ACTIVIDADES EN EL CAMPO DE LA INVESTIGACION BIOMEDICA.

Trabajo que presenta el Biol. Joaquín F. Herrera Muñoz ,
para optar al Grado de Maestro en Biología Experimental.

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La serie de trabajos de Investigación Publicados y aquí reunidos, fueron realizados en la Sección de Andrología de la Unidad de Investigación Biomédica del Centro Médico Nacional, del Instituto Mexicano del Seguro Social, bajo la Dirección del Dr. José Arturo Bermúdez y con la Colaboración y Asesoría del Dr. Gregorio Pérez Palácios.

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Agradecer tambien al Doctor José Arturo Bermúdez, al lado de quien he alcanzado el nivel que tengo en la actividad de la Investigación Biomédica.

A mi esposa e hijos, quienes tuvieron la paciencia para ver lograda ésta meta, a pesar de todo.

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I N T R O D U C C I O N .

Se ha presentado la oportunidad de que por medio de la exposición de mis actividades en el campo de la Investigación pueda optar a graduarme en la Mastría en Biología Experimental, por lo que a continuación expongo en forma breve, cuales han sido las areas en las que me he desarrollado, así como algunas de las motivaciones que me han llevado a ellas.

Mis actividades en la Investigación se iniciaron en el año de 1969, en el laboratorio del Dr. Oscar Domínguez V., en donde a través de los estudios del Metabolismo Esteroideo, se formaron en mi mente varias preguntas a las que he tratado de dar respuesta mediante varios modelos experimentales. Las preguntas fueron: ¿ Cual es el contenido endógeno de los esteroides que - participan en los estudios del metabolismo glandular ? , ¿ Cual es su papel en los niveles fisiológicos ? , ¿ Como se modifican durante condiciones fisiológicas o experimentales ?. Dar respuesta a tales preguntas, requirió de un entrenamiento intenso en técnicas de alta sensibilidad, como lo es el radioinmunoanálisis. Después de haber adquirido el entrenamiento técnico, se realizó una serie de estudios acerca de " Las pozas metabólicas de los esteroides en la glándula adrenal de la rata", los resultados de tales estudios mostraron un aspecto diferente del funcionamiento de dicha glándula.y fueron publicados en su oportunidad. Se cambió de modelo experimental al testículo, encontrando en éste órgano aspectos que incrementaron la motivación para estudiar su funcionamiento. Con éste modelo, se inició con una correlación de estudios morfológicos y la secreción de andrógenos en el testículo de cerdo, posteriormente, se realizó una investigación en testículos de ratas alimentadas con harina de semilla de algodón completa, en la que se buscó el efecto de uno de sus componentes, el Gosipol. En estos estudios se correlacionaron la esteroidogénesis con la cantidad y movilidad de los espermatozoides y a su vez con la capacidad fertilizante de los animales. Se llevaron al cabo tambien, otros estudios

complementarios en los que se utilizaron ratas a las que se les provocó desnutrición por restricción proteínica.

De los últimos estudios mencionados surgieron algunas otras preguntas que no presentaban una clara respuesta, así el interés inicial por las pozas metabólicas esteroideas ha ido transformandose , en un interés por el mayor conocimiento y mejor entendimiento del funcionamiento del testículo. Aunadas a la pregunta inicial, han surgido preguntas referidas entre otras, a la intercomunicación entre los compartimentos testiculares, a la presencia y función de receptores y otras proteínas con capacidad de unir esteroides y a los cambios en las funciones celulares por efecto de los esteroides.

En el área de la Investigación Clínica mi colaboración se ha dirigido a proyectos en los que se pretende estudiar, por una parte a pacientes con transtornos genéticos o congénitos que afectan las vias esteroidogénicas tanto de la glándula suprarrenal, como de las gonadas; en este tipo de estudios, la determinación cuantitativa de los esteroides precursores, intermediarios y hormonales, tanto en condiciones basales, como durante pruebas de funcionamiento glandular, permiten localizar o definir "la falla enzimática" dentro de la vía esteroidogénica, auxiliando así a los médicos a conocer la causa del problema clínico y coadyuvando también al mejor y más rápido diagnóstico de problemas similares. Por otra parte he colaborado en estudios de grupos de pacientes en los que el funcionamiento esteroideo se encuentra alterado en forma secundaria, por efecto de otro padecimiento aparentemente independiente; estudios que pueden ser tomados como base para el establecimiento de una terapia que mejore en parte las "condiciones generales" de los pacientes.

Tanto en los estudios de Investigación Básica, como en los de Investigación Clínica se pretende un mayor conocimiento y una mejor comprensión de la Fisiología de las glándulas esteroidogénicas.

Los planteamientos de intención en mis actividades de Investigación han sido el poder Plantear, Realizar, Desarrollar e Interpretar protocolos de Investigación para estudiar los aspectos naturales y/o experimentales, normáles y/o patológicos, básicos y/o clínicos de la Fisiología glandular esteroidogénica, por lo que he requerido de una preparación interdisciplinaria en los aspectos teóricos y técnico-experimentales . En ambos aspectos el haber cursado la Maestría en Biología Experimental me ha permitido un mejor y más amplio desarrollo dentro del campo de la Investigación Biomédica.

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SUBCELLULAR METABOLIC POOLS IN THE RAT ADRENAL GLAND. *IN VIVO* EFFECT OF ACUTE STIMULATION WITH ACTH ON STEROID BIOSYNTHESIS

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SUMMARY

To determine the metabolic pool changes during acute ACTH infusion, pregnenolone, 17-hydroxypregnenolone, dehydroepiandrosterone, progesterone, 17-hydroxyprogesterone, corticosterone and cortisol were measured by RIA in subcellular fractions from rat adrenal glands. The *in vivo* effect of ACTH on steroid biosynthesis was deduced from correlations between subcellular fraction measurements and serum concentrations values, permitting a dynamic interpretation of results. Twenty six Sprague–Dawley male rats, received 0.1 I.U./g of body weight of ACTH I.P. while eighteen (control group) received isotonic saline solution. Rats in both groups were decapitated immediately and at 5, 10 and 20 min after injection. Results showed the highest serum concentrations of corticosterone at time zero. Pregnenolone and corticosterone concentrations were highest in the mitochondrial fraction while progesterone was in the microsomal fraction. Exogenous ACTH depleted-pregnenolone and progesterone pools increasing the corticosterone pool as well as its serum concentration, with minor modifications in other steroids. Its maximal effect occurred at 10 min. In contrast with previous reports, it was found that 17-hydroxylated compounds such as cortisol, revealing 17-hydroxylase enzyme activity. It is concluded that ACTH not only stimulates pregnenolone synthesis but also increases the enzymatic activity of other systems which use this compound as a substrate to produce corticosterone.

INTRODUCTION

A better understanding of the physiological sequences and reactions in various metabolic systems has been achieved by labeling certain substances within the systems. For example, the relationship between steroid hormone biosynthesis and the mechanism of ACTH action has been investigated by perfusion of the whole organ incubation of tissue preparations [1–5] using *in vitro* and *in vivo* techniques in steroidogenic organs. In all these approaches, one or several labeled steroids are added to the medium and the final transformations of these radioisotopes are analyzed. By means of such studies all the metabolic routes of steroid biosynthesis, the enzymatic system for each reaction, as well as the cofactors required have been determined in the gland during anabolic phase, in the target cells, and in the specific deactivating organ. However, in the application of these techniques several factors, such as modification of cellular architecture, changes in membrane permeability and the intracellular relationship of the steroids have been disregarded. In addition, it would appear that exogenous radioactive steroids are handled differently from endogenous ones with regard to the enzymatic systems involved in some experiments employing these techniques. Thus, it is not surprising to find contradictory interpretations of a particular metabolic phenomenon such as those reached by Vinson and Whitehouse in 1969[6, 7], Stachenko *et al.*[8] and other groups [9, 10]. Moreover, Vinson and Whitehouse, observing incubations of rat adrenal gland in the presence of [^{3}H]-pregnenolone and [^{14}C]-progesterone and measuring the products formed and the transformation precentage of the mass by gas chromatography found differences in the transformation of mass depending on the radioactive material used. Stachenko *et al.*[8] on the other hand, used specific activity to measure the products and demon-

Trivial and systematic names Cholesterol, 5-cholest-en-3β-ol; 20α-hydroxycholesterol, 5-cholest-en-3β,20α-diol; Pregnenolone, 3β-hydroxy-5-pregnen-20-one; Progesterone, 4-pregnene-3,20-dione; 17-hydroxypregnenolone, 3β, 17-dihydroxy-5-pregnen-20-one; 17-hydroxyprogesterone, 17-hydroxy-4-pregnen-3,20-dione; Desoxycorticosterone, 21-hydroxy-4-pregnen-3,20-dione; desoxycortisol, 17,21-dihydroxy-4-pregn-ene-3,20-dione; Corticosterone, 11β,21-dihydroxy-4-pregnene-3,20-dione; Cortisol, 11β,17,21-trihydroxy-pregn-4-en-3,20-dione; Dehydroepiandrosterone, 3-hydroxy-5-androst-en-17-one.

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strated a difference in utilization by the enzymatic systems of radioactive steroids and of those endogenous ones already produced in the adrenal tissue.

Highly precise and accurate quantification of submicrogram amounts of steroids has become possible with RIA. Thus, it is feasible to measure metabolic pools of intracellular compartments which participate in the metabolism of the steroid hormones in a given organ. In the present study metabolic pools in some subcellular fractions of the rat adrenal gland were measured before and after acute *in vivo* stimulation with ACTH. The following steroids were studied: (a) precursors: pregnenolone (Preg), 17-hydroxypregnenolone (17OH Preg), and dehydroepiandrosterone (DHEA); (b) intermediate products: progesterone (Prog) and 17-hydroxyprogesterone (17OH Prog); and (c) the final active compounds: corticosterone (B) and cortisol (F).

MATERIAL AND METHODS

Biological material

Forty-four Sprague–Dawley male rats weighing approximately 200 g were used. The adrenal glands were stimulated with ACTH (Organon Laboratories, ampules of 25 I.U., lot 11720).

Reagents and equipment

Tris buffer (hydroxymethylaminometane lot B1370, Sigma Chemical Company), Sucrose (E. Merck), sodium chloride and sodium hydroxide (Baker Chemical Company) were used. All the RIA reagents have been described previously [11, 12]. Precoated silica gel plates with a fluorescence marker (254 nm) (0.25 mm thick from E. Merck, lot 147451) were employed in steroid purification. All solvents were distilled before use. The standard for protein determinations was bovine serum albumin (BSA) fraction V from Sigma Chemical Company, lot 119 B0250. A Beckman DK-2A spectrophotometer was utilized in the protein measurements. The subcellular fractions were obtained with following centrifuges: International U.V. Model, Head 240, Sorvall Rc.2B, Rotor SM24 and a Beckman Model L ultracentrifuge with a 50 Ti rotor. Radioactivity was measured with a Model 3320 Packard liquid scintillation spectrometer. The purity of the subcellular fractions was confirmed with a Phillips, Model EM-300 transmission electron microscope.

METHODS

Animals

The rats were divided in a control ($n = 18$) and stimulated group ($n = 26$). The stimulated lot received 0.1 I.U. per gram of body weight of ACTH dissolved in isotonic saline solution (10 I.U. per ml), intraperitoneally. The control group received 0.01 ml per gram of body weight of saline solution by the same route. Animals were sacrificed at 0,5,10 and 20 min post injection. Blood was collected for serum acquisition, and adrenal glands were immediately removed and weighed. They were homogenized in Tris–buffer (Tris 10 mM, sucrose 0.25 M, EDTA 0.05 mM, pH 7.4) at 1:10 weight/volume ratio. The reactions were stopped by adding 0.1 M NaOH and 0.15 M NaCl to the homogenate until the total mixture reached a volume of 3 ml. The time between death and inhibiton of the enzymatic reactions was less than 7 min. The whole procedure was performed at 4°C.

Subcellular fractions

Based upon techniques previously described [13, 14] the subcellular fractions were obtained as follows. First, the whole homogenate was centrifuged at 800 *g* for 15 min. Pellet I was made up of connective and not homogenized tissue, nuclei and some blood cells. Supernatant I, called "homogenate" was divided into 3 aliquots, one for protein determination (0.1 ml), the second (1 ml) for steroid determinations and the third (approximately 1.9 ml) for obtaining the subcellular fractions. This last portion was centrifuged at 10,000 *g* for 30 min and thus provided pellet II which, after washing and resuspension to its original volume, was called the mitochondrial fraction. Supernatant II was centrifuged at 105,000 *g* for 60 min to obtain pellet III. Following similar washing and resuspension, this was considered the microsomal fraction. Supernatant III was called the soluble fraction. The mitochondrial, microsomal, and soluble fractions were divided into aliquots of 0.25 ml for protein determinations and of 1.65 ml for steroid evaluations. All samples were kept frozen until studied. The mitochondrial and microsomal fractions from one of the pilot experiments were studied with an electron microscope (Philips EM-300) to verify the purity of the subcellular fraction. Both fractions were first fixed in glutaraldehyde 2.5% followed by 0.1 M osmium tetroxyde, pH 7.4, included in araldite, and finally sectioned with a Porter Blum Mt-2 ultramicrotome. The method of Lowry *et al.* [15] was used for protein quantification, and the reference for the standard curve in quintuplicate was the BSA with readings at 660 nm. All samples were assayed in triplicate.

The steroid measurements were made using RIA methods already described for the Δ^5-3β hydroxy series [11] as well as for Prog and 17OH-Prog [12]. The determinations of B and F were performed following techniques similar to those described by Ruder *et al.*[16]. The basic difference from the Ruder *et al.*[16] technique was that methylene chloride was used for extraction. The chromatographic system for thin layer chromatography was methylene chloride, methanol, and water in proportions of 100:6.0:0.3. The RIA calculations for all the steroids were processed in an IBM 1130 computer, programmed at a G level in Fortran. Quality control results for all the RIA assays were comparable to recent reports [11, 12] with accuracy of 98%. The intra-assay coefficient of variation was less than 8% and the minimal detect-

Table 1. Steroid metabolic pools in the rat adrenal gland after acute ACTH stimulation

