

Delay of Parturition in Rats by Various Progestational Steroids. (31626)

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It has been known for over thirty years that progestational agents will delay or prevent parturition when administered to various species of mammals at about the expected time of delivery(1). Although this delay suggested a relatively simple assay for progestational activity, the approach has been employed only rarely to compare the activities of various progestagens, although Stucki and Forbes(2) studied the effects of 17 α -hydroxyprogesterone esters. This lack of interest is surprising, since active compounds might be considered candidates for the therapy of threatened abortion. In the present communication the effects of 3 new, totally synthetic steroids with progestational activities(3) are com-

pared with those of various other steroids.

Materials and methods. Adult female Charles River rats (8) were caged in colonies with males (3-4). Vaginal smears were examined daily and the day sperm appeared in the smear was designated as day 1 of pregnancy. Treatment of the isolated pregnant females was begun on day 15 and continued until parturition or until autopsy, usually on day 25. Animals were examined for delivery up to 17:00 hours each day; any delivery after that time was scored for the following day. Of 36 control, oil-treated rats, 34 delivered their litters on days 22 or 23 of pregnancy; one delivered on day 24, and one was not pregnant at autopsy. Therefore, delivery

TABLE I. Delay of Parturition with Various Progestational Steroids.

Compounds	Dose (μ g/day)	Route	N	Delivery day			No. non-pregnant
				Normal	Equivocal delay	Significant delay	
Oil		SC	36	34	1		1
Progesterone	100	SC	8	8			1
	300	SC	8	7			
	1000	SC	8	3		5	
	3000	SC	8		1	7	
	10,000	SC	8			8	
	30,000	SC	8			8	
<i>dl</i> -13 β -Ethyl-17 α -ethynyl-17 β -hydroxygon-4-en-3-one	10	SC	8	8			2 2 1
	30	SC	8	8			
	100	SC	8	2	2	4	
	300	SC	8	1		5	
	1000	SC	8			6	
	3000	SC	8			8	
	100	IG	8	7			
	300	IG	8	8			
	1000	IG	7	7			
	3000	IG	8	3	4	1	
<i>dl</i> -17 α -Chloroethynyl-13 β -ethyl-17 β -hydroxygon-4-en-3-one	3	SC	8	7			1
	10	SC	8	4			4
	30	SC	8	2		6	2
	100	SC	8			8	
	300	SC	8			8	
	1000	SC	8			6	
	3000	SC	8			8	
	100	IG	8	8			
	300	IG	8	7	1		
	1000	IG	8	2	3	2	1
3000	IG	8	2	4	2		

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TABLE I (continued).

Compounds	Dose ($\mu\text{g}/\text{day}$)	Route	N	Delivery day			No. non- pregnant
				Normal	Equivocal delay	Signifi- cant delay	
<i>dl</i> -13 β , 17 α -Diethyl-17 β -hydroxygon-4-en-3-one	10	SC	8	5			3
	30	SC	8	7	1		
	100	SC	8	2	2	4	
	300	SC	8		1	7	
	1000	SC	8			8	
	3000	SC	8			8	
	100	IG	8	8			
	300	IG	8	8			
	1000	IG	8	7	1		
Testosterone	3000	IG	8	2	1	4	1
	10	SC	8	8			1
	30	SC	8	7			
	100	SC	8	7			
300	SC	8	8				
17 α -Ethinyl-19-nortestosterone	10	SC	8	8			1
	30	SC	8	8			
	100	SC	8	8			
	300	SC	8	5	2		
	1000	SC	8	1	3	4	
	3000	SC	8			8	
6 α -Methyl-17 α -acetoxyprogesterone	10	SC	8	7			1
	30	SC	8	8			
	100	SC	8		6	2	
	300	SC	8		3	5	
	1000	SC	8			8	

on days 22 or 23 was considered normal, delivery on day 24 was considered an equivocal delay, and delivery on day 25 or beyond was considered a significant delay in parturition. Since pregnancy prolonged beyond day 25 usually resulted in material and fetal death, most rats were sacrificed on that day.

Results and discussion. Progesterone subcutaneously at daily doses of 100 and 300 μg failed to produce significant delay in time

of parturition, while doses above 3000 μg had a significant effect in virtually all treated rats (Table I). The 1000 μg dose delayed delivery significantly in 5 of 8 rats, suggesting that this level was the approximate ED₅₀. Testosterone was inactive at doses up to 300 μg daily, although the propionate ester has been reported to be active at higher doses (2). The remaining compounds were all active subcutaneously and were less potent by gavage

TABLE II. ED₅₀ Values (Daily Dose in μg) and Potencies of Various Progestational Steroids in Delay of Parturition Test and Clauberg Test (4).

