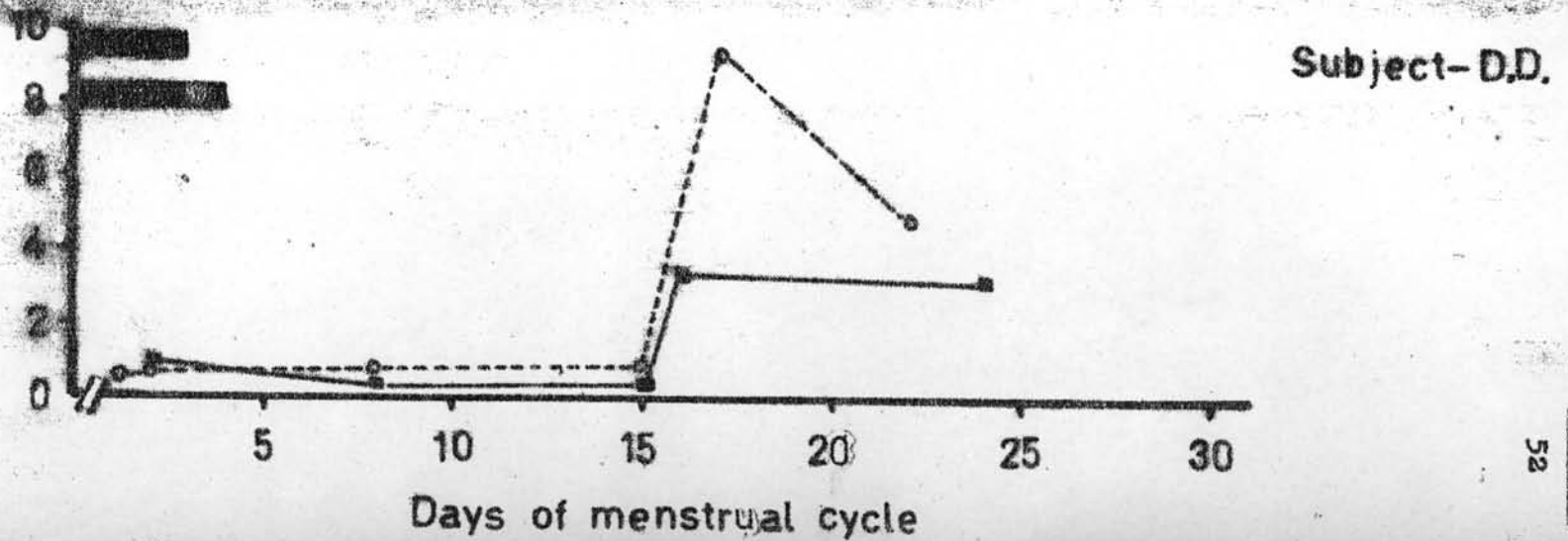


Table 7 Serum progesterone and estradiol levels in
Norgestrel-treated subjects.

Subject	II			VI			XII		
	Days of cycle	P ng/ml	E ₂ pg/ml	Days of cycle	P ng/ml	E ₂ pg/ml	Days of cycle	P ng/ml	E ₂ pg/ml
PJ ₉	7	0.33	53	7	0.27	97	9	0.31	145
	14	0.37	273	14	0.98	81	15	0.62	282
	21	0.31	313	21	0.69	111	22	0.32	150
	25	0.21	98	26	0.36	86	25	0.28	54
Cycle length		(27 days)		(27 days)		(26 days)			
BK ₉	7	0.34	84	7	-	-	6	0.23	-
	18	0.36	84	14	0.30	93	13	0.30	51
	21	0.31	37	21	0.30	51	20	0.28	46
	25	-	-	26	0.33	-	25	-	-
	32	-	-	32	0.33	-	32	-	-
Cycle length		(34 days)		(33 days)		(32 days)			
TrK ₉	6	0.41	31	7	-	-	7	0.40	57
	13	0.43	65	15	0.53	97	14	0.36	110
	21	0.51	59	22	0.58	66	21	0.77	688
	25	0.76	122	25	0.78	78	25	0.65	511
	32	-	-	32	-	-	32	0.49	138
Cycle length		(45 days)		(27 days)		(34 days)			

- indicates undetectable level

II, VI and XII = cycle 2, 6 and 12 after Norgestrel treatment

Table 7 Serum progesterone and estradiol levels in
Norgestrel-treated subjects. (Cont.)