	HOMOGENATE ng/mg Prot*				MITOCHONDRIAL ng/mg Prot				MICROSOMAL ng/mg Prot				SOLUBLE ng/mg Prot				SERUM ng/ml			
TIME (MINUTES)	0	5	10	20	0	5	10	20	0	5	10	20	0	5	10	20	0	5	10	20
PREG (1)	63±1.1	147±2.0	216±3.5	77±1.1	133±1.8	703±4.9	2715± 14	215±3.0	74±1.0	401±4.3	143±1.2	177±3.7	16±0.4	34±0.6	42±0.9	30±1.0	3.1±0.4	8.4±0.2	15±1.0	10±0.5
(2)		140±2.0	** 99±2.4	** 58±1.4		** 559±4.9	** 1071± 13	** 174±2.9		** 359±3.4	** 257±3.8	** 223±2.5		29±1.1	** 29±0.8	** 17±0.7		8.6±0.4	** 5.5±0.3	** 3.2±0.3
17OHPREG	2±0.1	1.8±0.2	1.4±0.2	1.5±0.1	4.0±0.3	5.1±0.6	4.2±0.4	2.9±0.5	0.8±0.1	3.5±0.3	1.5±0.2	1.8±0.3	0.16±0.0	0.3±0.0	0.4±0.1	0.4±0.0	0.1±0.0	0.4±0.0	0.9±0.1	0.8±0.1
		1.7±0.2	1.3±0.3	1.8±0.1		5.2±0.4	4.6±0.4	2.5±0.1		3.3±0.4	** 3.0±0.4	2.2±0.2		0.3±0.1	0.4±0.0	0.5±0.1		0.3±0.1	0.7±0.1	1.0±0.1
DHEA	0.9±0.1	2.2±0.1	1.7±0.2	1.4±0.2	5.1±0.5	10.3±0.4	10.1±0.7	7.8±0.4	2.3±0.1	6.3±0.6	33±1.0	5.5±0.6	0.56±0.0	0.9±0.1	0.5±0.0	1.0±0.2	0.1±0.0	1.0±0.1	1.4±0.1	1.5±0.2
		1.7±0.2	1.3±0.2	1.2±0.1		7.3±0.3	** 6.1±0.3	** 5.9±0.4		** 10.1±0.3	** 13±0.7	7.2±0.5		0.8±0.0	0.6±0.1	0.8±0.1		** 0.5±0.0	** 0.8±0.1	1.3±0.1
PROG	58±1.3	56±1.2	59±1.0	54±1.1	166±1.7	252±2.5	274±2.4	251±1.8	186±2.0	217±2.9	330±1.7	174±2.8	31±1.0	43±0.6	34±0.4	32±1.0	2.4±0.1	10±0.5	11±0.6	12±0.7
		51±1.2	** 46±0.9	** 33±0.9	***	** 211±2.2	** 209±2.0	** 226±2.4		** 193±2.1	** 191±2.2	** 195±2.0		40±1.1	31±0.7	** 25±1.0		9.2±0.8	18±0.4	** 27±0.8
17OHPROG	0.3±0.1	0.5±0.2	0.9±0.1	0.9±0.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.4±0.1	0.8±0.2	1.3±0.1	1.2±0.2
		0.6±0.1	0.8±0.2	1.1±0.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		0.8±0.1	1.2±0.0	1.0±0.1
B	8.9±0.6	14±0.7	16±0.9	9±0.9	104±2.1	135±2.2	103±2.7	92±2.0	85±1.7	90±1.6	95±1.6	78±2.5	6.4 ±0.2	7.3±0.8	8.2±0.4	6.9±0.5	179±2.1	201±1.6	279±2.0	234±1.5
		** 24±0.4	** 30±0.7	** 20±0.5		** 160±1.9	** 229±2.1	** 115±2.1		** 107±2.5	** 157±2.3	87±2.0		9.1±0.3	** 12.1±0.9	** 10±0.9		** 232±2.2	** 419±3.6	** 340±2.1
F	5.9±0.8	7.8±0.2	9.0±0.5	7.1±0.4	*** ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	9.0±0.3	10±0.5	12±0.5	8.8±0.5
		8.3±0.3	10.5±0.7	8.6±0.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		13±0.9	** 20±0.4	** 15±0.5

(1) Control group.
(2) Stimulated group.
* Mean ± SD.
** Statistically different $P < 0.05$ between control and stimulated groups.
*** Not determined.

able dose was 100 pg. Student's *t*-test was used to analyze the differences between the values obtained at different times in each group, as well as to compare control and experimental group results. Five per cent confidence limits were employed.

RESULTS

At 30,000 × magnification the mitochondrial and microsomal fractions showed very little contamination. The metabolic pool values and the serum concentrations in the different subcellular fractions appear in Table 1. Pools are expressed in ng/mg of protein (ng/mg) and serum concentrations in ng/ml. The values represent the mean of eight determinations at time zero and of six determinations at 5, 10, 15 and 20 min in both stimulated and control groups.

Figure 1 depicts the initial size (time zero in Table 1) of the metabolic pools in the subcellular fractions. In the *homogenate* Preg and Prog had similar values (63.3 and 58.2 ng/mg, respectively) which comprised the largest metabolic pools. They were followed by 8.9 ng/mg of B, and 5.9 ng/mg of F. The pools of other 17-hydroxylated compounds and DHEA were quite small. In the *mitochondrial* fraction, Prog was the predominant steroid with 166.2 ng/mg, followed by Preg (133.5 ng/mg) and B (104.2 ng/mg); 17OH-Preg and DHEA had a small pool size. In the *microsomal* fraction, Prog had the greatest pool (188.6 ng/mg), followed by Preg and B (74.3 and 85.3 ng/mg, respectively). In the *soluble* fraction, the pool size for Prog was 31.8, for Preg, 16.5 and for B, 6.4 ng/mg. 17OH-Preg and DHEA values were below 1 ng/mg. In *serum*, B had the highest initial concentrations (179 ng/ml), followed by F (9.0) Preg (3.1) and Prog (2.4); the remaining steroids analyzed showed considerably lower values (below 1 ng/ml).

As we can observe in Table 1, almost all steroids in the subcellular fractions, as well as in the serum, tended to increase after time zero, reaching maximum values at 5 or 10 min in both experimental groups. Among the precursors, pregnenolone showed the largest pool size in the mitochondrial fraction. Values 20 times higher than time zero were noted in the control group. It is interesting to note that the differences between the groups were most evident in this fraction, with smaller pools in the stimulated group; however, there was a second increment at 20 min in the *microsomal* fraction. 17OH-Preg and DHEA behaved with a variable pattern, increasing at the beginning and decreasing thereafter. In the homogenate, where values were statistically similar for both groups of animals, Prog, the intermediary in the biosynthesis of B decreased rather than increased in the stimulated group. In the mitochondrial and microsomal fractions, progesterone was also higher than other values throughout all observation, its highest point at 10 min with 330 ng/mg P, was followed by a steep fall to lower values than time zero. In these fractions the highest values for Prog, as well as the most evident changes with respect to initial values, were found in the control group. 17OH-Prog, measured only in the homogenate and serum climbed rapidly and reached its apex at 10 min. At 20 min, values in both groups descended slightly. The final product B, increased at 5 min in all fractions and in both groups, with highest

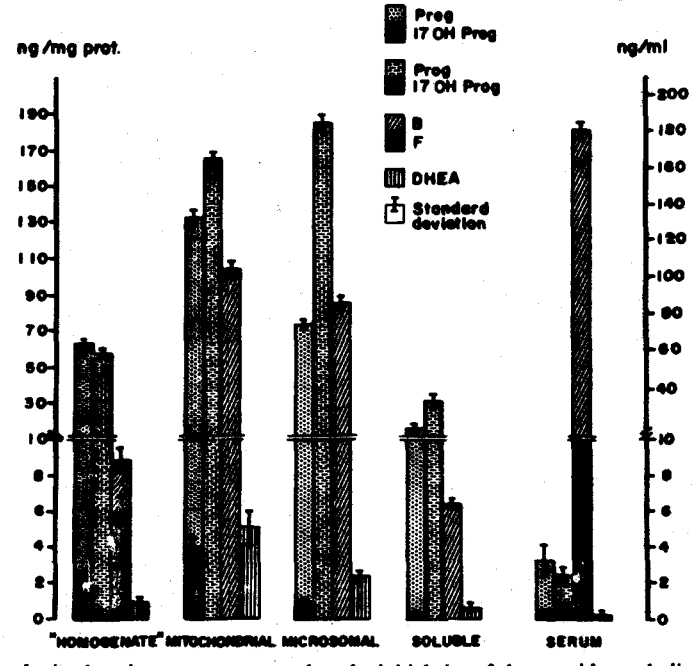

Fig. 1. Values obtained at time zero correspond to the initial size of the steroid metabolic pools in the subcellular fractions.

concentration at 10 min, followed by a moderate decline up to the end. The highest amount of B and its most evident changes were found in the mitochondrial fraction probably since it contains the 11β hydroxylase system.

So far, the analysis of results has been limited to the adrenal tissue response under the two experimental conditions. However, it seemed appropriate to compare the net effects of ACTH on the metabolic pools studied. In this context, we performed the following calculations: taking each control value at 0, 5, 10 and 20 min as 100%, we calculated the corresponding values of the stimulated group, and expressed the net effect of ACTH as per cent change. The results appear graphically in Figs 2–6.

Figure 2 shows the net changes induced by ACTH in the *homogenate fraction*. The Preg metabolic pool was diminished as a response to the stimulation, reaching its minimum at 10 min. The metabolic pool of the following steroid Prog, decreased continuously during the study, with the decrease becoming statistically significant by 10 to 20 min. This was most important at 20 min despite the recovery of the Preg pool. The double decrease was reflected in the metabolic pool of B, the final product of this metabolic route. The B pool was significantly increased throughout the sampling reaching its maximum at 20 min, at a level 2.21 times greater than the corresponding control. The rest of the steroids showed few changes.

Figure 3 portrays the changes in the *mitochondrial fraction*. Preg diminished to only 39% at 10 min of stimulation, with an increment at 20 min. There was a great metabolic pool rise of B, reaching over 220% of the control value within 10 min and coinciding with the maximum Preg response. Prog, with a pattern similar to Preg displayed smaller variations which can be considered a reflection of changes throughout the gland. DHEA had a similar pattern with a decrease at 5 min to a level similar to that observed in the majority of the other steroids.

The *microsomal fraction* (Fig. 4) synthesizes and transforms Prog. In this fraction, Preg showed a small but significant decline at 5 min. Subsequently it increased up to 180% and fell to 126% of control values. In the mitochondria Preg leaves faster than it is produced. These facts suggest an apparently ACTH dependent exit of Preg from the mitochondria with subsequent accumulation in the endoplasmic reticulum. At 10 min Prog dropped to less than 60% of the control pool and then increased to 110% at 20 min. DHEA had an extremely variable pattern. At 5 min it increased to its maximum value, almost 60% above the basal level, falling 40% below control at 10 min. The statistical outcome is similar to the control.

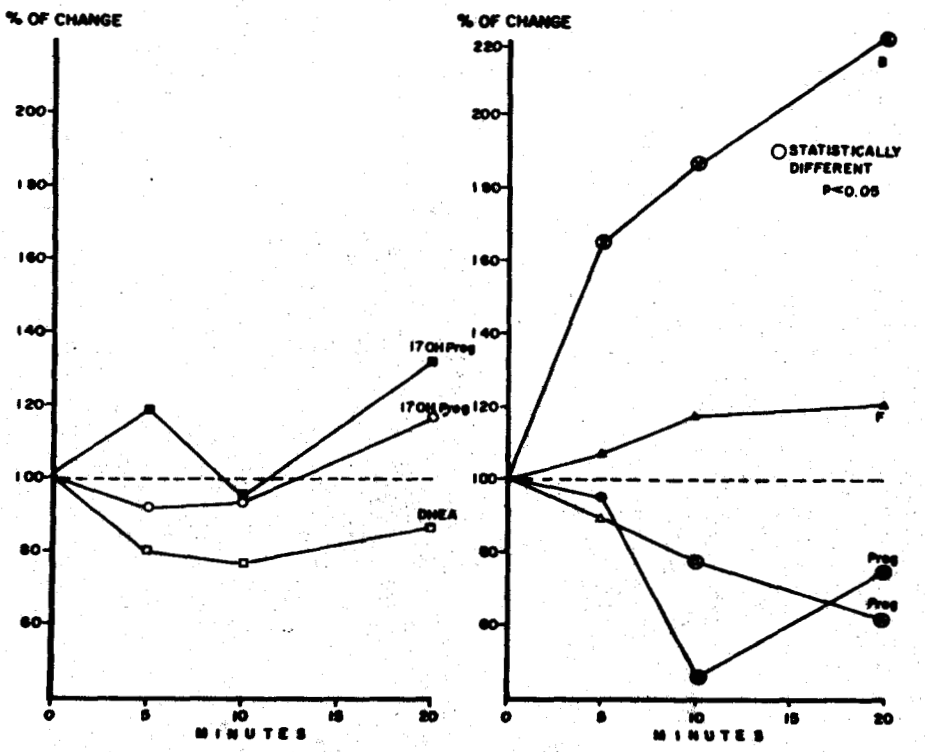

Fig. 2. Time course of the acute ACTH induced changes in the concentration of different steroids in the adrenal homogenate fraction. For each time point, control values were taken as 100% (see text). Pregnenolone: ● 17 OH-pregnenolone: ○ progesterone: △ 17 OH-progesterone: ■ dehydroepiandrosterone: □ compound B: × and Compound F: ▲

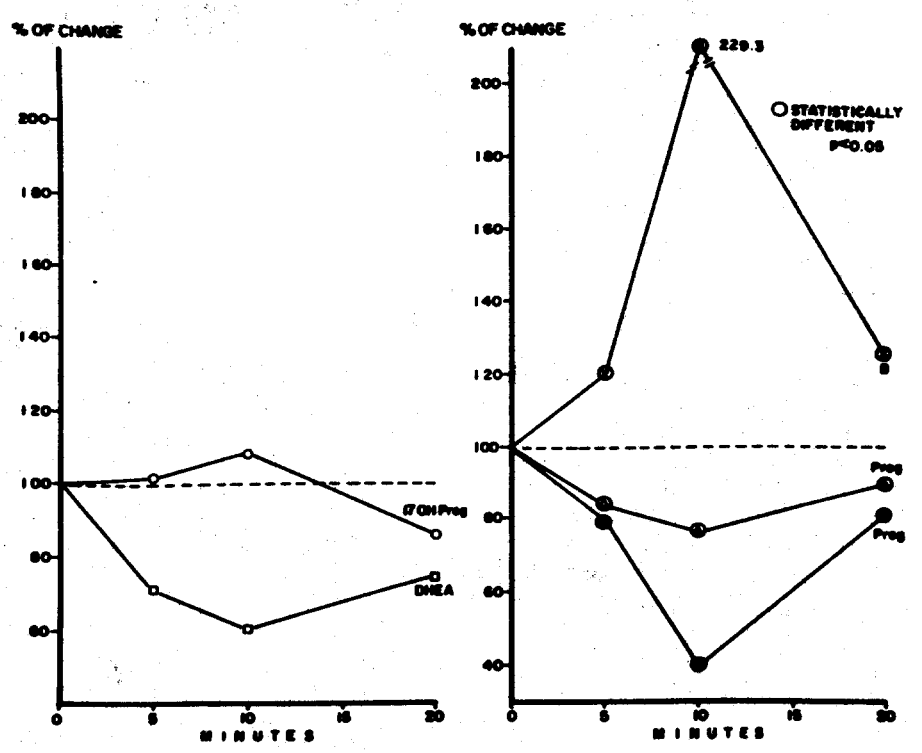

Fig. 3. Steroid concentrations changes with acute ACTH stimulation found in the mitochondrial fractions. Code as in Fig. 2.

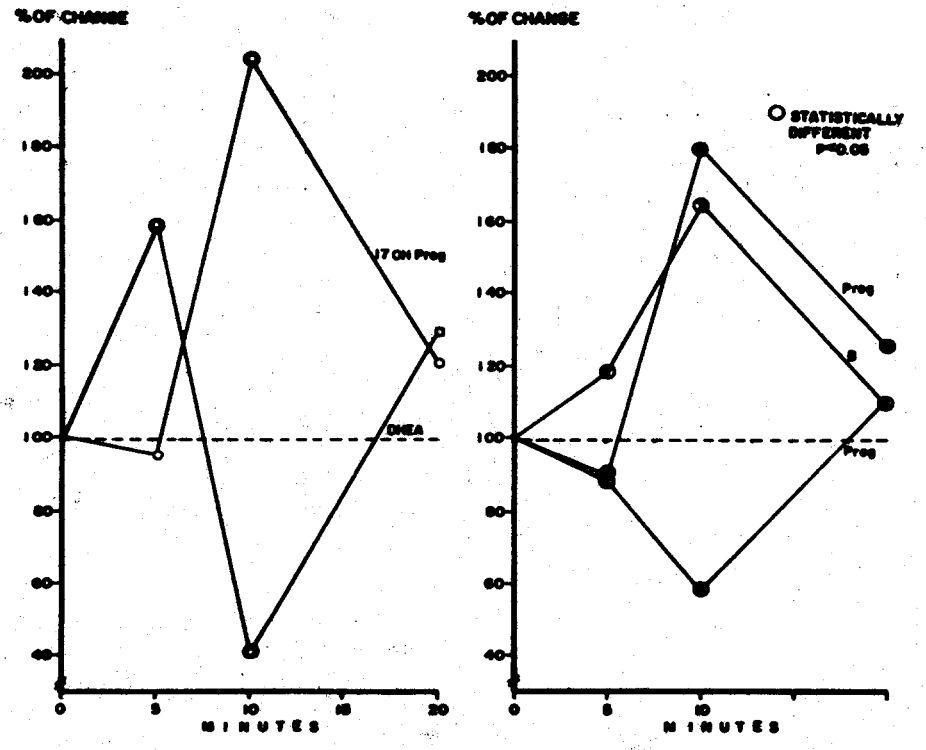

Fig. 4. Steroid concentrations changes with acute ACTH stimulation found in the microsomal fractions. Code as Fig. 2.