Compound	Route	Delay of parturition		Clauberg potency
		ED ₅₀	Potency	
Progesterone	SC	1000 μg	100%	100%
<i>dl</i> -13 β -Ethyl-17 α -ethinyl-17 β -hydroxygon-4-en-3-one	SC	100	1000	900
	IG	>3000		
<i>dl</i> -17 α -Chloroethinyl-13 β -ethyl-17 β -hydroxygon-4-en-3-one	SC	30	3000	1330
	IG	>3000		
<i>dl</i> -13 β , 17 α -Diethyl-17 β -hydroxygon-4-en-3-one	SC	100	1000	500
	IG	3000	30	
Testosterone	SC	>300		
17 α -Ethinyl-19-nortestosterone	SC	1000	100	8
6 α -Methyl-17 α -acetoxyprogesterone	SC	300	300	3500

(Table I). Although the data hardly lend themselves to precise quantification, crude ED₅₀ values were calculated and potency comparisons made (Table II). Of the series under study, the three synthetic gonanes (relatives of 19-nortestosterone) were the most potent; 17 α -ethynyl-19-nortestosterone was about equipotent with progesterone; and the acetoxypregesterone derivative was intermediate. Comparison of these results with Clauberg test data (Table II) shows that the activities parallel each other; the most apparent discrepancy is 6 α -methyl-17 α -acetoxypregesterone, which is the most potent compound in the series in its endometrial effects, but is only slightly more potent than progesterone in delaying parturition.

Summary. Various progestagenic steroids were studied for their effects in delaying parturition in pregnant female rats. 17 α -Ethynyl-19-nortestosterone was about as potent as

progesterone, the standard. 6 α -Methyl-17 α -acetoxypregesterone was 3 times more potent than progesterone, while 13 β -ethyl-17 α -ethynyl-17 β -hydroxygon-4-en-3-one and 13 β -17 α -diethyl-17 β -hydroxygon-4-en-3-one were both about 10 times the standard. 17 α -Chloroethynyl-13 β -ethyl-17 β -hydroxygon-4-en-3-one was the most potent compound of the series, being about 30 times more potent than progesterone.

1. Burrows, H., *Biological Actions of Sex Hormones*, 2nd. Ed., Cambridge Univ. Press, Cambridge, England, 1949, p516.

2. Stucki, J. C., Forbes, A. D., *Acta Endocrinol.*, 1960, v33, 73.

3. Edgren, R. A., Peterson, D. L., Jones, R. C., Nagra, C. L., Smith, H., Hughes, G. A., *Recent Prog. Hormone Res.*, 1966, v22, 305.

4. Edgren, R. A., Jones, R. C., Peterson, D. L., *Fertility and Sterility*, in press.

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Lipid Variations Caused by Pteridine Deficiencies in *Crithidia fasciculata** (31627)

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The first unconjugated pteridine growth factor was identified by Nathan and Cowperthwaite(1) in the course of defining the nutritional requirements of the trypanosomatid flagellate, *Crithidia fasciculata*. This organism temporarily became a biochemical oddity when it was shown(2) that it required for growth both a conjugated and unconjugated pteridine. It is now apparent that the double pteridine requirement is a characteristic of every member of the family Trypanosomatidae in which the nature of the pteridine requirement has been investigated (reviewed in Guttman(3)).

By the nutritional bypass method in which supplying the product of the metabolic chain obviated requirement for the intermediate, it

was shown that thymine is the growth-limiting product of conjugated pteridine (folic acid) metabolism in these organisms(2) and that a supplementary, non-limiting factor (a lipid?) is present in human serum(4).

Identification of products of unconjugated pteridine metabolism has been more difficult. Amount and kind of pteridine supplied to *C. fasciculata* alter the production of vitamin B₁₂ and its congeners(5) but supplementation of the culture medium with vit B₁₂ neither bypassed nor spared the unconjugated pteridine requirement. Similarly tyrosine, the product of an unconjugated pteridine-catalyzed hydroxylation of phenylalanine(6) neither bypassed nor spared the unconjugated pteridine requirement. Both phenylalanine and tyrosine are obligate nutritional requirements.

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