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THE EFFECT OF TESTOSTERONE AND OTHER ADRENAL STEROIDS ON PMS-INDUCED OVULATION IN THE IMMATURE RAT

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ABSTRACT

17α-hydroxyprogesterone at doses up to 200 μg/day and 11β-hydroxyandrostenedione at doses up to 500 μg had no effect on PMS-induced ovulation in intact immature rats. On the other hand, 10 μg/day of testosterone propionate on days 28-30 decreased the percent of animals ovulating and the average number of ova produced. This effect was observed also in adrenalectomized animals with or without corticosterone therapy. Three daily doses of 10 μg TP significantly increased the electrical threshold of the medial preoptic area in pentobarbital-blocked, PMS-stimulated animals. The effect of TP administration was also seen in a decreased rate of release and synthesis of FSH during the ovulatory surge, as well as in decreased storage and rate of release of LH. TP also inhibited the response to a large dose of HCG in the PMS-primed animal, suggesting a direct target organ effect.

In a previous study (1) it was shown that ovulation induced by the administration of pregnant mare's serum (PMS) to intact immature female rats could be blocked by the concurrent administration of ACTH. Subsequently, Feder et al. (2) observed the same effect of ACTH in cycling adult rats. In immature adrenalectomized rats (1) there was no interference with PMS-induced ovulation, suggesting that an adrenal product, rather than ACTH itself, was responsible. However, corticosterone administration over a dose range from 5 to 100 μg twice daily failed to block ovulation (1,3) and certain doses actually overcame pentobarbital blockade of ovulation (4). Thus, our attention was directed to other adrenal steroids. The ovulation-facilitating or inhibiting effects of progesterone have been studied extensively (5-11) in other contexts, and there is good evidence that this steroid is important to the ovulatory mechanism (12). 17α-hydroxyprogesterone and 11βhydroxyandrostenedione have not been examined. Another potentially important adrenal steroid is testosterone, which has been shown to have significant and complex effects on the gonadotropic mechanism. Early studies by Hohlweg (13) and Salmon (14,15) with repeated administration of milligram amounts of testosterone propionate (TP) to immature rats yielded evidence of ovarian follicle stimulation and occasional corpus luteum formation. However, Bradbury (16) gave 50-500 μg doses and found no effect on pituitary weight or gonadotropic potency. Gallo and Zarrow (6) observed that 500 μg TP failed to stimulate premature ovulation in the 30-day-old PMS-primed immature rat, and Kobayashi et al. (3) blocked ovulation with single doses of 500-5,000 μg in adult cycling rats. Klawon et al. (17) blocked ovulation and also the preovulatory LH spike induced by PMS with four

doses of 500 μg TP. Studies with smaller doses become increasingly difficult to interpret, in part because of the variety of experimental designs. Johnson and Naqvi (18) gave 25 or 100 μg doses of TP to rats at 22 days of age and observed a positive feedback effect, with three to five cyclic rises and falls in plasma FSH over the following 116 hours. Consistent with this finding is the earlier observation (19) that two to three doses of 50-100 μg TP accelerated the onset of puberty. Naqvi and Johnson (20) also showed that a single dose of 100 μg TP increased plasma FSH but not pituitary FSH, suggesting that both synthesis and release of FSH were stimulated. On the other hand, more prolonged administration of TP seems to have an inhibitory effect. Zarrow et al. (19) found that prolonged administration of 100 μg daily delayed rather than accelerated the onset of puberty; Boyd and Johnson (21) had earlier reported that 25 μg TP daily for seven days increased pituitary LH content and decreased plasma LH levels. Studies on castrated animals are consistent with this latter effect. Ramirez and McCann (22) showed that 25 μg TP/100 gm rat daily prevented or abolished the postcastration rise in plasma LH in male rats, while 10 μg TP/100 gm given to castrated immature female rats treated with estradiol benzoate (23) had no effect on either the high pituitary or low plasma levels of LH. Thus, TP, like estradiol benzoate and progesterone, seems to have a biphasic or triphasic action (7) depending on dose level, duration of exposure, and other parameters. This situation complicates efforts to evaluate the role of various adrenal steroids in the ovulation blockade produced by ACTH. Our initial studies on the effect of three such steroids are presented.