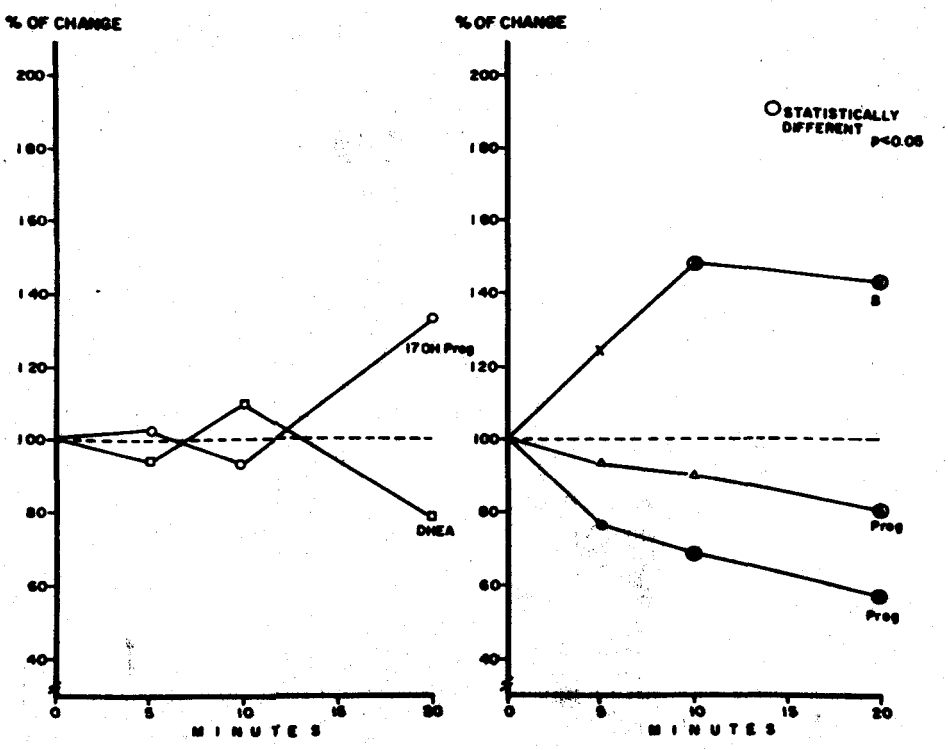

Fig. 5. Steroid concentrations changes with acute ACTH stimulation found in the soluable fractions. Code as Fig. 2.

The *soluble fraction* (Fig. 5) shows less important changes. Preg diminished significantly between min 10 to 20 and Prog fell most sharply at 20 min. B increased significantly at 10 min. The rest of the steroids analyzed showed minimal modifications.

The *serum* changes were important (Fig. 6). The precursor, Preg diminished considerably at 10 min, with a small further fall at 20 min. Simultaneously, Prog increased at 10 min reaching its peak concentration at 20 min, more than 225% of the control in 10 min. DHEA fell most at 5 min, recovering at the end of the study. F reached its maximum concentration at 20 min, 75% above the control.

DISCUSSION

Some of the first results concerning the mechanism of action of ACTH appeared in 1951. Hechter[17] reported an increase in steriodogenesis in bovine adrenal glands perfused *in vitro* with labeled precursors and stimulated with ACTH. Later, Karaboyas and Koritz[2] observed an increment in adrenal cAMP levels and located the steroidogenic increase in the mitochondrial step between cholesterol and pregnenolone. In 1959 Haynes, Peron and Koritz[18] reported ACTH-induced increases in steroidogenesis similar to those obtained in rat adrenal gland incubations with cAMP. With the same approach and purpose, Ferguson[19] found that parallel to the steroidogenic surge there was a marked influence on protein synthesis by ACTH, which was inhibited by puromicine even under ACTH or cAMP stimulation. In this context Garren, Ney and Davis[20] reported a slowing down in steroid biosynthesis following administration of cycloheximide during maximum stimulation with ACTH. They also found a rapid transformation of pregnenolone to corticosterone, thus confirming that the critical biosynthetic step was the conversion of cholesterol to pregnenolone. They suggested that one or several proteins with a very rapid metabolic turnover were needed and concluded that such levels regulate the velocity of steroid biosynthesis. Farese[21, 22] described two protein factors which interact by stimulation or inhibition of the critical step within the mitochondrion. Similar results, described by Schulster[1] in 1970 support these findings. Hirshfield and Koritz[3] analyzed some substances which produce "swelling" of the mitochondrion with a concomitant increase in the synthesis of pregnenolone. Koritz and Hall[23, 24] proposed an inhibitory allosteric feed-back regulation of pregnenolone which inhibits the cholesterol transformation to 20OH-cholesterol. Analyzing the cofactors involved in the hydroxylation reaction, Peron *et al.*[25] found that NADPH was required as a cofactor and suggested that cAMP controlled the electron flow to NADP. Similar findings have been reported by Hirshfield and Koritz[26]. However, Koritz and Kumar[4] provided data which argues against the relationship between protein synthesis and steroido-

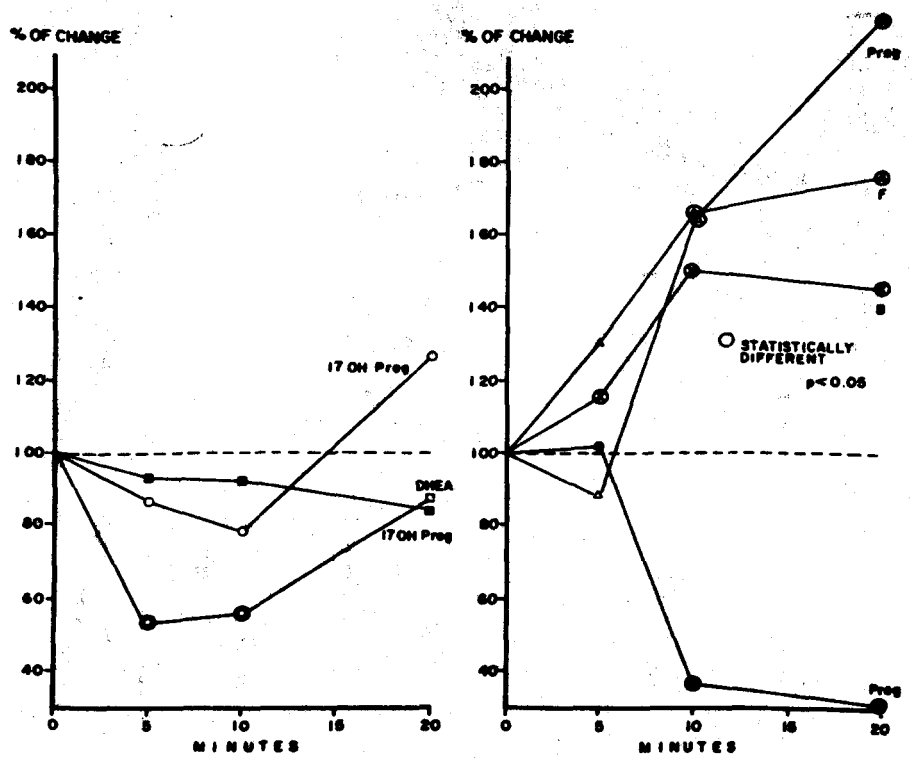

Fig. 6. Steroid concentrations changes with acute ACTH stimulation found in the serum fractions. Code as Fig. 2.

genesis and proposed a mechanism of action of ACTH which only implies changes in the permeability of the mitochondrial membranes. They argue that reduction of the inhibiting feed-back by pregnenolone, with an increase in the intake of NADPH, brings about a final increment in the total steroidogenesis.

To aid in the search for resolutions to the controversies mentioned above, we calculated the net effect of ACTH on the adrenal steroid metabolic pools. In the homogenate fraction, the fall in the Preg pool might mean that Preg is used as substrate more rapidly than it is produced. This should be reflected in the following compound, the intermediary Prog, which was affected by the ACTH and also was used faster than it was produced. If we consider that the homogenate fraction reflects the general changes occurring in the interior of the gland, an increment in the enzymatic activity leading to the formation of Preg would have been expected since its mass was increased. However, there was an activation of the main portions of the enzymatic systems that use this steroid as substrate transforming it to Prog. This would explain the decrease of the metabolic pool of the precursor. The Prog formed was also transformed into B more rapidly than it was formed. Since these findings were present in all the subcellular fractions, it is possible to suggest the existence of a preferential biosynthetic pathway: Preg → Prog → DOC → B, with possible alternate routes forming DHEA from 17OH-Preg and F from 17-hydroxylated compounds, another possible route could be the formation of 18-oxygenated steroids, intermediaries in the biosynthesis of aldosterone but their metabolic pools were not included in the study. However, in the microsomal fraction, the Prog pool diminished simultaneously with the maximum increase of Preg, suggesting a depletion of the Prog forming enzymatic systems since at 10 min a great quantity of substrate was not transformable to Prog. On the other hand, Preg is also a substrate for 17-hydroxylase in forming 17OH-Preg, which increased significantly at 10 min, when most steroids reached maximum values. It would appear that the synthesis was overcome by the velocity of transformation. Nevertheless, this fraction contains the enzymatic systems that formed Preg, as well as 11β hydroxylase whose final product is B.

Since DHEA and 17OH-Preg are related, we believe that the first increment of DHEA might arise from 17OH-Preg at the start of its formation. During the maximum response all the gland's "efforts" seem to go toward the main metabolic pathway without production of DHEA and accumulation of 17OH-Preg. Once the maximum effect of ACTH is exhausted the gland again produces DHEA, starting from 17OH-Preg. Thereafter, both steroids return to their control values. This would imply that a possible alternate pathway towards DHEA is not particularly important during acute ACTH stimulation.

In our model with ACTH stimulation, the serum

values reflect intraglandular changes. Preg is used as a precursor within the gland, diminishing its serum concentration. The glandular synthesis of Prog increases, augmenting its seric concentration. B, the final product of the main metabolic route, also increases at 10 min indicating the probable moment of maximum response to the stimulation with ACTH. F, the other final product increases but DHEA does not. This might indicate that between DHEA and F, the latter is more important in the rat adrenal gland. This might be due to a metabolic tendency using the enzymatic systems as the preferential metabolic route. It would also seem, that changes in the final product are less important than in the precursors and intermediaries, although it is necessary to remember that serum concentrations of Preg and Prog are 60 times lower than that of B, the end product.

In conclusion: I—Steroids metabolic pools and experimental changes can be determined by radioimmunoassay of steroidogenic tissues. II—The presence of 17OH-Preg, 17OH-Prog, F and DHEA in the rat adrenal gland implies the existence and functioning of the 17-hydroxylase enzymatic system in agreement with results found by Vinson *et al.*[27]. The proportion between these compounds and the non-hydroxylated ones in the same position underscores a preferential metabolic route formed by Preg → Prog → DOC → B. III—ACTH stimulation not only increases the production of the precursor Preg, but also that of the full biosynthetic route. IV—The net effect of ACTH was a depletion of both Preg and Prog pools. Apparently, both compounds are used faster than they are formed. Therefore, ACTH stimulates the enzymatic system producing Preg, as well as those using Preg as a substrate towards Prog and then DOC. V—The values found in blood serum reflect changes in the adrenal gland. The results obtained in the mitochondrial and microsomal fractions suggest that some factor transports mitochondrial Preg to the endoplasmic reticulum where it is transformed to Prog. Future experiments will be conducted to test this hypothesis.

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Estudio de la actividad ovárica pos-parto mediante la determinación de progesterona en ovejas Dorset, Suffolk y Tabasco[a]

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Resumen

Se estudió el reinicio de la actividad ovárica posparto en ovejas adultas, Dorset, Suffolk y Tabasco cuyos partos ocurrieron entre el 4 de enero y el 23 de febrero de 1978. Se utilizaron 10 animales de cada raza, los que fueron mantenidos en confinamiento. El trabajo se llevó a cabo en el Rancho Experimental de la Facultad ubicado en Tepotzotlán, Estado de México a 19° 44′ latitud norte y 99° 44′ longitud oeste. Se tomó como indicador del reinicio de la actividad ovárica después del parto, el incremento de los niveles circulantes de progesterona, cuya determinación fue hecha por el método de radioinmuno-análisis en muestras de suero obtenidas en los días 10, 20, 25, 30, 35, 40, 45, 50 y 55 posparto (Día 0 = día del parto). A los 30 días posparto, tres ovejas Tabasco, una Dorset y ninguna Suffolk mostraron evidencia de reinicio de la actividad ovárica. En el día 35, 40% de las ovejas Tabasco mostraron evidencia de cuerpo lúteo activo, mientras que sólo el 10% de las otras dos razas presentaron una elevación en los niveles de progesterona. Para el día 40, el 60% de las ovejas Tabasco mostraron indicio de actividad ovárica comparado con sólo 20% de las Dorset y Suffolk. Estos resultados, aunados a otras características reproductivas ventajosas de la raza Tabasco, señalan el potencial de estos animales para incrementar la producción ovina en el trópico.

Introducción

Los ovinos poseen ciertas cualidades que los sitúan en una posición ventajosa sobre otras especies animales, tales como su gran adaptabilidad, su condición de rumiante, su tamaño pequeño que hace que requiera un espacio reducido, su docilidad y fácil manejo y la existencia de gran número de razas adaptadas a diversidad de condiciones ambientales. Esto les permite aprovechar muchas zonas geográficas cuyas características climáticas o topográficas impedirían la introducción de otras especies.

Existe la necesidad y el interés creciente en mejorar y aumentar la producción ovina en México; para esto, es necesario desarrollar tecnologías de producción que permitan elevar al grado óptimo la utilización de los recursos disponibles. Entre los componentes de los sistemas de producción el aspecto reproductivo juega un papel importante. Cuanto mayor sea el número de crías obtenidas por borrega en una determinada unidad de tiempo, tanto más eficiente será la explotación.

Una forma de incrementar la eficiencia reproductiva es mediante el acortamiento de los intervalos entre partos. Para esto es necesario conocer la fisiología del posparto, particularmente el reinicio de la actividad ovárica. Es difícil establecer con exactitud cuándo se reinicia la actividad ovárica después del parto en la oveja; algunos autores aseguran que una elevada proporción de ovejas puede ovular y mostrar signos de estro en forma natural en el transcurso de los primeros 2 a 3 meses. Sin embargo, no es fácil distinguir los calores posparto, pues pueden ser silentes.[3]

De la extensa revisión bibliográfica hecha por Hunter[6] se desprende que el reinicio de la activi-

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dad ovárica posparto varía en función de factores genéticos y del medio ambiente. Se han encontrado diferencias notables entre razas. Entre los factores ambientales destacan el nivel nutricional y la presencia o ausencia del macho. La presencia del macho al final de la lactancia estimula la presentación del estro y ovulación. El amamantamiento, según algunos investigadores,[6] no influye sobre la duración del anestro posparto, mientras que según otros,[8] retarda el reinicio de la actividad ovárica. La época del año en que ocurre el parto puede también influir, sobre todo en razas con marcada estacionalidad reproductiva.

El objetivo de este trabajo es determinar el reinicio de la actividad posparto en la oveja y las diferencias entre razas.

