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Augmented Excretion of Urine Gonadotrophins During ACTH Administration. (19246)

RICHMOND W. SMITH, JR. (Introduced by O. H. Gaebler.)
From the Section of Endocrinology, Department of Medicine, Henry Ford Hospital, Detroit, Mich.

Observations on the effects of ACTH on renal excretion of pituitary gonadotrophin have been made by the author during the past two years. Recently, Sohval and Soffer(1) reported the results of a similar investigation in 22 patients receiving ACTH and/or cortisone. They called attention to the limited data on the subject, specifically those of Mason *et al.*(2) and Sprague *et al.*(3), who observed no change in urinary gonadotrophin titers of 2 young women. Sohval and Soffer (1) found enhanced titers in 9 patients of their series but they stated that the mechanism of this change remained unknown. The present report concerns the results of gonadotrophin analysis of 73 urine samples from 9 patients receiving ACTH. Methods of gonadotrophin isolation and bioassay were employed which differed from those used in the above-mentioned investigations.

Materials and methods. Pertinent data on the case material is presented in Table I where dosage, route and duration of the hormone administration are summarized with the gonadotrophin titers. All patients received ACTH and Case 5 received, in addition, a short course of oral cortisone therapy. Case 3 received 15 mg of ACTH intravenously over a 6-hour period while all others received the hormone intramuscularly in divided doses. Urine samples, collected over periods of from 12 to 24 hours, were preserved under toluene. Gonadotrophins were isolated by adsorption on activated kaolin(4). The gonadotrophins were dried under a stream of nitrogen, sealed in a test tube and kept at 3°C until assayed. At this time the powdered material was dissolved in isotonic saline and the bioassay carried out as reported by Lloyd *et al.*(5). This method employs 21-day-old female white mice of 8 to 10 g, using 2 mice at each of 4 dilutions per gonadotrophin sample. Reassay at the same or different dilutions was carried out when confirmation was desired or the end point was not obtained initially. In these instances, a total of 16 mice per sample were required. Volumes of 0.25 cc of gonadotrophin solution were injected subcutaneously twice daily for 3 days and the animals sacrificed 24 hours following the last injection. Throughout the assay period the solutions of gonadotrophins were kept at 3°C. Both ovarian and uterine weights were recorded but only the latter were used to determine the titers. Mice were assayed in lots of 50 of which 5 to 7 served as saline-injected controls. A positive result was recorded when the average of the uterine weights of the 2 gonadotrophin-injected animals at any level exceeded the average of the saline-injected controls by 100%. Toxicity of the gonadotrophin solutions was encountered infrequently and only 9 of more than 800 mice used in the study were lost. One group of 5 mice received a total of 4.1 mg of ACTH per mouse in 6 divided doses over a 3-day period to determine the direct effects of this hormone on the uterine, ovarian and adrenal weights (Table II).

Results. In all cases augmented gonadotrophin excretion of varying degree was observed during the period of ACTH adminis-

TABLE I. Clinical Data, ACTH Dosage* and Urinary Gonadotrophin Levels.†

Case	Age, sex	Gonadal status	Day of treatment 0	1	2	3	4	5	6	7	8	9	10	11	15	22
1	63 ♂	Normal								>16			>16			
			<8	<8	8	32			8	<32			<32	16	16	8
				65	75	60	50	40	50	60	50	40	40	40	20	0
2	57 ♂	”	16	32	32	16	16	16	8	<8	<8					
				80	80	50	40	40	35	10	0					
3	44 ♀	Regular menses	8	16	16											
				15§	0											
4	17 ♀	Primary amenorrhea	<8	<4	12	<4	<4	<4	6	<4	<4		<4			
				40	40	40	40	40	40	0	0	0	0			
5	79 ♀	Postmenopausal	—			> 64		>32			>16					
				32	32	<128	64	<64	64	32	<24	24	24			
				100	100	100	100	100	75	50	150‡	150‡	150‡			
6 (a)	63 ♀	”				>256										
			64	128	256	<512	256	64	<64		128	128		64		
				70	60	50	40	30	30	22.5	7.5	0	0	0		
(b)	—	—	—	>128				> 64								
				<256	64	512	512	<128				64				
				40	70	60	60	50	20	10	0	0				
7	50 ♀	Menopausal	—	> 64												
				<128	128	256	64									
				20	80	45	0									
8	54 ♂	Normal	4	16	16	16		<4	8							
				80	70	40	30	10	0							
9	32 ♀	Irregular menses	<8	64				64			8			8		
				100	100	100	75	75	75	75	75	50	0	0		

* Lower figure for each day is the total ACTH in mg per 24 hr.