MATERIALS AND METHODS

Immature female Sprague-Dawley rats were obtained at 22 days of age from the Holtzmann Co., Madison, Wisconsin. The animals were provided laboratory food and tap water ad lib. Temperature was controlled at 24° ± 1°C and lighting was provided for 14 hours starting at 5 a.m. PMS (Organon, West Orange, New Jersey) was dissolved in isotonic saline at a concentration of 50 IU/0.05 ml and injected intraperitoneally at 9 a.m. at 28 days of age. Steroids were dissolved in sesame oil and the concentrations adjusted so that the total dose was contained in a constant amount of 0.1 ml. The steroids were usually injected at 9:30 a.m. and 4 p.m. daily for three days beginning at 28 days of age. All animals were killed 72 hours after the PMS injection, and tubal ova were counted under the microscope. FSH and LH assays were performed using radioimmunoassay materials obtained from the National Institutes of Health. Nembutal blockade and electrical stimulation were carried out as previously described (1).

RESULTS AND DISCUSSION

While 17α-hydroxyprogesterone is not an intermediate in corticosteroid biosynthesis in the rat, it is nevertheless an important adrenal intermediate in many other species, and its effect, as well as that of another adrenal steroid, 11β-hydroxyandrostenedione, were investigated using the described procedure. The data are summarized in Tables 1 and 2. It is evident that up to 200 μg of 17α-hydroxyprogesterone and up to 500 μg

daily of 11β-hydroxyandrostenedione failed to influence PMS-induced ovulation.

Many studies of adrenal secretion indicate that androstenedione is a major $C_{19}O_2$ product. The biological activity probably derives from peripheral transformation into testosterone. The latter compound was therefore selected for initial studies. Table 3 summarizes the results when consecutive daily injections of TP were administered to PMS-primed rats. A statistically borderline (P = .06) decrease in the percentage of animals ovulating was seen at 7 μg/day; at 10 μg/day, the inhibition of ovulation (53% compared to the control of 93%) was highly significant (P = .0002). The average number of ova per rat was not significantly decreased until the 10 μg/day dose was reached. (A significant difference occurred in the comparison of the control group to the 3 μg/day group, but this appears to be due to the fortuitously low standard error of the 3 μg/day group.)

On the basis of the ovulation inhibition demonstrated by 10 μg TP per day, an investigation into the mechanism of the effect was undertaken. Since other steroids are known to have effects on CNS electrical thresholds of PMS-injected, pentobarbital-blocked animals, stimulation by stainless steel bipolar electrodes placed in the medial preoptic area according to the technique previously described (1) was undertaken. The results are summarized in Table 4. Increasing the current intensity in the control animals increased the number of animals ovulating. With a current of

110 μA, 82% of the animals ovulated and yielded an average of 6.2 ova per rat. In animals receiving three daily doses of 10 μg of TP there was far less effect of the electrical stimulation, so that even with 120 μA only 38% of the animals ovulated, yielding an average of only 3.3 ova per rat. These findings suggest that TP acts, at least in part, by increasing electrical thresholds in the CNS.

The effects of TP on pituitary function were examined by radioimmunoassays of FSH and LH in plasma and pituitary glands. These studies were performed in collaboration with Dr. S. M. McCann. Figure 1 shows the FSH concentration in plasma and pituitary glands of control and TP-treated animals. A fall in pituitary FSH content associated with a spike in plasma FSH is evident on day 30 in the control animals. In the TP-treated animals, a delayed but otherwise similar fall in pituitary FSH occurred. On the other hand, the plasma FSH levels throughout the period from day 29 to day 31 were consistently lower and the ovulatory spike was lower and of shorter duration than the controls. These findings can only be explained by a decreased rate of release of FSH as well as a diminished rate of synthesis before and during the time of FSH discharge. The timing of the ovulatory FSH surge seems to have been unaffected. A repetitive, cyclic discharge of FSH, as observed by others (18) after larger doses of TP, could probably not have been detected with the sample-timing used in this protocol.

The events in plasma and pituitary LH are shown in Figure 2. In the control group, the pituitary LH level showed a steep preovulatory decline by noon of day 30, and this was accompanied by a surge in the plasma LH level. In the TP-treated animals, the pituitary LH level was much lower than in the controls, but a decline in the level was still evident between noon and 3 PM on day 30, accompanied by a small surge in the plasma level. Thus, TP treatment produced a decreased storage and a low level of release of LH into the plasma. The preovulatory surge was still clearly present.