Material y métodos

Animales. Se utilizaron ovejas adultas de las razas Dorset, Suffolk y Tabasco (también conocida con el nombre de Pelibuey), en número de 10 de cada una. Estos animales fueron mantenidos en el Centro Nacional para la Enseñanza, Investigación y Extensión de la Zootecnia, rancho 4 Milpas de la Facultad de Medicina Veterinaria y Zootecnia de la UNAM, ubicado en Tepotzotlán, Edo. de México, a 19° 44′ latitud norte y 99° 44′ longitud oeste, bajo confinamiento en corrales. Su alimentación fue a base de heno de alfalfa a discreción y un suplemento de concentrado. Las ovejas parieron entre el 4 de enero y el 23 de febrero de 1978.

Toma de muestras. La determinación del reinicio de la actividad ovárica se basó en la elevación de los niveles circulantes de progesterona, como indicación de la presencia de cuerpo lúteo activo.

Para ello se tomaron muestras de sangre de cada animal a intervalos definidos después del parto; la primera se tomó en el Día 10 (Día 0 = día del parto), la segunda en el Día 20, y las siguientes a intervalos de 5 días hasta el Día 55. Las muestras de sangre, 10 ml en cada caso, se recolectaron por punción yugular con jeringas estériles. Los sueros fueron separados por centrifugación de las muestras coaguladas, y guardados en congelación a —10°C hasta su procesamiento.

Determinación de progesterona. Se llevó a cabo en los laboratorios de la División de Biología de la Reproducción, Unidad de Investigación Biomédica del Instituto Mexicano del Seguro Social.

El método usado fue el de radioinmunoanálisis (RIA) según la técnica descrita por Bermúdez *et al.*[1] Todas las muestras fueron procesadas en un ensayo único al mismo tiempo y bajo similares condiciones.

Análisis estadístico. Las comparaciones entre razas, en lo que respecta al reinicio de la actividad ovárica a diferentes etapas posparto, se hicieron mediante la prueba de X^2 (Ji — cuadrada).[9]

Resultados

Las concentraciones individuales de progesterona en el suero sanguíneo de las ovejas Tabasco, Suffolk y Dorset, con presencia aparente de cuerpo lúteo, se presentan en las figuras 1, 2 y 3.

En la figura 1 se puede observar que la oveja 493 de la raza Tabasco, tuvo 489.9 pg/ml de progesterona a los 25 días posparto, esta cantidad aumentó

FIGURA 1. NIVELES DE PROGESTERONA SERICA EN BORREGAS TABASCO CON PRESENCIA APARENTE DE CUERPO LUTEO.

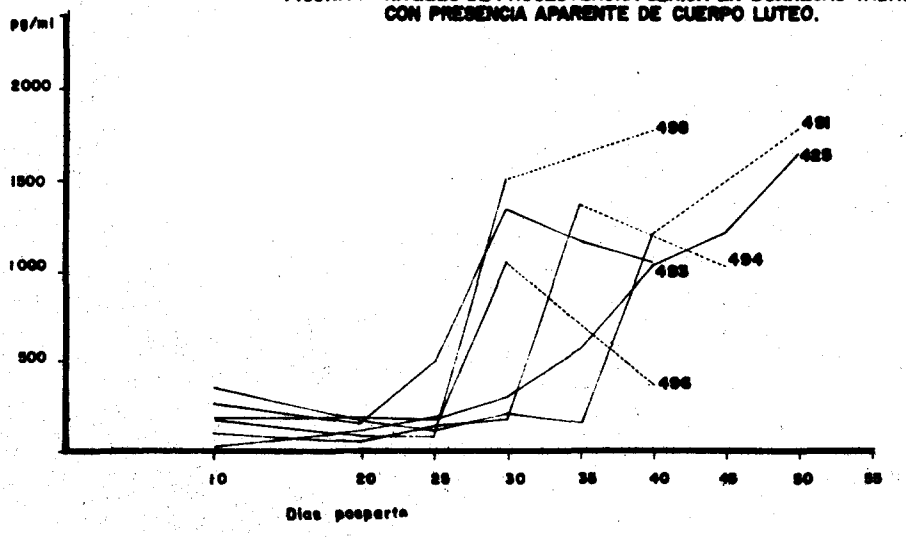


FIGURA 2. NIVELES DE PROGESTERONA SERICA EN BORREGAS SUFFOLK CON PRESENCIA APARENTE DE CUERPO LUTEO.

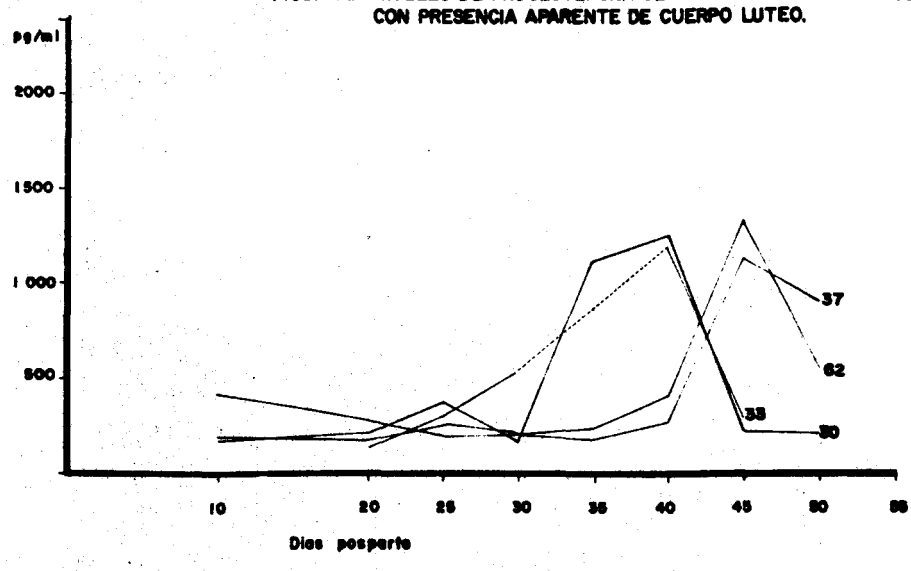
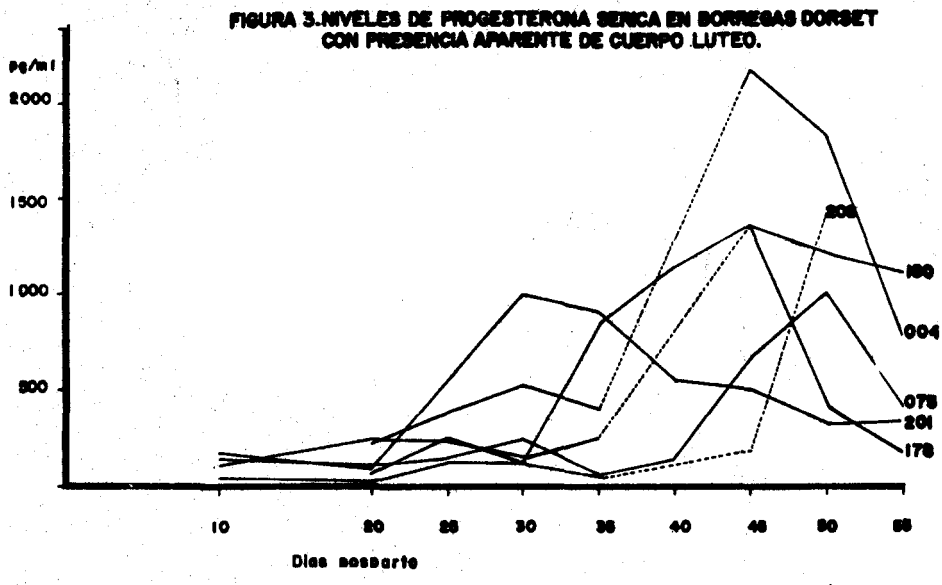


FIGURA 3. NIVELES DE PROGESTERONA SERICA EN BORREGAS DORSET CON PRESENCIA APARENTE DE CUERPO LUTEO.



a 1337.2 pg/ml hacia el día 30, lo que indica que probablemente hubo ovulación antes del día 25. En el día 30, dos ovejas más (496 y 498) mostraron niveles altos de progesterona, lo que se interpreta como indicio de presencia de cuerpo lúteo activo.

Según la figura 2, la primera indicación de presencia de cuerpo lúteo activo en ovejas Suffolk ocurrió el día 35 cuando la oveja núm. 30 mostró una marcada elevación del nivel de progesterona.

En la raza Dorset (figura 3) la primera elevación del nivel de progesterona ocurrió en una oveja (núm. 201) el día 25, con un aumento mayor en el día 30; esto indica que probablemente hubo ovulación antes del día 25.

En el Cuadro 1 se presenta un resumen del número de hembras con supuesta actividad ovárica a diferentes intervalos después del parto, tomando como base los cambios en los niveles de progesterona circulante. En el día 25 solamente una oveja Dorset dio muestras de presencia de cuerpo lúteo activo, mientras que en el día 30 tres borregas Tabasco mostraron actividad en comparación con 1 Dorset y ninguna Suffolk. La diferencia entre Suffolk y Tabasco fue estadísticamente significativa al 10% de probabilidad ($X^2 = 3.52$, $P < 0.10$). La diferencia entre Tabasco y las otras dos razas en el día 40 fue igualmente significativa al 10% ($X^2 = 3.34$, $P < 0.10$).

CUADRO 1

NÚMERO DE OVEJAS QUE MOSTRARON ACTIVIDAD OVÁRICA EN DIFERENTES ETAPAS POSPARTO, CON BASE EN LOS NIVELES DE PROGESTERONA CIRCULANTE

	Tabasco		*Suffolk*		*Dorset*	
Días posparto	*n*	*Activas*	*n*	*Activas*	*n*	*Activas*
10	10	0	10	0	10	0
20	10	0	10	0	10	0
25	10	0	10	0	10	1
30	10	3	10	0	10	1
35	10	4	10	1	10	1
40	10	6	10	2	10	2
45	(a)	—	10	4	10	4
50	(a)	—	(a)	—	10	6

(a) No se dispuso de muestras para determinar los niveles de progesterona en los animales restantes.

DISCUSIÓN

En la borrega, la fuente principal de progesterona durante el ciclo estral son las células luteínicas del cuerpo lúteo, aunque esta hormona también se secreta en la corteza suprarrenal, pero en concentraciones muy bajas.[12]

Según Pant *et al.*[7] y Walton *et al.*[12] los niveles plasmáticos de progesterona durante la fase luteínica del ciclo estral varían de 3 a 5 ng/ml; algunos investigadores[18] han encontrado cifras mayores de 6 ng/ml; sin embargo, Thorburn *et al.*[10] dan valores de 0.4 ng/ml de progesterona durante los primeros 4 días del ciclo (niveles basales) y de 1.5 a 2.5 ng/ml entre los días 4 a 9 del ciclo, permaneciendo estos valores durante la fase lútea hasta declinar hacia el día 14 o 15 del ciclo estral. Estos últimos valores coinciden con los obtenidos en el presente trabajo. Con base en estos datos se puede considerar que las elevaciones de los niveles de progesterona correspondieron a presencia de cuerpo lúteo activo.

Los resultados obtenidos indican que el reinicio de la actividad ovárica posparto en ovejas Tabasco ocurrió más tempranamente que en las de raza Suffolk y Dorset. Esto concuerda con la observación de Valencia *et al.*[11] quienes encontraron un intervalo de 25 a 60 días entre el parto y el primer estro en ovejas Tabasco en el trópico de México. Por otro lado Hulet[5] informa intervalos entre partos de 198 días en ovejas de esta misma raza, lo que indicaría un promedio de 48 días abiertos. González y De Alba[4] encontraron intervalos promedio de 41.5 ± 15.9 entre parto y primer estro y de 225.7 ± 50.3 días, entre partos.

En el rebaño experimental de ovejas Tabasco del Centro de Investigación, Enseñanza y Extensión en Ganadería Tropical de la Facultad de Medicina Veterinaria y Zootecnia de la UNAM, en Martínez de la Torre, Veracruz, se encontró un intervalo promedio entre partos de 175 días en las primeras ocho ovejas que tuvieron dos partos (Fernández-Baca S. 1979. Comunicación personal). Las ovejas en este Centro se mantienen con macho en forma continua y a base de pastoreo solamente. Todo lo anterior indica la gran potencialidad de las ovejas de la raza Tabasco para su utilización en la producción intensiva de carne.

El comportamiento de las ovejas Suffolk y Dorset fue similar. Pese a que el reinicio de la actividad ovárica posparto en ambas fue más tardía que en Tabasco, fue interesante observar que a los 45 días el 40% de las ovejas se encontraron ciclando. Esto contrasta con lo informado por Bostedt *et al.*[27] quienes determinaron la actividad ovárica posparto de ovejas Merino Württemberg por medio de los niveles de progesterona y encontraron una marcada estacionalidad. Las ovejas que parieron entre enero y junio, y entre octubre y diciembre iniciaron su actividad ovárica más tardíamente (60-80 días posparto) que aquellas que parieron entre julio y septiembre (50-60 días posparto).

SUMMARY

Postpartum ovarian activity was studied in Dorset, Suffolk and Tabasco ewes that lambed from January 4 to February 23, 1978. Ovarian activity was assessed through changes in the circulating levels of progesterone determined by RIA. Blood samples from 10 ewes of each one of the 3 breeds were taken on Days 10 and 20 postpartum (Day 0 = day of lambing) and at 5-day intervals thereafter until Day 55. All animals were maintained under confinements at the Veterinary Faculty's Experimental Station at Tepotzotlán, Estado de México (near Mexico City) located at 19° 44' North and 99° 44' West. On Day 30 postpartum 3 Tabasco ewes showed a marked increase in progesterone levels indicating presence of active corpus luteum, as compared to one Dorset and no Suffolk ewes. On Day 35 the percentages of ewes with elevated levels of the steroid were 40, 10 and 10 for Tabasco, Dorset and Suffolk, respectively. On Day 40 the corresponding figures were 60, 20 and 20. Differences were statistically significant at the 10% level of probability. Results indicate that

resumption of postpartum ovarian activity in Tabasco ewes occurred sooner than in the other two breeds. This explains the short lambing intervals which seems to be one of the outstanding characteristics of this breed.

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Endocrine Profile in Patients with Chronic Renal Failure Under Zinc Replacement

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The plasma levels of androstenedione (A), testosterone (T), dihydrotestosterone (DHT), follicle stimulating hormone (FSH), luteinizing hormone (LH), and prolactin (PRL) were studied in 15 men (aged 24–50 years) with chronic renal failure under periodic peritoneal dialysis, before and after 50 mg of elemental zinc (Zn) orally, twice a day for three weeks. Before treatment, they were divided into three groups: group I, plasma A above normal and PRL < 100 ng/ml; group II, low or normal A levels with PRL < 100 ng/ml; and group III, normal or high A levels with PRL > 100 ng/ml. After oral Zn, plasma FSH, LH, and PRL were unchanged in all groups; however, in groups I and II plasma A was within normal T and DHT rose significantly, the A/(T+DHT) ratio decreased to normal, and the T/DHT ratio rose above normal. In group III plasma androgens remained low and androgen ratios were unchanged. Oral Zn seems to improve the conversion of A to T and also uncovered the possibility that plasma PRL levels > 100 ng/ml might cause a blockade in the 5 alfa-reductase.

Key Words: Androgens; Prolactin; Zinc treatment; Uremia.

INTRODUCTION

A low plasma Zinc (Zn) concentration has been documented as one of the biochemical alterations present in patients with chronic renal failure (CRF) [2]. A negative correlation between plasma Zn levels and the biosynthesis of androgens, indirectly expressed by the ratio of androstenedione (A) to testosterone (T) plus dihydrotestosterone (DHT), has been reported, suggesting a possible role of Zn in the conversion of A to T [9]. The present study describes the effects of oral Zn administration on androgen plasma concentrations in patients with CRF and its relation with prolactin (PRL).

MATERIALS AND METHODS

Fifteen men with terminal CRF (creatinine clearance < 3 ml/min) clinically stable, aged 24–50 years, undergoing periodical peritoneal dialysis were studied. Anabolics and hypotensive drugs were withdrawn 15 days before the study. The control group included 12 clinically healthy men volunteers, aged 28–46 years.