Subject	II			VI			XII		
	Days of cycle	P ng/ml	E ₂ pg/ml	Days of cycle	P ng/ml	E ₂ pg/ml	Days of cycle	P ng/ml	E ₂ pg/ml
SG ₉	6	0.71	78	7	0.81	76	7	0.86	72
	13	0.58	154	14	0.67	-	14	-	61
	20	2.70	317	20	19.30	151	20	0.80	271
	25	3.90	265	24	16.30	167	26	11.90	241
	32	-	-	-	-	-	33	6.00	141
Cycle length		(31 days)		(29 days)			(36 days)		
CB ₉	7	0.33	130	7	-	-			
	13	0.39	181	16	0.37	241			
	21	2.00	232	21	8.90	221			
	25	2.60	111	25	2.40	184			
Cycle length		(29 days)		(29 days)					
DN ₉	7	-	-	8	0.37	58			
	14	0.65	97	14	0.44	84			
	21	0.67	87	21	0.50	100			
	25	0.88	119	25	0.74	110			
	31	0.79	72	32	0.88	73			
	38	0.35	76	39	0.47	72			
	45	0.43	61	49	0.49	79			
Cycle length		(60 days)		(60 days)					

Table 7 Serum progesterone and estradiol levels in Norgestrel-treated subjects. (Cont.)

Subject	II			VI		
	Days of cycle	P ng/ml	E ₂ pg/ml	Days of cycle	P ng/ml	E ₂ pg/ml
KC ₉	7	0.17	48	9	0.15	-
	14	0.19	-	14	0.11	-
	21	-	-	21	0.32	84
	25	0.18	75	25	0.20	-
	32	0.18	-	-	-	-
Cycle length		(32 days)		(26 days)		
Subject	Days of cycle	Progesterone Values ng/ml				
		II	VI			
MN ₉	6	0.25	-			
	7	-	0.34			
	13	0.37	-			
	14	-	0.70			
	21	0.14	-			
	24	0.15	-			
	25	-	0.20			
	31	0.78	-			
	32	-	0.12			
	38	1.10	-			
39	-	0.13				
Cycle length		(40 days)	(40 days)			

Table 7 Serum progesterone and estradiol levels in
Norgestrel-treated subjects. (Cont.)

Subject	Days of cycle	Progesterone Values ng/ml	
		II	VI
BV ₉	7	0.45	-
	14	0.65	
	21	0.52	
	25	0.85	
	32	0.85	
Cycle length		(49 days)	
SS ₉	8	0.44	-
	15	1.60	
	22	0.91	
	25	1.20	
Cycle length		(26 days)	

estradiol of more than 3 ng/ml and 150 pg/ml respectively during the luteal phases of her 3 cycles.

9. Studies on Pregnancy level

Two groups of subjects were studied.

Group 1. Normal pregnancy. Random blood samples were collected from 29 pregnant subjects at different stages of gestation (4 weeks of gestation up to 40 weeks).

Group 2. Postpartum periods. Random blood samples were drawn from 9 women after 17, 21, 24, 27, 39, 45, 46, 70 and 116 hours of delivery.

Table 6, page 50 and figure 17, page 58 indicated that progesterone level in pregnant subjects varied from 9.01 to 22.79 ng/ml during 4-8 weeks of gestation, 28.92 to 116.15 ng/ml between 16-28 weeks of gestation, 107.66 to 225.33 ng/ml during 32-38 weeks of gestation and 173.39 to 182.11 ng/ml for full term pregnancy.

Progesterone level during the postpartum period (17 hours to 116 hours after the delivery) decreased rapidly from 19.20 to 1.65 ng/ml (table 8, page 59 and figure 18, page 60).