† Upper ” ” ” ” ; expressed in mouse units per 24 hr.

‡ Oral cortisone acetate in mg per 24 hr.

§ Intravenous ACTH.

< No assay performed at lower dilution.

$\gtrless$ Increase in average of uterine weights over controls approached 100% at the higher dilution.

TABLE II. Comparison of Body, Uterine, Ovarian and Adrenal Weights of Gonadotrophin, ACTH and Saline-Injected Mice.

Group	No. of mice	Mean body wt	Mean uterine wt	Mean ovarian wt	Mean adrenal wt
Saline-inj.	9	11.2 g	15.6 mg	2 mg	2 mg
Gonadotrophin-inj.	12	11.6	55.8	4.8	2.4
ACTH-inj. (4.1 mg total)	5	11.2	18.2	2.5	3.6

tration. In Case 4, however, an increased titer was found only on the second day of treatment and, thereafter, the level did not exceed the control. Although a 2-fold increase in titer was observed in Case 3, the control urine in this instance was collected 6 days prior to the ACTH administration. Lack of a control urine in Case 5 prevents an interpretation of the magnitude of increase that can be attributed to the effects of the corticotrophin. Highest titers in this patient were observed, however, from the 3rd through the 6th day of therapy when ACTH dosage was maximal, while the lowest titers were obtained during the period of oral cortisone administration. Exitus of the patient during cortisone therapy prevented a post-treatment control observation. In Case 7, therapy was instituted 4 hours prior to completion of the first day of urine collection and this specimen, for the most part, may be considered as a control. Two to 8-fold increases were observed in the remaining patients, the greatest in Case 6 and 9. Changes were often abrupt as demonstrated by the increased titers of Cases 2, 6, 8 and 9 during the first 24 hours of therapy and by the decreases in Cases 1, 2, 7 and 8 when the dosage was reduced or the hormone discontinued. In Case 6(a), however, higher titers returned when ACTH was discontinued and for at least 24 hours thereafter. In general, highest titers were found during periods of most intensive hormonal therapy, but no close correlation to ACTH dosage can be made. Where positive results were obtained using uterine weights, similar interpretations could be made from ovarian weight increases which, in the lower dilutions of the gonadotrophin solution, often were marked. The ovaries, if enlarged, characteristically were pale and occasionally ovarian hemorrhages and hyperemia were observed only at the lower dilutions. In the mice receiving ACTH (Table II), ovarian and uterine weights did not differ enough from the controls to be considered positive by the criteria above mentioned, yet the adrenal weights nearly doubled. In 12 gonadotrophin-injected animals chosen at random among positive reactors, marked ovarian and uterine weight increases were found while adrenal weights were increased slightly.

Discussion. The results presented here are supplementary to those which have been reported recently by Sohval and Soffer(1). The fact that the methods of gonadotrophin isolation and bioassay have differed in the two studies adds further significance. The extent of the increases in the excretion of gonadotrophins in the two series is similar as the above investigators(1) found changes of approximately 2 to 10-fold. Whereas they demonstrated the gonadotrophic nature of the substance by failing to obtain uterine weight increases in ovariectomized mice, the present investigator has achieved a similar result by observing significant increases in ovarian weights. That the predominant gonadotrophin was FSH is suggested by the observation that the enlarged ovaries, with few exceptions, were pale. Ovarian hyperemia and hemorrhagic corpora lutea were seen only as isolated occurrences at low dilutions of the injected material. The above mentioned investigators made early observations in only one patient who was found to have a titer of 180 mouse units on the second day of ACTH therapy. They were unable to determine the duration of the augmented excretion primarily due to the scatter of specimens assayed and the frequent toxicity of the preparations injected. In the present series, 4 (Cases 2, 6, 8 and 9) were found to have their control titers at least doubled during the first 24 hours of hormonal therapy. Whether increased levels continued throughout therapy appears to have