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Each patient received 50 mg of elemental Zn in sulfate form, orally twice a day for three weeks. Before and after treatment fasting heparinized blood samples were obtained at 8:00 AM always 12 hr after the last dialysis. The plasma was separated and frozen (−20°C) until the following duplicate determinations were carried out: Zn, follicle stimulating hormone (FSH), luteinizing hormone (LH), and PRL as well as A, T, and DHT [4, 9].

Results were expressed as mean ± SEM. The differences between groups were determined by the Mann–Whitney's "U" test [11] for unrelated samples and for paired samples the Wilcoxon's tests was used. The correlation analysis was done by the Kendall's coefficient.

RESULTS

Before Zn therapy the patients were divided in three groups: group I, plasma A above normal and PRL < 100 ng/ml; group II, low or normal A levels and PRL < 100 ng/ml; and group III, normal or elevated A levels and PRL < 100 ng/ml.

After three weeks of oral Zn administration, the plasma Zn levels clearly rose in all groups ($p < 0.05$); however, no significant changes in FSH, LH, or PRL were seen in any group. Nevertheless, in group I this treatment caused a decrease in A and a clear rise in T, both within normal limits; DHT significantly increased, but still below normal. The previously elevated A/(T + DHT) ratio became normal and the normal T/DHT ratio increased. In group II, A remained normal but T levels markedly increased and plasma DHT rose to nearly normal. The A/(T + DHT) and T/DHT ratios exhibited changes similar to those seen in group I. In group III, A remained elevated, T and DHT low. Consequently, the A/(T + DHT) and T/DHT ratios remained unchanged. Combining the three groups, a significant positive correlation was observed between T concentrations after Zn replacement and PRL concentrations ($t = 0.49$, $p < 0.01$).

DISCUSSION

The Zn defficiency associated with CRF [2, 8] has been at least partially related to the abnormal androgen biosynthesis present in uremic men. The observation that Zn administered by the dialysis bath improves plasma testosterone supports this hypothesis [1]. However, the precise role of Zn on androgen metabolism remains obscure as yet. The present results demonstrated that after oral Zn supplementation T increased significantly in group I (elevated plasma A) and in group II (normal plasma A), which was also reflected by the increment in the A/(T + DHT) ratio in both groups. These findings suggest that Zn may facilitate the 17 beta-OH steroid dehydrogenase enzyme activity and thus enhances the conversion of A to T.

Another interesting observation was that only in groups I and II (both with plasma PRL < 100 ng/ml) did plasma T become normal after Zn treatment. Thus, high plasma PRL concentrations (> 100 ng/ml) may also play an important role in testosterone biosynthesis in CRF, similar to those cases with a pituitary adenoma [3], since the blocking action of prolactin on the 5 alpha-reductase enzyme activity is well documented [7]. This in turn, could explain in part the persistent low levels of DHT in all patients and the elevation of the T/DHT ratio after Zn treatment only in groups I and II. The presence of hyperprolactinemia in CRF is fairly well known [5, 6]; although there are

discrepancies about its frequency and origin it seems not to be due to blood accumulation secondary to the decreased renal clearance rate [10].

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Testicular Function in Men with Chronic Renal Failure and Transplant Recipients Under Bromoergocriptine Therapy

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Plasma zinc (Zn), follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), and androgens concentrations were determined in 15 male patients with chronic renal failure who were successful recipients of a kidney transplant. After 25 ± 8.5 months of the renal transplant, Zn levels were (88 ± 4 g/dl) lower than in the control group (116 ± 5 μg/dl). Normal concentrations for androstenedione (A) (0.63 ± 0.05 ng/ml) and testosterone (T) (3.31 ± 0.15 ng/ml) were found. Dihydrotestosterone (DHT) levels (0.38 ± 0.01 ng/ml) were lower than normal (1.11 ± 0.09 ng/ml), suggesting a blockade in the conversion of T to DHT. Eleven of the 15 patients showed higher PRL levels (9.5 ± 0.8 ng/ml) in contrast with the normal group (3.6 ± 0.3 ng/ml). Ten patients received orally 2-α-bromoergocriptine (BEC) 2.5 mg/day for ten days. Plasma PRL decreased to 2.6 ± 1.0 ng/ml ($p<0.001$), but A and T levels did not significantly change; however, DHT increased from 0.38 ± 0.02 ng/ml to 0.72 ± 0.04 ng/ml ($p<0.01$). All patients showed an increase in both gonadotropins before BEC without significant changes after treatment. The high PRL levels may be responsible for the impaired conversion of T to DHT, possibly by interference with the enzyme 5 α-reductase.

Key Words: Zinc; Prolactin; Gonadotropins; Androgens; Kidney transplant; Bromoergocriptine.

INTRODUCTION

The impairment in androgen biosynthesis is partially related to a zinc (Zn) deficiency and high prolactin levels in patients with chronic renal failure (CRF). The oral administration of Zn improves their plasma androgen levels depending on the magnitude of the hyperprolactinemia [2, 19]. However, all these hormonal changes are frequently reversed also when a kidney transplant is performed [1, 3, 9, 11, 21]. The present study was undertaken in a group of patients who had undergone successful kidney transplants in order to study the effect of the improvement in kidney function upon the plasma Zn, prolactin (PRL), gonadotropins, and androgen concentrations before and after bromoergocriptine administration.

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MATERIALS AND METHODS

Fifteen adult men (aged 32 ± 2.3 years) with CRF and a subsequent successful renal transplant were studied. In 13, the initial diagnosis was chronic glomerulonephritis, 1 had polycystic kidneys, and 1 had a chronic pyelonephritis. The graft source was from a living donor in eight cases and from cadavers in the other seven. The average posttransplant period at the time of the study was 25 ± 8.5 months, all the patients being treated with prednisone (5–12.5 mg/daily). The average creatinine clearance rate at the time of study was 99 ± 5 ml/min. All antihypertensive drugs were eliminated for at least 15 days prior to the study. After the initial studies were carried out, 10 out of the 15 patients received orally 2-α-bromoergocriptine (BEC) 2.5 mg/day over a period of 10 days. Twelve healthy age-matched men were used as the control group.

In all subjects a heparinized blood sample was obtained at 8:00 AM, and the plasma separated and kept frozen at −20°C until the following duplicate determinations were carried out: Zn concentration was measured by atomic absorption spectrophotometry [8]; follicle stimulating hormone (FSH), luteinizing hormone (LH), PRL, androstenedione (A), testosterone (T), and dihydrotestosterone (DHT) concentrations were measured by previously described radioimmunoassays [2, 19]. Statistical analysis was done by means of the Mann–Withney U-test for independent samples and the correlation coefficients were established by the least square method [20].

RESULTS

Before BEC the transplanted group had a lower plasma Zn concentration than the control group (Table 1) ($p<0.01$). FSH, LH, and PRL concentrations were higher in the transplanted patients than in controls ($p<0.01$). No significant differences in A and T levels existed between both groups; however, DHT was lower in the transplanted than in the control group ($p<0.01$). The A/(T + DHT) ratio was similar in both groups, but the T/DHT ratio was higher in the transplanted than in the control group ($p<0.01$). When the control subjects and the transplanted patients before BEC therapy were analyzed together a logarithmic correlation was found between PRL concentrations and the T/DHT ratio ($r=0.78$, $p<0.01$).

After 10 days of BEC therapy, the only significant changes observed were a significant

TABLE 1 Hormonal Profiles in Controls and Transplanted Patients Before and After 2-α-Bromoergocriptine Therapy (BEC). Mean ± SEM

Group	Zn (μg/dl)	FSH (ng/ml)	LH (ng/ml)	PRL (ng/ml)	A (ng/ml)	T (ng/ml)	DHT (ng/ml)	A/(T+DHT)	T/DHT
Controls									
$n=12$	116±5	301±51	108±9	3.6±0.3	0.56±0.05	3.3±0.1	1.1±0.1	0.13±0.01	3.2±0.3
Transplanted									
Before BEC	88±4	942±219	235±36	9.5±0.8	0.6±0.1	3.8±0.2	0.3±0.02	0.15±0.01	10.1±0.7
($n=15$)	*a*	*a*	*a*	*a*	NS	NS	*a*	NS	*a*
After BEC	85±3	725±172	203±31	2.6±1.0	0.8±0.2	4.0±0.3	0.7±0.04	0.17±0.01	5.61±0.42
($n=10$)	NS	NS	NS	*b*	NS	NS	*b*	NS	*b*

[a] $p<0.01$ compared with controls.
[b] $p<0.001$ compared before therapy.

decrease in PRL levels ($p<0.01$), as well as in the T/DHT ratio ($p<0.01$), the latter as a consequence of the significant rise in plasma DHT ($p<0.01$).

DISCUSSION

It has been postulated that the decreased T concentrations in patients with CRF are indirect evidence of a decreased convertion of A to T, secondary to the uremic state [5] and a Zn deficiency [18]. The fact that in our transplanted patients the A and T levels (and thus, the A/T ratio) were normal despite a Zn level lower than normal might be due to (a) a threshold in the plasma Zn concentrations above which A-to-T conversion begins to normalize (we have provided information suggesting that such threshold in plasma Zn levels was between 80–90 μg/dl [2, 19]), or (b) factors other than the above mentioned that may influence A-to-T conversion.

In the transplanted patients, plasma DHT continued to be low, suggesting a partial block in the conversion of T to DHT. No information exists about a persistent decrease in DHT in spite of a successful kidney transplant and normal plasma levels of T. This

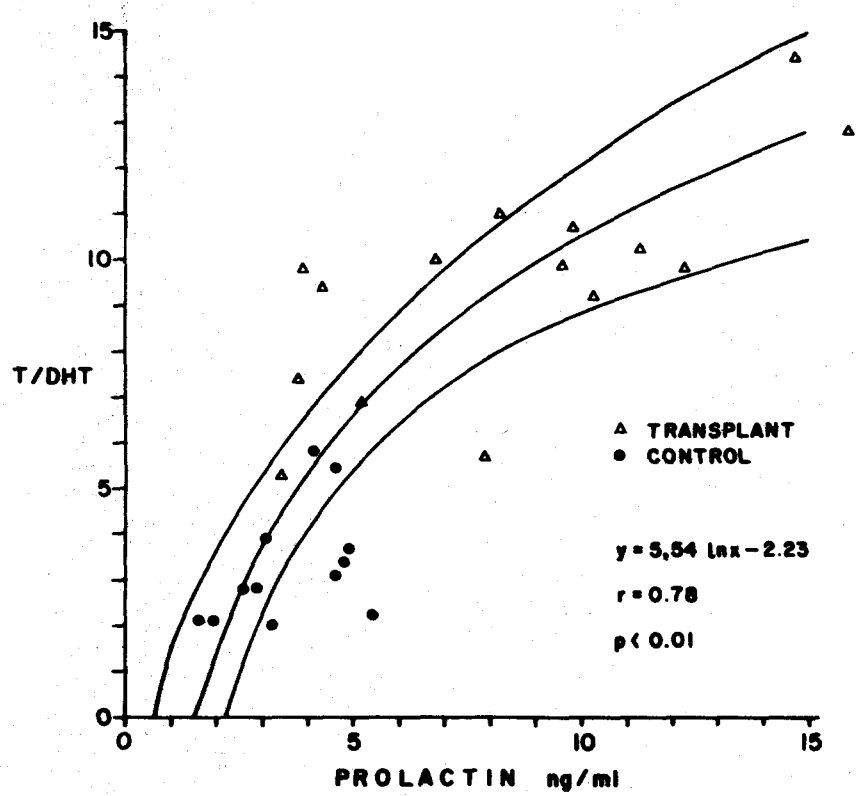

FIGURE 1. Correlation coefficient observed between plasma prolactin levels and the T/DHT ratio in control subjects and in transplanted patients before 2-α-bromoergocriptine treatment. The lines represent the mean ± SD of the regression equation.

apparent slowing in the activity of 5 alpha reductase might be related to the elevated PRL levels in this group of patients, since its activity is reduced by high PRL levels [15]. The clear improvement in plasma DHT and the T/DHT ratio after BEC therapy observed in the transplanted patients would further support this viewpoint. There is a high incidence of hyperprolactinemia among patients with CRF [4, 6, 7, 8], although its cause remains to be elucidated. Eleven out of 15 successfully transplanted patients remained with elevated prolactin levels, which cannot be explained on the basis of PRL accumulation since the renal function was normal in all our transplanted subjects. However, after BEC therapy there was a significant decrease in prolactin levels, simultaneous with a clear rise in DHT concentrations; consequently the T/DHT ratio significantly decreased. This observation supports the hypothesis on the blocking effect of high PRL levels upon the activity of the 5 alpha reductase enzyme [2] and thus, a more prolonged therapy with BEC might be useful to correct this abnormality.

The persistent elevations of FSH and LH in the transplanted patients before and after BEC have not been clearly explained, although an exaggerated pituitary response to gonadotropin releasing hormones has been reported to exist in these patients [16, 17].

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Zinc, Prolactin, Gonadotropins, and Androgen Levels in Uremic Men

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This study correlates plasma levels of Zinc (Zn) and some pituitary and testicular hormones in 20 uremic men (aged 17–58 years) on a weekly peritoneal dialysis program. Patients were compared to 12 healthy male volunteers (aged 28–40 years). In uremic men, plasma androstenedione (A) was elevated, while testosterone (T), dihydrotestosterone (DHT), and Zn were low. On a group basis, plasma follicle stimulating hormone (FSH) and luteinizing hormone (LH) were normal while prolactin was increased. A negative correlation was observed between plasma A and LH levels, as well as between the A/T + DHT ratio and plasma Zn concentrations. Our results suggest a diminished A-to-T conversion and point to the possible role of Zn in the enzyme activity of the 17 β-hydroxysteroid dehydrogenase.

Key Words: Zinc; Pituitary hormones; Testicular hormones; Uremia.

INTRODUCTION

Several endocrine abnormalities have been described in patients with chronic renal failure (CRF), such as a decrease in plasma concentrations of testosterone (T) and dihydrotestosterone (DHT) in the face of normal plasma luteinizing hormone (LH) levels, despite the normal pituitary reserve present in these patients [2, 5, 6, 7, 14, 18]. Also a decrease in plasma zinc (Zn) levels has been reported in CRF [4, 9]. The oral administration of Zn is followed by an improvement both in plasma T levels and in sexual potency [1].

The present study was undertaken to analyze the plasma levels of Zn and some pituitary and testicular hormones in uremic men, attempting to offer some possible explanations for the hormonal abnormalities present in patients with CRF.

MATERIAL AND METHODS

Subjects. Twenty men aged 17–58 years with terminal CRF (creatinine clearance < 3 ml/min) on a weekly peritoneal dialysis program were studied. Two weeks before the study, anabolics, α-methyl dopa, and propranolol were withdrawn. The results were compared to those obtained in 12 normal adult male volunteers, aged 28–46 years. In both groups fasting heparinized blood

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samples were taken at 8:00 AM. In CRF patients, this sample represented 12 hr after the last dialysis. The plasma was separated and stored at −20°C until assayed.

Analytical Methods. On each plasma sample the following duplicate determinations were carried out: Zinc [8]; FSH, LH, and prolactin (PRL) by commercially available kits (International CIS-SORIN, France) and the results were expressed in ng/ml (IRE-STANDARD), with 1 ng equivalent to 180 ng LER 907 for FSH, to 45 ng LER 907 for LH, and to 30.4 UI MRC 75/504 for PRL. A, T, and DHT were measured by radioimmunoassays previously described [12, 13]. For the statistical analysis the results were expressed as mean ± SEM and the differences between groups were determined by the Mann-Whitney's "U" test for unrelated samples and the correlation coefficients by the least squares approach. The level of statistical significance considered was $p < 0.05$ [20].