Figure 17

PROGESTERONE LEVELS DURING PREGNANCY

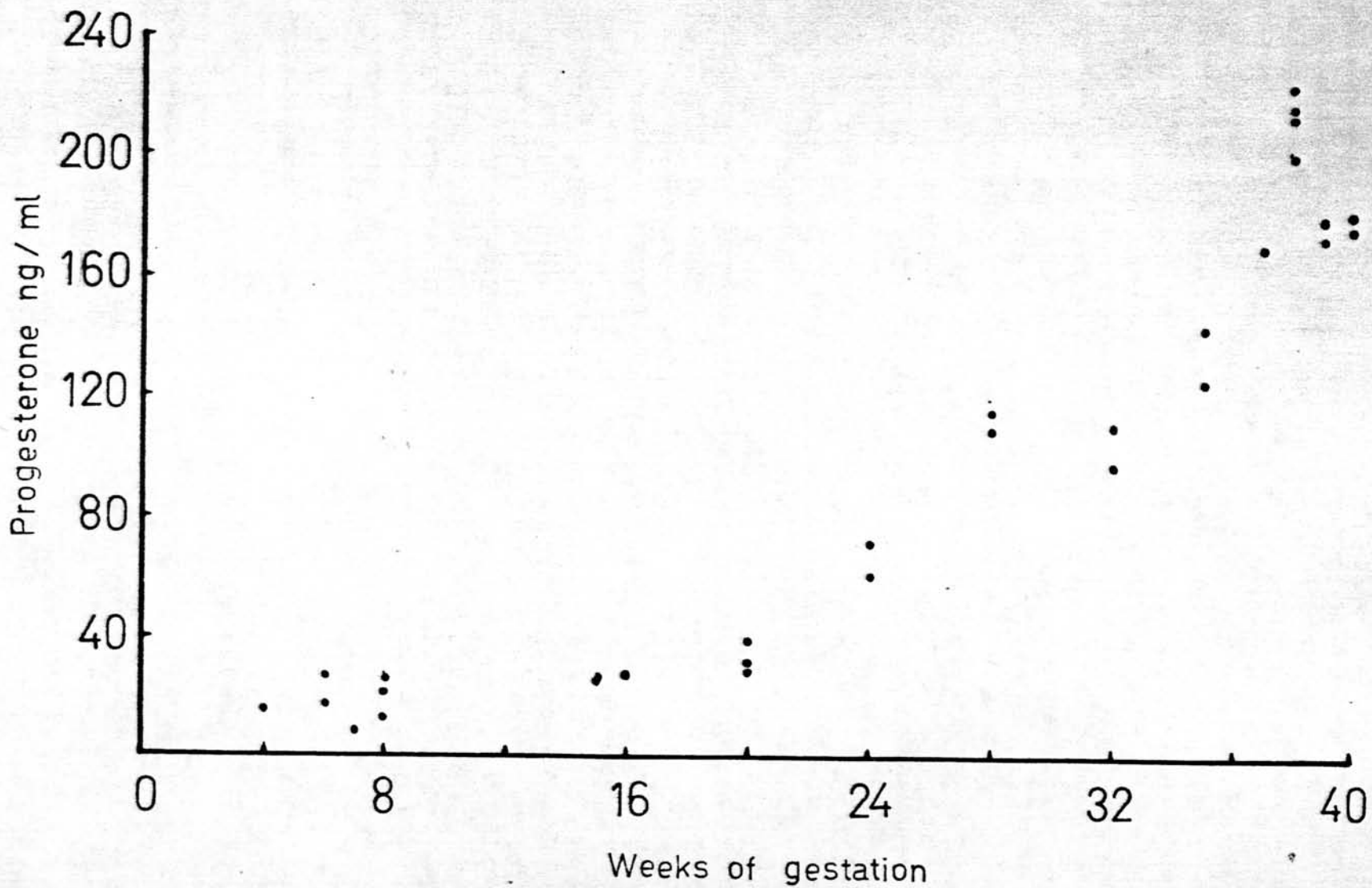


Table 8 Levels of serum progesterone during the post partum period.

Hours after delivery	Progesterone levels (ng/ml)
17	19.19
21	16.19
24	13.07
27	11.37
39	8.13
45	6.47
46	6.53
70	3.21
116	1.68

Note: Each value represents single sample.