Having demonstrated both a CNS and a pituitary effect of TP in the PMS-primed rat, the possibility of a direct effect of TP on ovarian sensitivity was investigated using the response to a luteinizing stimulus (10 IU HCG) as an index (7). As shown in Table 5, a single injection of HCG on day 30 produced 100% ovulations on day 31 in control animals with an average of 60.5 ova per rat. When three consecutive injections of 10 μg TP were added to the regimen, the number of rats ovulating was reduced (although the difference was not significant at the 0.05 level) and the number of ova per rat was greatly reduced (from 60.5 to 9.2; $P = <0.01$). Thus, there appeared to be some evidence of a direct effect of TP on ovarian response.

The role of testosterone in the absence of the adrenal was investigated further. However, adrenalectomy itself has an adverse effect on ovulation

(Table 6); only part of this effect was reversed by the immediate post-adrenalectomy administration of a total of 60 μg corticosterone daily.

Adrenalectomized rats without corticosterone treatment were used to study the action of TP on the ovulatory mechanism in the absence of all endogenous adrenal hormones. As shown in Table 7, the effect of TP on ovulation frequency was already evident at the 5 μg/day level. An effect on the average number of ova produced per rat could not be demonstrated, presumably because of the low yield already evident in the control animals.

The role of a permissive action of corticosteroids was explored further in adrenalectomized rats supplemented with a total of 30 or 60 μg corticosterone daily started immediately or three days after the adrenalectomy and given a subthreshold dose of TP. In no instance did the corticosterone augment the suppressive effect of a subthreshold dose of TP. Thus, these two sets of experiments do not support the notion of a permissive role of the adrenal or of corticosterone itself in the ovulation-inhibiting effect of small doses of TP.

It may therefore be concluded that testosterone exerts a multiplicity of effects on the PMS-induced ovulatory mechanism of the immature female rat. The inhibitory action of 10 μg/day is also observed in the adrenalectomized animal (which may be a trifle more sensitive to TP); replacement of adrenocortical function by various dosage levels of corticosterone appears to have no effect, thus militating against synergistic or permissive effects

of this corticosteroid. The decreased effectiveness of stimulation of the medial preoptic area in the pentobarbital-blocked, PMS-stimulated rat is indicative of an increase in electrical threshold, as has already been demonstrated in intact animals treated with ACTH (1). Thus, a CNS site of action of testosterone appears to be at least one mechanism by which the observed inhibition might occur. Whether these effects are related to the changes in gonadotropin secretion produced by testosterone is unclear. In any event, the testosterone-treated animal shows a decreased rate of release of LH. Others have demonstrated complex effects of testosterone on plasma gonadotropin levels using different blood sampling regimens. Finally, testosterone inhibited the response to a luteinizing stimulus (HCG), suggesting that testosterone might exert its effects also by a direct inhibitory action at the target organ. Since androstenedione is a known adrenal secretory product, and since this is converted to a significant degree to testosterone in the periphery, the latter steroid might well play a role in the normal CNS-pituitary-ovarian mechanism. It remains to be demonstrated whether the testosterone levels used in the present studies are relevant to physiological or only to pharmacological events.