RESULTS

The individual values, as well as the mean ± SEM in healthy subjects and patients with CRF, are shown in Fig. 1. The patients with CRF showed a considerable reduction in plasma Zn concentrations in comparison to control values ($p < 0.05$). However, on a group basis, no differences were found neither in FSH or LH. There was an obvious increment in PRL plasma concentrations in the CR patients as compared to control ($p < 0.01$). Testosterone and DHT levels were much lower in the CRF group than in the controls ($p < 0.01$). An inverse pattern for A was found with higher values in CRF than in controls ($p < 0.05$). This increment in plasma A was more evident when expressed as the A/T + DHT ratio, which was found higher in the former than in the latter group ($p < 0.01$). A significant negative quadratic correlation was disclosed between Zn plasma levels and A/T + DHT ratio ($r = 0.65$, $p < 0.05$) (Fig. 2a). A similar correlation was found between plasma A and LH levels, $r = 0.6$, $p < 0.01$ (Fig. 2b). No correlation was found in plasma T or DHT and LH levels. The T/DHT ratio was 0.83 in CRF significantly lower than in the control group 3.26 ± 0.37 ($p < 0.01$).

DISCUSSION

The low levels in plasma T concentrations have been considered as a determinant factor for impotence and decreased libido. Although some reports have suggested a primary testicular disorder [10, 11], there is not enough information in relation with the effect of uremia on steroidogenesis. Recently, it has been questioned if the reduced T values are a direct consequence of CRF or are indirectly caused by the Zn depletion [1, 4, 9]. Our results showed a distinct elevation of plasma A along with a diminution of T and DHT, which suggest a block in the enzymatic conversion of A to T. This possibility seems to be partially supported by the observed negative correlation between A/T + DHT ratio and Zn concentration as well as the low T/DHT ratio. With these data it seems likely that Zn may play an important role in the diminished convertion of A to T, although the mechanism remains unclear as yet. However, there are two possible explanations: First, Zn may have interactions with the 17β-hydroxysteroid dehydrogenase (17βOHSD) as metaloenzyme or acting as an activator [17]. In the dwarf mice,

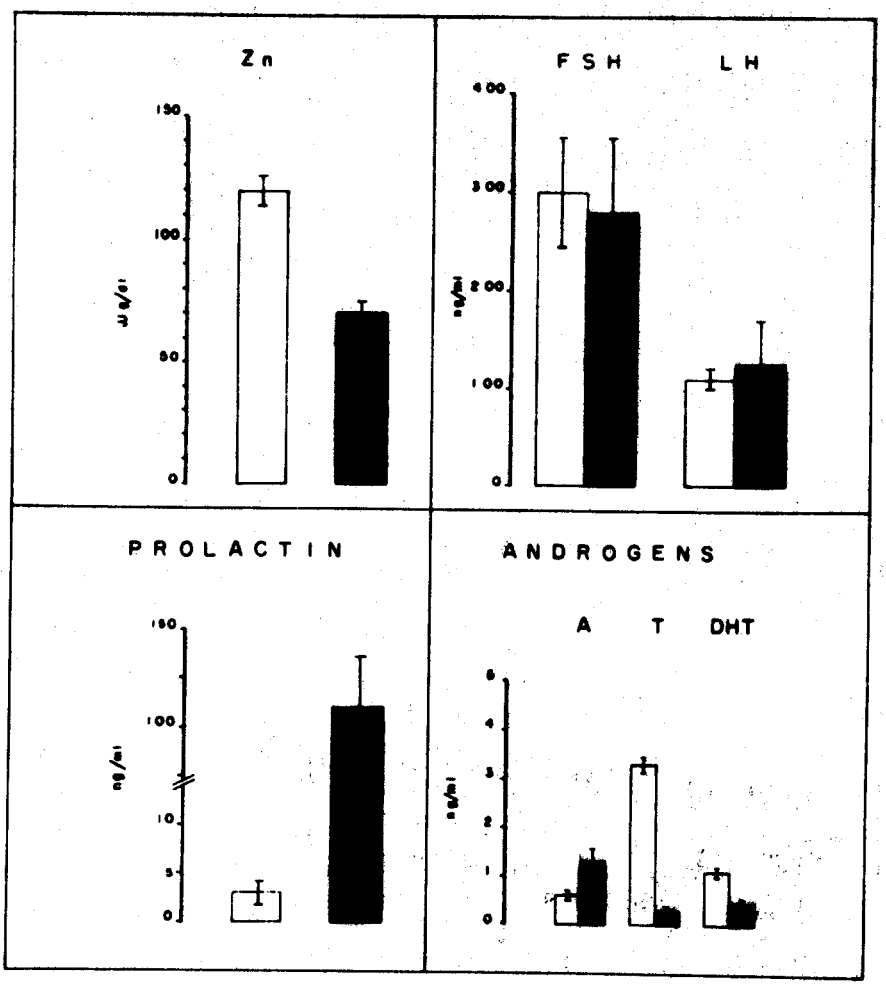

FIGURE 1. Plasma concentrations observed in the CRF (dark bars) and in the control groups (light bars). Values represent mean ± SEM.

prolactin administered subcutaneously, increases the 17βOHSD activity [15] and since the CRF patients studied had high plasma prolactin levels it is likely that the low Zn concentration was critical to evidence the reduced enzyme activity in androgen synthesis. The second possibility takes into account the role of Zn as a membrane protecting agent, giving support to the membranal enzymes, as is the case for the 17βOHSD [3]. It was found an inverse correlation between plasma A and LH levels, which could explain the presence of normal plasma LH levels in the presence of usually low T and DHT concentrations, since the antigonadotrophic effect of A in other species has been previously documented [16, 19].

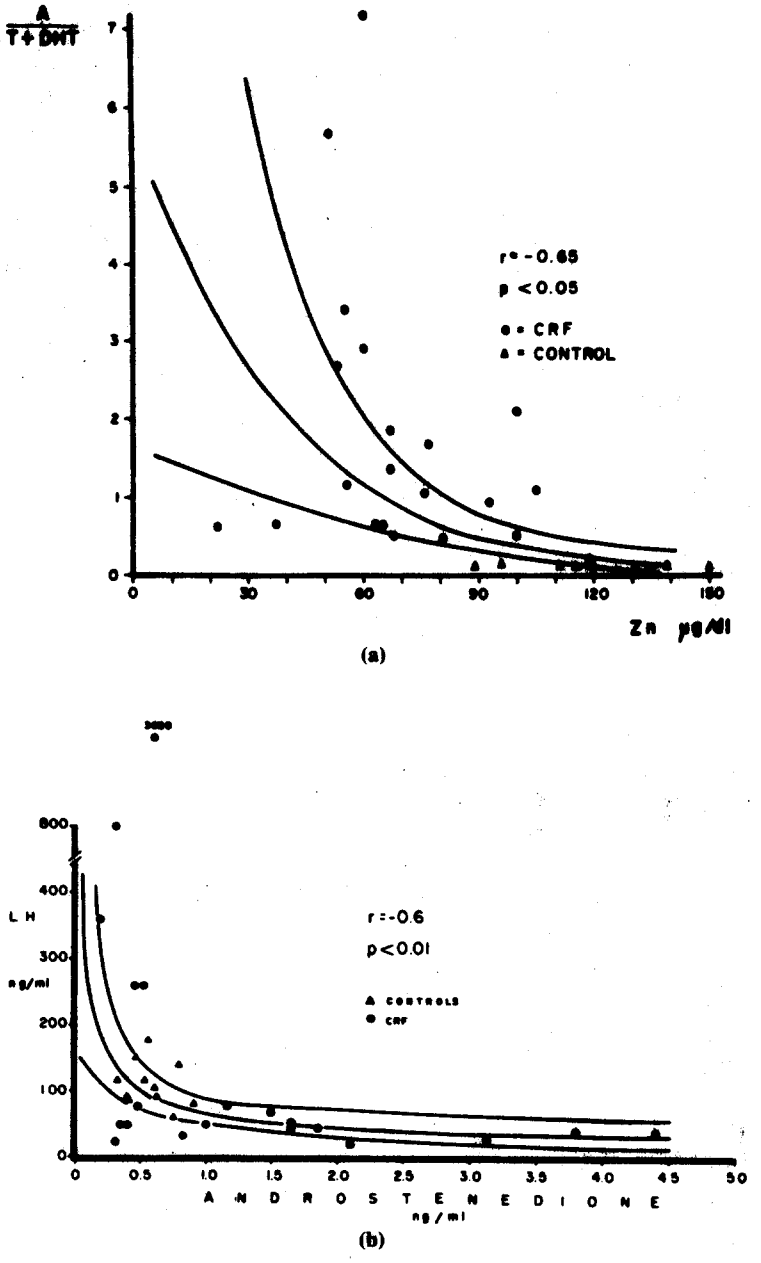

FIGURE 2. a. Negative correlation between plasma zinc and the A/T + DHT ratio. The solid lines represent the mean and the 95% confidence limits interval. b. A nonlinear negative regression line between plasma A and LH levels was obtained.

Acknowledgments: Thanks are due to Dr. Adalberto Parra for his suggestions to the manuscript.

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CORRELACION ENTRE CINC, PROLACTINA, GONADOTROFINAS Y ANDROGENOS PLASMATICOS EN HOMBRES CON INSUFICIENCIA RENAL CRONICA

CORRELATIONSHIP BETWEEN ZINC, PROLACTIN, GONADOTROPHINS AND PLASMA ANDROGENS IN MEN WITH CHRONIC RENAL FAILURE

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RESUMEN

En 20 hombres urémicos en programa de diálisis peritoneal intermitente (DPI) se estudiaron las concentraciones plasmáticas de cinc (Zn), hormona estimulante del folículo (FSH), hormona luteinizante (LH), prolactina (PRL), testosterona (T), dihidrotestosterona (DHT) y androstenediona (A). Se encontró una importante disminución de Zn, T y DHT, con aumento de A y PRL en relación a 12 controles sanos. FSH y LH estuvieron normales. Los resultados se interpretaron como

ABSTRACT

Zinc (Zn) plasma concentration, follicle stimulating hormone (FSH), luteinizing hormone (LH), prolaction (PRL), testosterone (T), dihidrotestosterone (DHT) and androstenedione (A), were measured in 20 uremic males undergoing an intermitent peritoneal dialysis program. Zn, T and DHT were lower than normal and significant increments in A and PRL were found. FSH and LH were similar to normal. This results indicate a deficience in A to T steep which

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un deficiente paso de A a T que parece relacionado a la deficiencia de Zn puesto que el índice A/T+DHT correlacionó con Zn, asimismo la elevación de A explica la normalidad de LH puesto que tiene efecto inhibitorio en la hipófisis.

was statistically related to serum Zn. Normal LH may be explained by A increment since hypophyseal inhibitory efect has been demonstrated.

Introducción

Existen importantes alteraciones endocrinas en varones con insuficiencia renal crónica en estado-terminal, entre ellos se ha enfatizado la disminución de T y DHT con una aparente paradójica normalidad de LH con reserva hipofisiaria también normal.[2,5,6,7,14,18] Aunque mucho se ha elucubrado acerca del origen de estas alteraciones, pocos avances prácticos se habían logrado hasta que se relacionó la deficiencia de Zn comúnmente vista en sujetos urémicos y las alteraciones sexuales,[4,9] relación que fue confirmada cuando al administrar Zn en el líquido de diálisis se observó mejoría en la concentración de T y en la potencia sexual.[3]

Hasta el momento se desconoce el mecanismo de acción de Zn, por esta razón aquí se estudia la correlación de varias hormonas del eje hipotálamo-hipófisis-testículo, tratando de dilucidar el papel de Zn en las alteraciones endocrinas sexuales de la IRC.

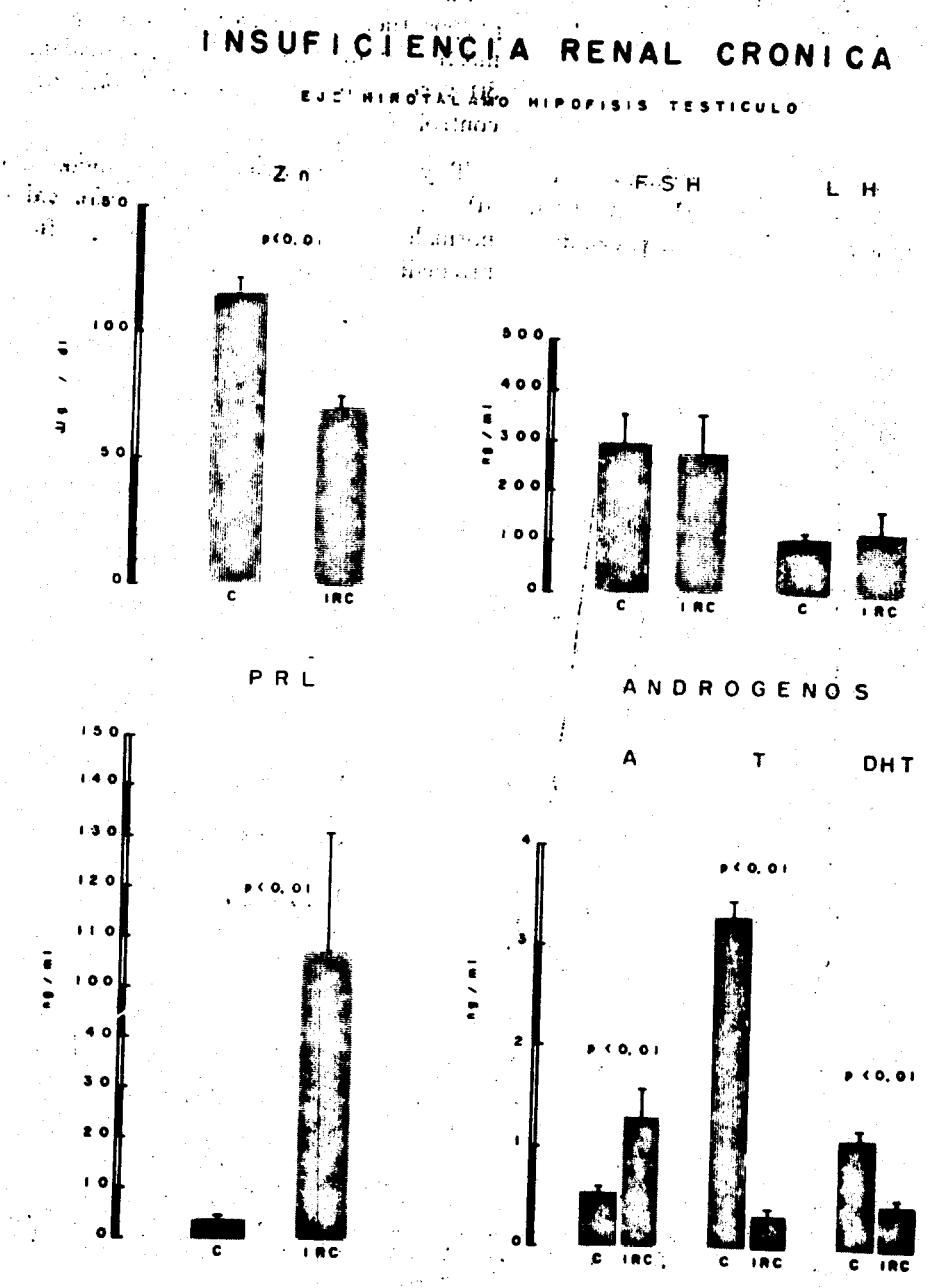
Material y Métodos

Se estudiaron 20 pacientes masculinos con IRC, sus edades fluctuaron entre 17 y 58 años. Al momento del estudio sus depuraciones de creatinina fueron inferiores a 3 ml/min. y recibían tratamiento dialítico intermitente de 24 baños de 2 l cada uno por semana. Todos habían tenido vida sexual activa previa a la enfermedad renal por lo que en las dos semanas anteriores al estudio se suspendió toda medicación con efectos conocidos en los niveles hormonales, especialmente anabólicos, metildopa y propranolol. Para comparación se efectuaron los mismos estudios hormonales en 12 hombres sanos con edades similares (28-46 años).