been more a matter of a critical dosage than the duration of treatment, as suggested in Cases 2, 5, 6 and 8. Discrepancies appeared, however, in that Case 6(a) was found to have enhanced titers 24 hours following ACTH withdrawal and Case 9 was observed to have an 8-fold reduction in titer during 4 days of a fixed ACTH dosage. The possibility of a pituitary rebound effect in the former instance might be considered. With these irregularities and the known limitations of bioassay, it would be inappropriate to suggest a quantitative relationship between dosage and gonadotrophin excretion. It is apparent that similar gonadotrophin responses were elicited in male and female patients and that the degree of augmentation was not related to the gonadal status or the initial level of gonadotrophin excretion. This suggests that the explanation for the enhanced titers is less likely to be found in direct hormonal interrelationships than in a possible renotrophic effect of the adrenal steroids. Significant increases in glomerular filtration following ACTH administration have been reported(6-8). These changes, approximating 30%, would not account for the 2 to 8-fold increases in gonadotrophin titers recorded here and the 2 to 10-fold increases previously reported(1). Increased proteinuria has been observed during ACTH administration(9) and enhanced glomerular permeability might account for additional renal loss of the trophic hormones.

The observations on the dwarf with intrasella calcification (Case 4) suggest that increased pituitary function is essential for the maintenance of the enhanced titers. From clinical study and from an evaluation of 4 months of vaginal smears, the patient was considered to have incomplete sexual maturation with no significant variations in the low level of ovarian function. With a calcified anterior hypophysis and a fixed release of gonadotrophins (less than 4 m.u.) she was unable to sustain the transient response to the renotrophic effects of ACTH by releasing more gonadotrophin from the pituitary. It is questionable whether the results in Case 3 can be attributed to ACTH. The control urine specimen was collected 6 days prior to the day of intravenous ACTH which was administered on the 12th day of the menstrual cycle.

Absence of significant changes in ovarian and uterine weight of intact mice receiving ACTH in large amounts (Table II), essentially excludes the possibility that recovery from the urine of this hormone was partly responsible for the enhanced gonadotrophin titers during its administration. There is some evidence, furthermore, that the kidney is unimportant in the disappearance of ACTH from body fluids(10,11).

Further data are necessary before results herein presented can contribute to an understanding of the aberrations of gonadal function which have been observed during or following intensive ACTH therapy. It is possible, however, that increased renal loss of FSH during ACTH therapy might divert enough trophic hormone from the gonads to disturb their function. The possibility is recognized that a similar increased excretion of other trophic hormones might lead to a temporary aberration of end-organ function, such as the ACTH-induced hypothyroidism previously reported(12,13).

Summary. Augmented excretion of urine pituitary gonadotrophins has been demonstrated in 8 of 9 patients receiving ACTH for the treatment of several clinical entities. In the one exception, the doubling of the control gonadotrophin titer could not be attributed directly to the effects of the ACTH. Increases of 2 to 8-fold were observed in the remaining patients. The augmentation appeared to be independent of age and sex, was not proportional to the dose of ACTH, and showed irregularities in time of appearance or persistence. In the patient with hypophyseal calcification, the effect was observed on the second day of therapy only which suggested that normal hypophyseal function was essential for the increased excretion. The known renotropic effect of the adrenal steroids was considered the most probable explanation for the observed changes.

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Effects of Growth Inhibitors on Response of Rat's Uterus to Estrogen.* (19247)

JOSEPH T. VELARDO. (Introduced by Frederick L. Hisaw.)

From the Biological Laboratories, Harvard University.

Hertz and Sebrell(1) first reported the failure of estrogens to induce growth of the female reproductive tract in the absence of folic acid in the chick, and subsequent studies followed on the frog(2), rat(3), and monkey (4,5). In all of these studies the main objective was centered around gross morphological observations and weight relationships. The experiments reported here represent an attempt to examine the physiological nature of the inhibitory process produced by folic acid antagonists on the growth response of the uterus to estrogen, and to determine in what way this may differ from similar inhibitory effects produced by other compounds such as beryllium(6), cadmium(7), and strychnine. These comparisons are made on the basis of inhibitory effects on the imbibition of water by the uterine tissue in response to estrogen and changes in nitrogen, fat, and glycogen content.

Materials and methods. A total of 56 100-day-old female albino rats, of an inbred strain developed from Wistar stock, weighing 175-200 g, were used for these experiments. A folic acid antagonist (Aminopterin†), and beryllium, cadmium, and strychnine were given concurrently with estradiol.‡ The estradiol was dissolved in sesame oil, and the other drugs in physiological saline. All injections were made subcutaneously and the dosage of beryllium, cadmium, and strychnine was such that the animals given such treatment would survive for at least two weeks without loss of weight. The animals given aminopterin for 3 to 4 days lost little or no weight. Nitrogen determinations were made colorimetrically(8), while the tests for fat and glycogen were made by histochemical

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