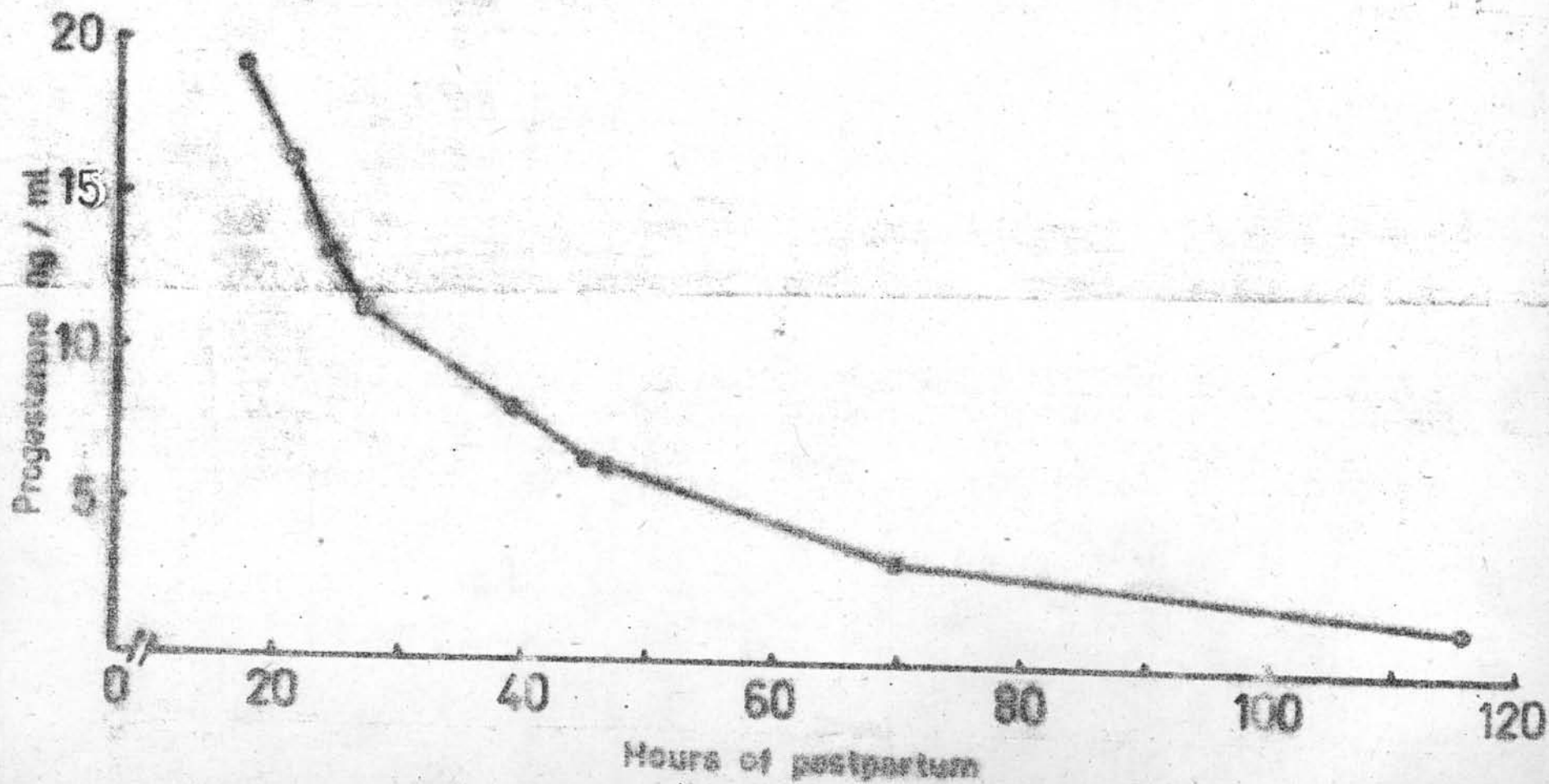


Table 9 Serum progesterone levels in normal menstruating subjects (during follicular and luteal phase) and in different stages of pregnancy observed in this study compared to those observed by other investigators using similar technique.

Physiological conditions	Abraham's (1971) (ng/ml)	Mikhail's (1972) (ng/ml)	Progesterone levels obtained in this study (ng/ml)
follicular phase	0.54±0.10	0.50±0.44	0.54±0.04
luteal phase	8.56±4.66	12.00±4.81	6.31±2.47
weeks of gestation			
4-8	-	-	17.20±6.42
16-18	48.40±18.00	-	62.00±36.10
28-30	98.00±28.00	-	-
32-38	-	-	169.48±52.71
38-40	178.50±48.00	-	177.76±4.36

Figure 18

PROGESTERONE LEVELS DURING THE POSTPARTUM PERIOD



(Each point represents one single sample)