FIGURE I

PLASMA AND PITUITARY FSH CONCENTRATION
IN PMS-TREATED IMMATURE FEMALE RATS

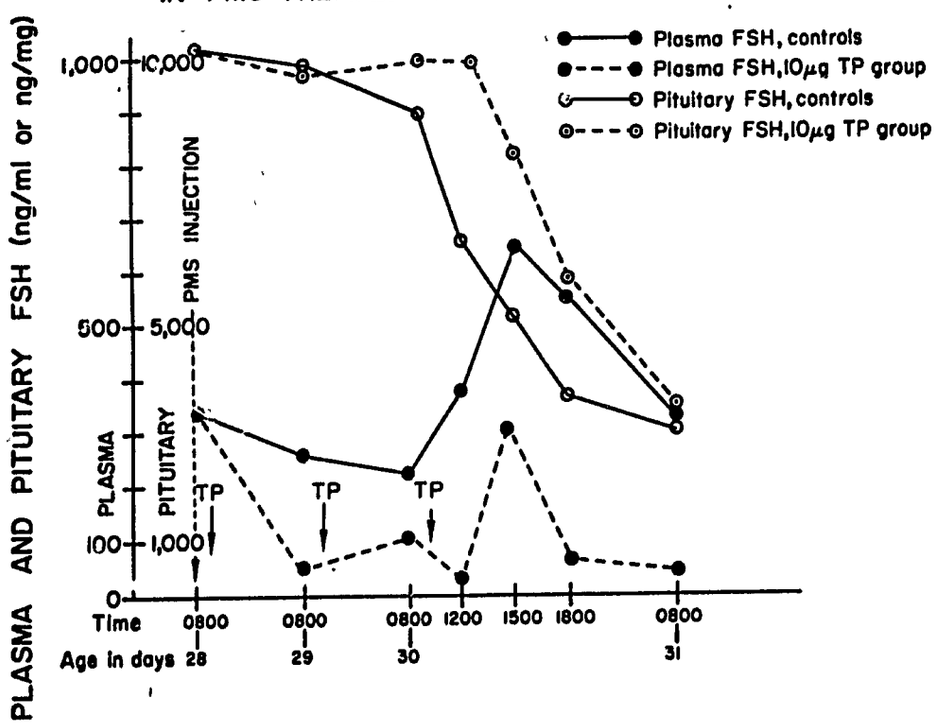

FIGURE 2

PLASMA AND PITUITARY LH CONCENTRATION
IN PMS-TREATED IMMATURE FEMALE RATS

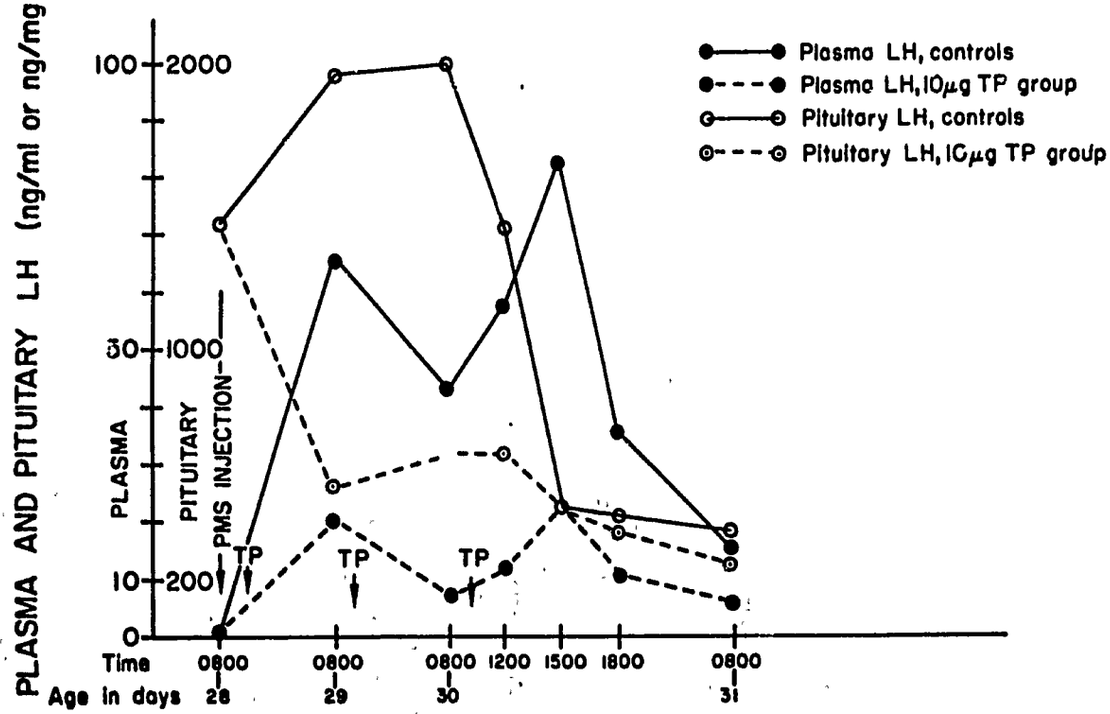

TABLE 1

EFFECT OF 17-OH PROGESTERONE ON PMS-INDUCED OVULATION IN THE RAT

Treatment Regimen							Rats Showing Tubal Ova on Day 31		
Agent	Dose	Treatment Day 28	29	30	Time	No. Rats	No. Rats Ovulating	% Ovulation	Average no. Tubal Ova
PMS	50IU	+	-	-	9 AM	10	9	90	19.8
PMS	50IU	+	-	-	9 AM	10	9	90	11.3
170HP	200μgx2	+	+	+	8 AM, 3 PM				
PMS	50IU	+	-	-	9 AM	10	9	90	13.8
170HP	200μgx2	-	-	+	9 AM				