Las muestras para estudio se tomaron a las 8 a.m., 12 horas después del último procedimiento dialítico y consistieron en 20 ml de sangre venosa heparinizada de donde se separó el plasma que se mantuvo en congelación hasta efectuar las mediciones.

Por duplicado se midió por espectrofotometría de absorción atómica la concentración de Zn[8] y por RIA con equipos comerciales (CIS-SORIN-Francia) FSH, LH y PRL. La T, DHT y A se midieron también mediante RIA previa extracción y cromatografía en placa fina según técnicas descritas previamente.[12,13]

Los resultados se expresan como valor medio±E E y las pruebas estadísticas aplicadas fueron: "U" de Mann Whitney para muestras independientes, y mínimos cua-


INSUFICIENCIA RENAL CRONICA
EJE HIPOTALAMO HIPOFISIS TESTICULO
Zn
µg / dl
p<0.01
C
IRC
F S H
L H
ng / ml
PRL
ANDROGENOS
A
T
DHT
p<0.01

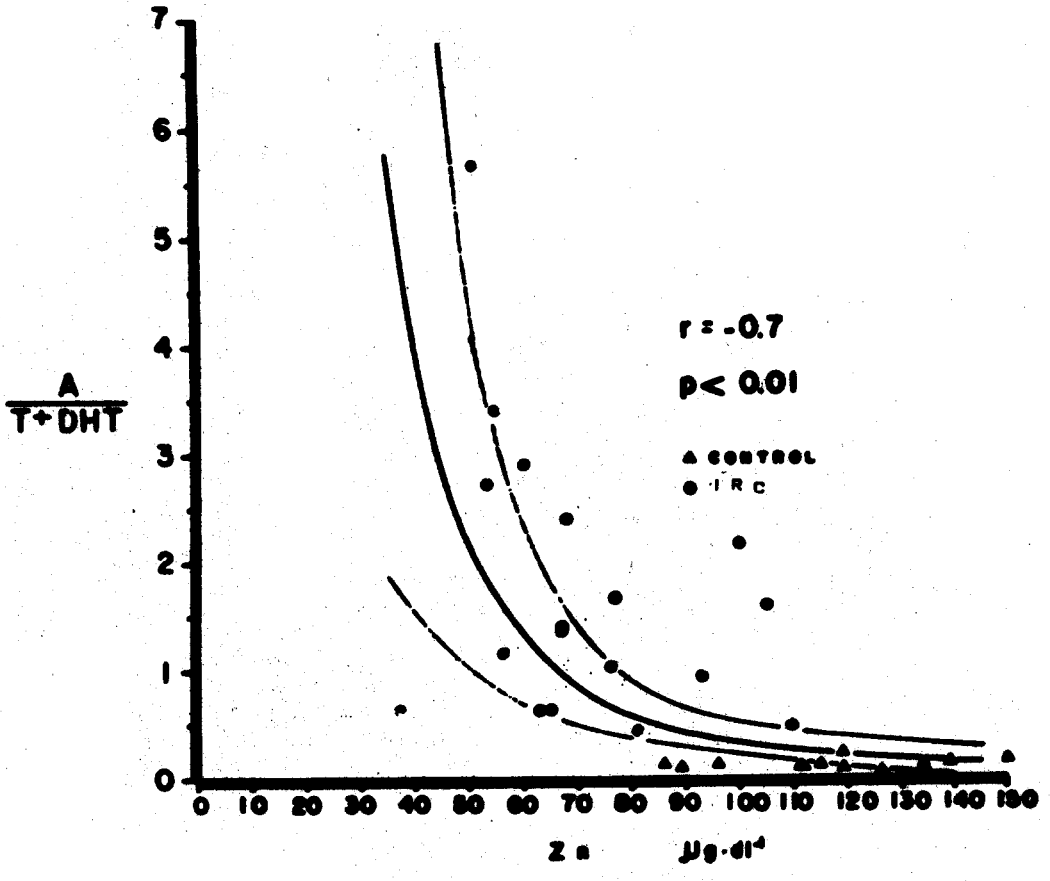
drados para la regresión y correlación.[20] El nivel de significancia fue fijado en 0.05.

Resultado

La figura 1 resume los resultados obtenidos. Los pacientes con IRC tuvieron una importante reducción del Zn plasmático ($p<0.01$).

Las hormonas FSH y LH en los dos grupos fueron similares, sin embargo PRL mostró un incremento de aproximadamente 30 veces sobre los niveles de los sujetos control ($p<0.01$).

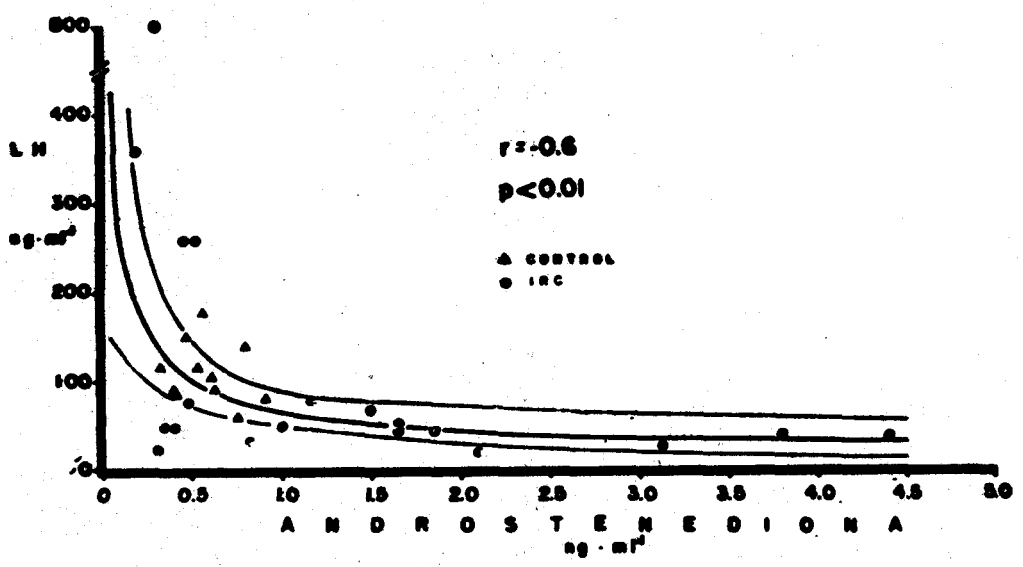
T y DHT estuvieron disminuidos a un 10 y un 40% respectivamente de los valores normales ($p<0.01$), en cambio A fue en promedio el doble del control.



En la figura 2 se muestra la relación logarítmica entre Zn y el índice A/T + DHT que resultó significativo ($r = -0.6$ $p<0.01$). No se encontró correlación entre las demás hormonas analizadas entre sí ni con las concentraciones de Zn.

Discusión

La mayor parte de la literatura relacionada con este tema indica que la alteración hormonal principal radica en el testículo [10,11] señalando como responsable entre otros a fallas enzimáticas causadas por el estado urémico o bien a la desnutrición de estos pacientes o a la hiperprolactinemia. Es conocida desde hace tiempo la depleción de Zn de estos pacientes, [4,9] y también se sabe que la carencia de este elemento produce regresión o no aparición de los caracteres sexuales secundarios. [17] Ambas premisas llevaron recientemente a probar si la administración de Zn mejora la potencia sexual y la concentración de T circulante, encontrándose efectos positivos en los dos parámetros. Nuestros resultados sugieren que el sitio de acción del Zn está en el paso de A a T, puesto que la primera está elevada y la segunda disminuida, así como por la correlación establecida entre Zn y el índice A/T+DHT. No podemos sin embargo precisar el mecanismo de acción de Zn aunque tomando en cuenta que es parte del sitio activo de varias enzimas cabe la posibiilidad de que la encargada de la transformación de A a T sea una metaloenzima Zn dependiente. No hay hasta la fecha información sobre este hecho. La enzima mencionada (17 B hidroxiesteroide dihidrogenasa) se sabe que es modulada por PRL, de manera que una modulación positiva deficiente no puede argumentarse porque contamos con hiperprolactinemia en estos pacientes. Toda hormona protéica, una vez



que se une al receptor específico e induce actividad es desprendida de la membrana por peptidasas específicas que la liberan como Hormona Receptor. Recientemente se ha documentado que el Zn estabiliza el complejo LH-Receptor en testículo de rata y le protege de las peptidasas pudiendo de esta manera mejorar la calidad de estimulación de LH.[21] Este mecanismo alterado en el urémico, es una probable explicación a nuestros hallazgos. Otra posibilidad de acción del Zn menos específica, sería a través de su papel como protector de membrana[3] dado que la 17 B hidroxiesteroide dhidrogenasa es una enzima de membrana.

Una observación interesante es la correlación entre LH y A. Si consideramos que A tiene actividad antigonadotrófica[16,17] se puede explicar la poca o nula elevación de LH en presencia de T baja y la respuesta normal de LH a la hormona liberadora.[18]

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EFECTO DE LA ADMINISTRACION DE CINC SOBRE LOS ANDROGENOS EN EL HOMBRE CON INSUFICIENCIA RENAL CRONICA

EFFECT OF ZINC ADMINISTRATION ON PLASMA ANDROGEN OF MEN WITH CHRONIC RENAL FAILURE

R. Paniagua,* F. Arreola,** A. Pérez,* J. Herrera,** S. Díaz,** L. Mondragón,** S. Villalpando,** J. A. Bermúdez *** y E. Exaire *

RESUMEN

Se estudió en 15 pacientes masculinos con Insuficiencia Renal Crónica (IRC) y depuración de creatinina inferior a 3 ml/min, mantenidos en tratamiento de diálisis peritoneal intermitente, el efecto de la administración oral de sulfato de cinc (Zn) sobre los andrógenos, gonadotrofinas y prolactina (PRL). Después de 3 semanas de ingerir el elemento se observó un franco incremento del Zn plasmático en todos los enfermos, con aumento de la testosterona (T) hasta cifras normales en aquellos pacientes en los que la PRL no

ABSTRACT

Zinc (Zn) administration effect on plasma androgens, gonadotrophins and prolactin (PRL) was estudied in 15 males patients with chronic renal failure with creatinine clearence lower than 3 ml/min, undergoing an intermitent peritoneal dialysis program. After 3 weeks of Zn administration, serum Zn level rises in all patients as well as T, in those subject with no extreme hiperprolactinemia. There was no sig-

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estaba extraordinariamente elevada. No se obtuvo cambio significativo en los valores de androstenediona (A), Dihidrotestosterona (DHT), gonadotrofinas ni en la PRL. Estos resultados son sugestivos de que el Zn actúa como protector de membrana favoreciendo la síntesis de T, y de que la PRL tiene un efecto negativo en la síntesis de andrógenos, actuando sobre la 5a reductasa.

nificant changes in A, DHT, gonadotrophins or PRL. Results, sugest than Zn acts as a membrane protecting agent improving T syntesis and a negative effect of PRL on androgen syntesis, specifically on 5a reductase.

Introducción

A las muchas alteraciones bioquímicas observadas en la IRC se ha sumado una deficiencia real o relativa de Zn.[1,2] En un informe previo hemos documentado que existe relación, al menos estadística, entre la concentración plasmática de Zn y la síntesis alterada de andrógenos en la IRC cuando se expresa como el cociente A/T+DHT, que traduce una conversión deficiente de A a T y DHT.[3] Estos datos concuerdan con el conocimiento previo de que en la deprivación experimental de Zn en hombres sanos, disminuye el nivel sérico de T4 y que la administración de suplementos de Zn incrementa T sérica y la líbido en hombres urémicos.[5]

En este estudio damos a conocer el efecto de la administración oral de Zn sobre varias hormonas del eje hipotálamo-hipófisis-testículo de hombres con IRC.

Material y Métodos

El estudio se realizó en 15 pacientes masculinos con IRC bioquímicamente estables que recibían tratamiento de diálisis peritoneal intermitente semanaria con función renal residual menor de 3 ml/min de depuración de creatinina. Sus edades variaron de 24 a 50 años.

Dos semanas antes del estudio se suspendieron todos los medicamentos con acción conocida sobre la síntesis de andrógenos y niveles de PRL en especial antihipertensivos y anabólicos. Como grupo control se tomaron 12 sujetos sanos de edades comparables.

Los pacientes recibieron sulfato de Zn equivalente a 50 mg de Zn elemental por vía oral dos veces al día por 3 semanas. Antes y después del tratamiento se obtuvieron 15 ml de sangre venosa heparinizada. Todas las muestras se tomaron a las 8 a.m. En los pacientes se obtuvo entre 12 y 18 horas después de concluida la última diálisis. El plasma se separó por centrifugación y se mantuvo congelado a —20°C hasta que se efectuaron las mediciones.

El Zn se cuantificó mediante espectroscopía de absorción atómica.[6] Las hormonas folículo estimulante (FSH), luteini-

zante (LH) y PRL se midieron con equipos comerciales de radioinmunoanálsis (Internacional CISSORIN, Francia).

Los resultados se expresan en ng/ml. El coeficiente de variación intraensayo fue menor del 4%. La A, T y DHT se midieron por radioinmunoensayo con técnicas previamente descritas [7,8,9] con coeficientes de variación intra ensayo de 5%. Todas las lecturas se hicieron por duplicado.

Los resultados se expresan como promedio±un error estandar. Las diferencias entre grupos se analizaron con la prueba "U" de Mann-Whitney para muestras independientes.

Para muestras pareadas se utilizó la prueba de Wilcoxon. [10] La correlación entre T y PRL se calculó con el coeficiente de Kendall. El nivel de significancia se estableció en 0.05.

Resultados

De acuerdo a las concentraciones basales de hormona esteroides y PRL los pacientes se dividieron en tres grupos: el grupo I con A superior al promedio normal y PRL menor a 100 ng/ml; grupo II con A igual o menor al promedio normal y PRL menor a 100 ng/ml y grupo III con A normal o elevada y PRL superior a 100 ng/ml.

En la gráfica 1 pueden observarse los resultados basales de Zn que fueron de 51± 7.4; 47.6±3.9 y 51.0±6.0 ug/dl en los grupos I, II y III respectivamente y se elevaron después de la administración del elemento a 103.2±21; 100.2±11.7 y 86.0± 9.9 ug/dl en el mismo orden. En todos los casos la elevación de Zn fue significativa ($p<0.05$).

La figura 2 muestra los resultados de FSH, LH y PRL.

Estos fueron FSH: 176.8±56.4 y 164.0 ±46.4 ng/ml antes y después del Zn para el grupo I; 69.8±9.7 y 106.6±24.5 ng/ml para el grupo II y 57.8±6.9 y 73.8±15.6 ng/ml para el grupo III.