TABLE 2

EFFECT OF 11β-OH-Δ^4-ANDROSTENEDIONE ON PMS-INDUCED OVULATION IN THE RAT

Treatment Regimen							Rats Showing Tubal Ova on Day 31		
Agent	Dose	Treatment Day 28	29	30	Time	No. Rats	No. Rats Ovulating	% Ovulation	Average no. Tubal Ova
PMS	10IU	+	–	–	9 AM	56	53	94	13.4
PMS 11OHΔ	10IU 25μgx2	+ +	– +	– +	9 AM, 4 PM	10	9	90	9.4
PMS 11OHΔ	10IU 50μgx2	+ +	– +	– +		10	9	90	10.6
PMS 11OHΔ	10IU 100μgx2	+ +	– +	– +		22	19	86	11.5
PMS 11OHΔ	10IU 150μgx2	+ +	– +	– +		12	12	100	11.4
PMS 11OHΔ	10IU 250μgx2	+ +	– +	– +		12	12	100	9.9

TABLE 3

EFFECT OF TESTOSTERONE ON OVULATION

Treatment Regimen						Rats Showing Tubal Ova on Day 31					
Agent	Dose	Treatment Day 28	29	30	Time	No. Rats	No. Rats Ovulating	% Ovulation	P Value	No. Ova Per Rat (Mean ± SE)	P Value
Intact											
PMS	50IU	+	-	-		30	28	93		16.4 ± 1.9	
PMS	50IU	+	-	-							
TP	3μg	+	+	+	9 AM	10	9	90		10.7 ± 0.8	<.01
PMS	50IU	+	-	-							
TP	5μg	+	+	+	9 AM	20	17	85		11.5 ± 2.6	
PMS	50IU	+	-	-							
TP	7μg	+	+	+	9 AM	18	13	72	.06	16.2 ± 1.2	
PMS	50IU	+	-	-							
TP	10μg	+	+	+	9 AM	36	19	53	<.01	9.0 ± 1.7	

TABLE 4

ELECTRICAL THRESHOLD OF ANTERIOR HYPOTHALAMUS IN TESTOSTERONE TREATED, PENTOBARBITAL-BLOCKED RATS

Treatment Regimen							Rats Showing Tubal Ova on Day 31		
Agent	Dose	Treatment Day 28	29	30	Time	No. Rats Current (μA)		% Ovulation	Average No. Ova Per Rat
PMS	50IU	+	-	-	9 AM				
Stim *		-	-	+	2 PM	50	7	14	1.0
PMS	50IU	+	-	-	9 AM				
Stim		-	-	+	2 PM	80	7	57	6.5
PMS	50IU	+	-	-	9 AM				
Stim		-	-	+	2 PM	110	6	82	6.2
PMS	50IU	+	-	-	9 AM				
TP	10μg	+	+	+	9 AM				
Stim		-	-	+	2 PM	50	5	0	0
PMS	50IU	+	-	-	9 AM				
TP	10μg	+	+	+	9 AM				
Stim		-	-	+	2 PM	80	8	0	0
PMS	50IU	+	-	-	9 AM				
TP	10μg	+	+	+	9 AM				
Stim		-	-	+	2 PM	120	8	38	3.3

* Electrical Stimulation

TABLE 5

EFFECT OF TESTOSTERONE ON PMS+HCG-STIMULATED OVULATION

Treatment Regimen						Rats Showing Tubal Ova on Day 31		
Agent	Dose	Treatment Day 28	29	30	Time	No. Rats	% Ovulation	Average No. Ova Per Rat (Mean ± SE)
PMS	50IU	+	-	-	9 AM			
HCG	10IU	-	-	+	5 PM	10	100	60.5 ± 5.38
PMS	50IU	+	-	-	9 AM			
TP	10μg	+	+	+	9 AM			
HCG	10IU	-	-	+	5 PM	15	80	9.2 ± 1.8

TABLE 6

EFFECT OF ADRENALECTOMY ON OVULATION

Treatment Regimen						Tubal Ova on Day 31				
Agent	Dose	Treatment Day 28	29	30	Time	No. Rats	No. Rats Ovulating	% Ovulation	No. Ova Per Rat (Mean ± SE)	P Value
Intact										
PMS	50IU	+	-	-	9 AM	30	28	93	16.1 ± 1.9	
Adrenalectomy										
PMS	50IU	+	-	-	9 AM	25	18	72	8.1 ± 1.5	<.01
PMS	50IU	+	-	-	9 AM					
*Cpd B	20μg	+	+	+	9 AM	10	9	90	9.0 ± 2.5	<.05

*Cpd B was administered immediately after adrenalectomy in daily doses of 20 μg.

TABLE 7

EFFECT OF TESTOSTERONE ON OVULATION IN ADRENALECTOMIZED RATS

Treatment Regimen						Rats Showing Tubal Ova on Day 31					
Agent	Dose	Treatment Day 28	29	30	Time	No. Rats	No. Rats Ovulating	% Ovulation	Value	No. Ova Per Rat (Mean ± SE)	P Value
Adrenex PMS	50IU	+	-	-		25	18	72		8.1 ± 1.5	
PMS	50IU	+	-	-	9 AM						
TP	5μg	+	+	+	9 AM	17	5	30	<.01	7.0 ± 2.9	
PMS	50IU	+	-	-	9 AM						
TP	7μg	+	+	+	9 AM	13	6	46		4.3 ± 1.5	
PMS	50IU	+	-	-	9 AM						
TP	10μg	+	+	+	9 AM	25	8	32	<.01	5.5 ± 1.5	

TABLE 8

EFFECT OF ADRENALECTOMY, COMPOUND B, AND TESTOSTERONE ON OVULATION

Treatment Regimen						Tubal Ova on Day 31					
Agent	Dose	Treatment Day 28	29	30	Time	No. Rats	No. Rats Ovulating	% Ovulation	P Value	No. Ova Per Rat (Mean ± SE)	P Value
PMS	50IU	+	-	-							
TP	5μg	+	+	+		17	5	30		7.0 ± 2.9	
PMS	50IU	+	-	-	9 AM						
TP	5μg	+	+	+	9 AM						
Cpd B	10μg	+	+	+	9 AM	10	5	50		6.6 ± 3.1	
PMS	50IU	+	-	-	9 AM						
TP	5μg	+	+	+	9 AM						
Cpd B[1]	10μg	+	+	+	9 AM	8	5	62		3.4 ± 0.8	
		+	+	+	4 PM						
PMS	50IU	+	-	-	9 AM						
TP	5μg	+	+	+	9 AM						
Cpd B[2]	10μg	+	+	+	9 AM	9	6	66		8.6 ± 3.2	
		+	+	+	4 PM						

[1] Administration delayed for 3 days.
[2] Cpd B administered immediately after adrenalectomy in 2 daily doses of 10 μg.

1. Hagino, N., M. Watanabe, and J. W. Goldzieher, Endocrinology 84: 308, 1969.

2. Feder, H. H., K. Brown-Grant, and C. S. Corker, J. Endocrinol. 50: 29, 1971.

3. Kobayashi, F., K. Hara, and T. Miyake, Endocrinol. Jap. 16:493, 1969.

4. Ying, S.-Y., and Meyer, R. K., Endocrinology 84: 1466, 1969

5. Redmond, W. C., Endocrinology 83: 1013, 1968.

6. Gallo, R. V., and M. X. Zarrow, Endocrinology 86: 296, 1970.

7. Hagino, N., and J. W. Goldzieher, Endocrinology 86: 29, 1970.

8. Naqvi, R. H., and D. C. Johnson, Endocrinology 87: 4818, 1970.

9. Terasawa, E., and C. H. Sawyer, Exp. Neurol. 27: 359, 1970.

10. Caligaris, L., J. J. Astrada, and S. Taleisnik, Endocrinology 89: 331, 1971.

11. Kaasjager, W. A., D. M. Woodbury, J. A. M. J. van Dieten, and G. P. van Rees, Neuroendocrinology 7: 54, 1971.

12. Nequin, L. G., and N. B. Schwartz, Endocrinology 88: 325, 1971.

13. Hohlweg, W., Klin. Wochenschr. 16: 586, 1937.

14. Salmon, U. J., Proc. Soc. Exp. Biol. Med. 37: 488, 1937.

15. Salmon, U. J., Endocrinology, 23: 779, 1938.

16. Bradbury, J. T., Endocrinology 41: 501, 1947.

17. Klawon, D. L., S. Sorrentino, Jr., and D. S. Schalch, Endocrinology 88: 1131, 1971.

18. Johnson, D. C., and R. H. Naqvi, Endocrinology 85: 881, 1969.

19. Zarrow, M. X., R. H. Naqvi, V. H. Denenberg, Endocrinology 84: 14, 1969.

20. Naqvi, R. H., and D. C. Johnson, J. Endocrinol. 45: 29, 1969.

21. Boyd, R., and D. C. Johnson, Acta Endocrinol. (Kbh) 58: 600, 1968.

22. Ramirez, V. D., and S. M. McCann, Endocrinology 76: 412, 1965.

23. Ramirez, V. D., and C. H. Sawyer, Endocrinology 76: 1158, 1965.