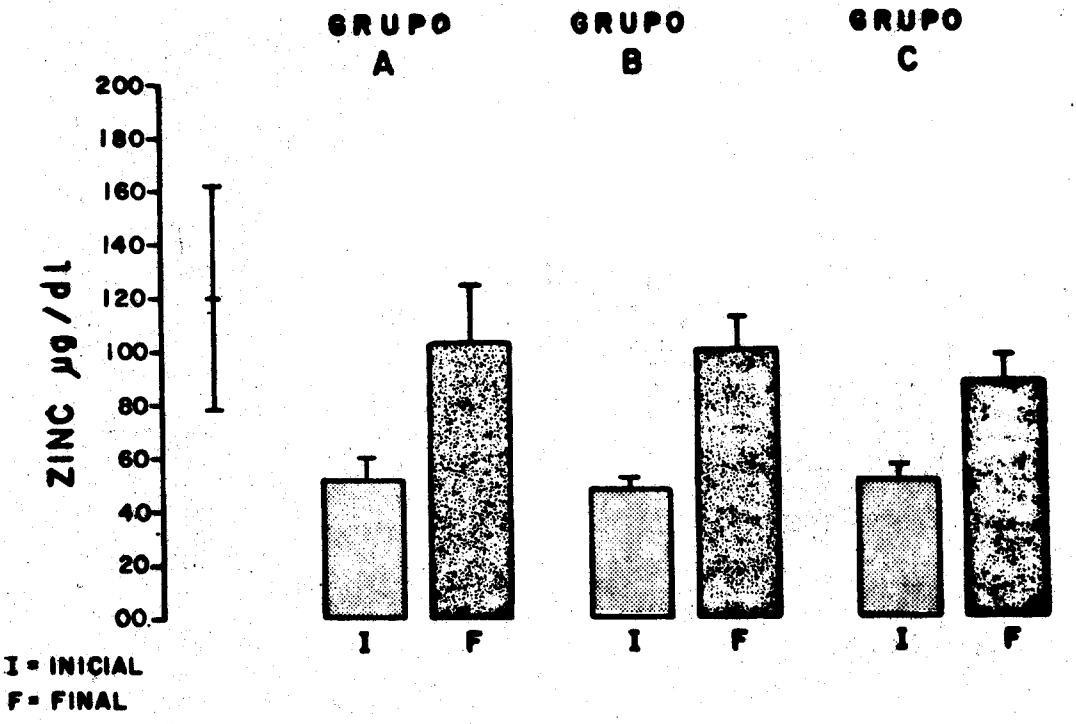
Para LH y en el orden anterior los resultados fueron:

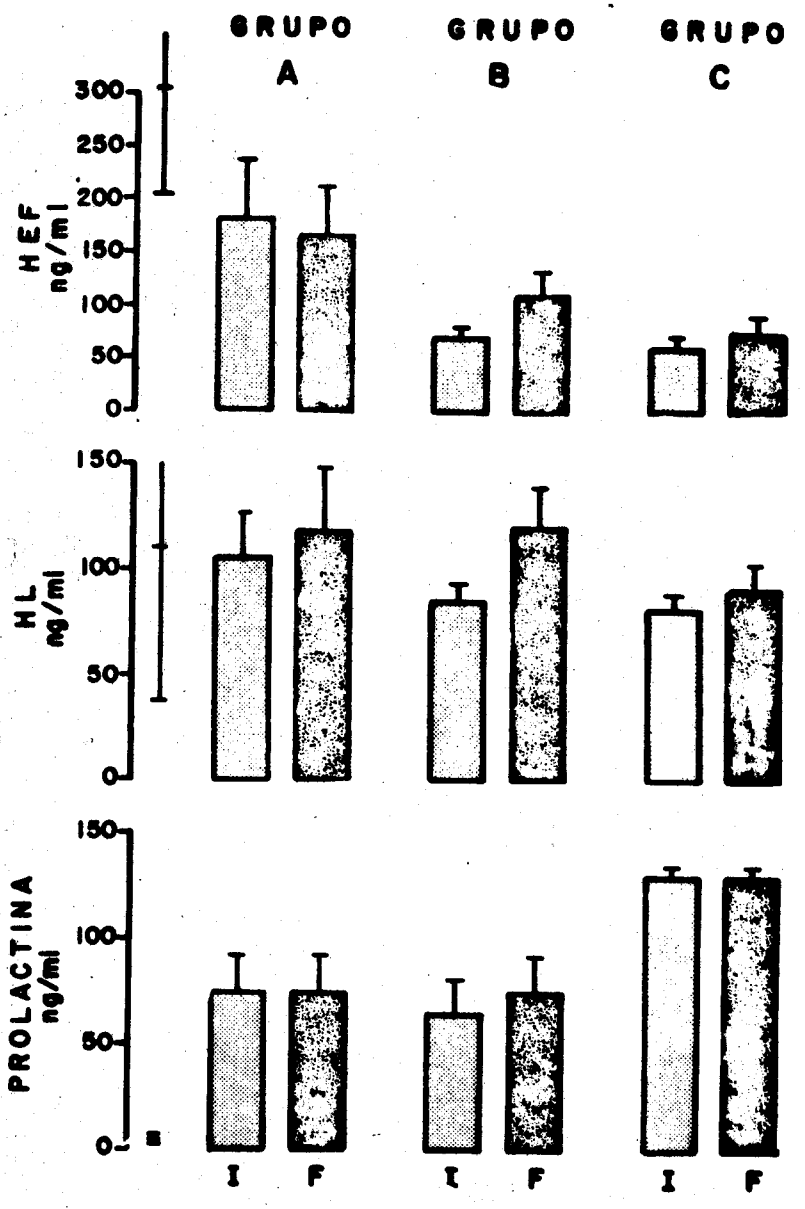
Grupo I 103.2±21.2 y 117.0±29.1 ng/ml, grupo II 84.6±8.0 y 121.4±19.2 ng/ml y grupo III 81.4±7.3 y 91.2±13.4 ng/ml.

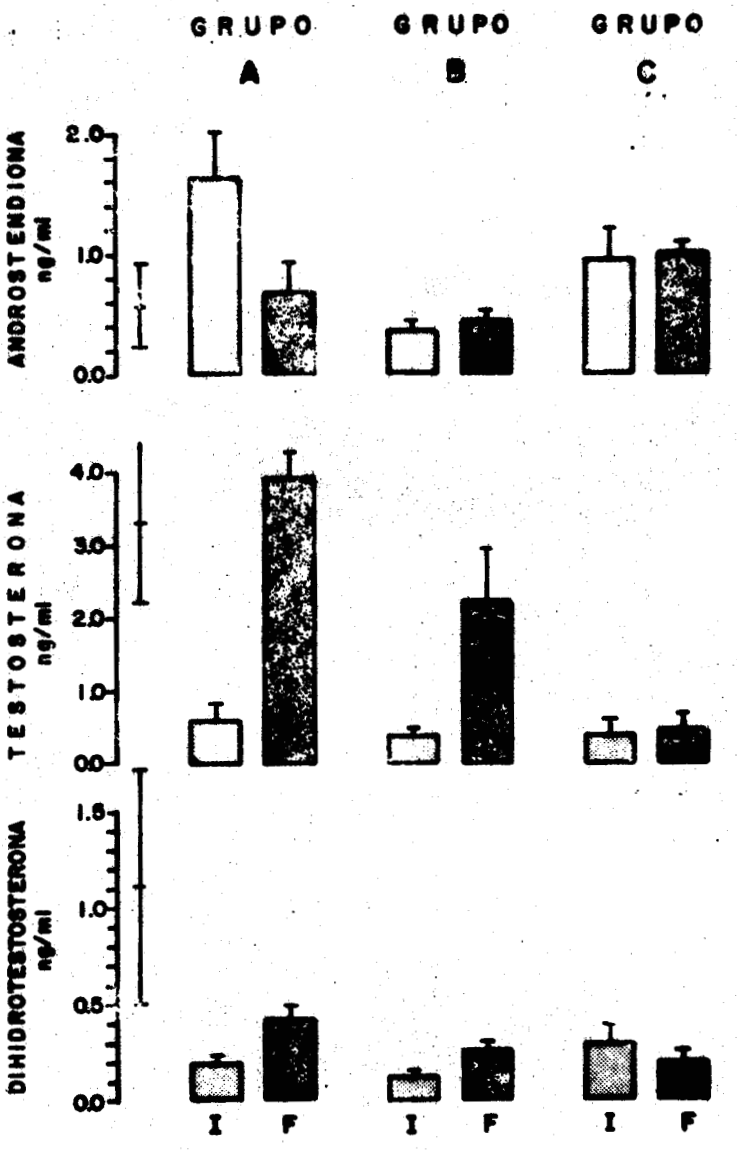
Los niveles de FSH y LH fueron similares a los del grupo control y no se modificaron con el Zn.

La concentración de PRL fue: Grupo I 74.6±16.9 y 74.0±171; Grupo II 66.2± 15.8 y 74.8±16.6; grupo III 131.4±3.1 y 131.6±1.8 ng/ml. En los tres grupos los valores basales no difirieron significativamente los controles; en el grupo III la concentración del PRL fue mucho mayor que en los ojos dos y la administración de Zn no tuvo efecto alguno.

En la figura 3 se encuentran representados los valores de A, T y DHT. En el grupo I la A basal fue de 1.62±0.36 ng/ml y 0.68±0.24 ng/ml final. El valor basal fue mayor que el control ($p<0.05$) y el final indica un descenso significativo ($p<0.05$) a cifras normales (0.56±0.05 ng/ml). En el grupo II fue de 0.36±07 y 0.42±0.08 ng/ml y en el grupo III 0.94±0.26 y 1.02 ±0.08 ng/ml ambos similares al control y sin efecto apreciable del Zn. La T estuvo


GRUPO A
GRUPO B
GRUPO C
ZINC µg/dl
200
180
160
140
120
100
80
60
40
20
00
I
F
I
F
I
F
I = INICIAL
F = FINAL


GRUPO A
GRUPO B
GRUPO C
HEF ng/ml
300
250
200
150
100
50
0
HL ng/ml
150
100
50
0
PROLACTINA ng/ml
150
100
50
0
I
F
I
F
I
F


GRUPO A
GRUPO B
GRUPO C
ANDROSTENDIONA ng/ml
2.0
1.0
0.0
TESTOSTERONA ng/ml
4.0
3.0
2.0
1.0
0.0
DIHIDROTESTOSTERONA ng/ml
1.5
1.0
0.5
0.0
I F I F I F

significativamente baja en la etapa inicial: 0.58±0.19; 0.36±0.09 y 0.40±0.18 ng/ml para los grupos I, II y III respectivamente. Después del tratamiento ascendieron los niveles en el grupo I a 3.90±0.34 ng/ml y en el grupo II a 2.22±0.69. En ambos casos la diferencia con los valores iniciales fue significativa ($p<0.05$) y los finales cayeron en rango normal (3.31±0.16 ng/ml) en el grupo I y ligeramente abajo en el grupo II. El grupo III no sufrió alteración alguna permaneciendo inferior a lo normal. Tomados en conjunto los 3 grupos, se encontró correlación ($p<0.01$) entre la concentración de T final y PRL.

La DHT aunque se elevó con el tratamiento en los grupos I y II, el incremento no tuvo importancia práctica puesto que, no alcanzó en ningún momento a entrar en cifras normales (1.11±0.09 ng/ml). Los datos numéricos fueron: grupo I 0.18±0.04 y 0.42±0.06, grupo II 0.12±0.02 y 0.26±0.04 y grupo III 0.28±0.11 y 0.21±0.08 ng/ml.

Discusión

En hombres sanos existe correlación lineal entre T y Zn [12] y cuando hay carencia de este último tanto en animales como en humanos la T disminuye. [11,4] En un pequeño grupo de pacientes se ha podido observar que el tratamiento con Zn mejora la líbido y las concentraciones séricas de T. [5]

Sin embargo este no es un hallazgo constante. [13]

En un informe previo [3] mencionamos que el Zn puede actuar de manera inespecífica como protector de membrana. Los resultados aquí presentados apoyan esta posibilidad ya que dentro en el grupo I con A elevada como en el grupo II con A normal se incrementó la T.

Es importante hacer notar que en el grupo III con prolactiva excesivamente alta no se modificó T, y que hubo correlación entre T y PRL. Es posible que PRL tenga efecto negativo en la síntesis de andrógenos igual que ocurre en sujetos con adenomas hipofisiarios. [14] Esta misma hiperprolactinemia puede ser responsable de la falta de respuesta en los niveles de DTH puesto que la enzima 5a reductasa es modulada negativamente por la cantidad de PRL circulante. [15]

La hiperprolactinemia de la IRC es un hecho conocido [16,17] si bien se ignora su causa. La pobra depuración renal de PRL no lo explica por sí sola. [18] Informes previos han sugerido que en pacientes con hipoparatiroidismo y función renal normal la infusión aguda de hormona paratiroidea eleva la PRL circulante, [19] igualmente la paratiroidectomía mejora la concentración de T en pacientes con IRC. [20,21] La causa del aumento en PRL no está esclarecida y la hormona paratiroidea puede tener una influencia importante en relación con la función gonadal y las alteraciones de la líbido en IRC.

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Cottonseed Flour Effects on Androgen Testicular Content and Serum Levels in Rats

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Natural gossypol present in the whole cottonseed flour (CSF) has clear antifertility properties, decreasing the number and motility of spermatozoa. In this study the endocrine function of the testes was evaluated, measuring by specific radioimmunoassays the testicular content and the serum levels of androstenedione (A), testosterone (T), and 5α-dihydrotestosterone in male rats fed with different and nontoxic concentrations of CSF along with Purina chow for 4 weeks, followed by 6 weeks of a recovery period. The testicular content of A, T, and DHT decreased 63%, 90%, and 38%, respectively, in comparison with their control. The serum levels of the three androgens diminished as follows: A 74%, T 95%, and DHT 60%. All the steroids measured in the recovery period showed a tendency towards the control values in both testicular content and serum. The most affected steroid was testosterone, which, after the recovery period, did not reach the control values. A also decreased, suggesting a blockade in testicular steroidogenesis caused by the initial diet.

Key Words: Gossypol; Testis; Androgens; Biosynthesis.

INTRODUCTION

The antifertility effect of gossypol and its effectiveness at doses below the cytotoxical ones have been investigated [1, 10]. The existence of specific changes in quantity, motility, and structure of spermatozoa has been shown in studies performed in vivo, as well as in vitro [3, 5, 9, 12]. However, its effect on testicular steroidogenesis is still unclear, as neither changes in androgen levels [1, 10] nor diminution of plasmatic testosterone values, according to a dose-effect relationship and an unspecific blockade in the steroidogenic pathways, has been demonstrated [8]. Gossypol was used in these studies either in its gossypol–acetic acid or its pure forms. A similar antifertility effect was found in rats fed with whole cottonseed flour (CSF) as a natural gossypol source [11]. To correlate those findings with changes in the endocrine testicular function, plasmatic and testicular contents of three active androgens—androstenedione (A), testosterone (T), and 5α dihydrotestosterone (DHT)—were measured using specific radioimmunoassays (RIA) in male rats fed with CSF-supplemented diet.

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MATERIALS AND METHODS

Preparation and evaluation of CSF-supplemented diets have been described [11]. Diets containing 10% total protein complemented with four different CSF concentrations, were prepared as follows: I—100% protein defatted CSF; II—whole CSF–Purina Chow 25%:75%; III—Whole CSF–Purina Chow 35%:65%; IV—Whole CSF:Purina Chow 50%:50%; and a control (C) diet with 100% protein Purina Chow. The experimental procedure has also been described [11]. Briefly, fifty Sprague–Dawley male rats with an average weight of 300 g were divided into five groups of ten animals each and fed with the diets already described over a period of 4 weeks. At the end of the exposure period, four animals from each group were killed by decapitation, their blood was collected, and the testes separated; the remaining animals were fed with a Purina Chow diet during the following 3 or 6 weeks to allow their recovery, prior to their being killed.

Serum was obtained by centrifugation at 1,500 *g*/10 min. Testes were decapsulated and homogenized in buffered saline to a final volume of 5 ml. Aliquots of the homogenates were kept frozen until the assay was performed. Radioimmunoassay methods were similar to those previously described [2, 6, 7]. To serum or homogenate aliquots, 1000 cpm of each steroid were added as a tracer, steroids were extracted with 10 ml ethylic ether, and the aqueous phase was frozen in acetone–dry-ice in order to completely separate the organic phase. After solvent evaporation, residues were applied to silica thin layer chromatographic plates and developed in each one of the following systems: (a) bencene, (b) bencene–ethyl acetate (7:3), and (c) bencene–methanol (9:1). The steroids were eluted with ethylic ether–methanol (9:1) in order to recover the tracer and proceed with specific RIAs. The antibodies used were prepared in our laboratory by using the A, T, and DHT 19-hemisuccinate-BSA complexes as antigens, and were further evaluated for their specificity and titer [2]. All antibodies were used to a final dilution of 1:10,000.

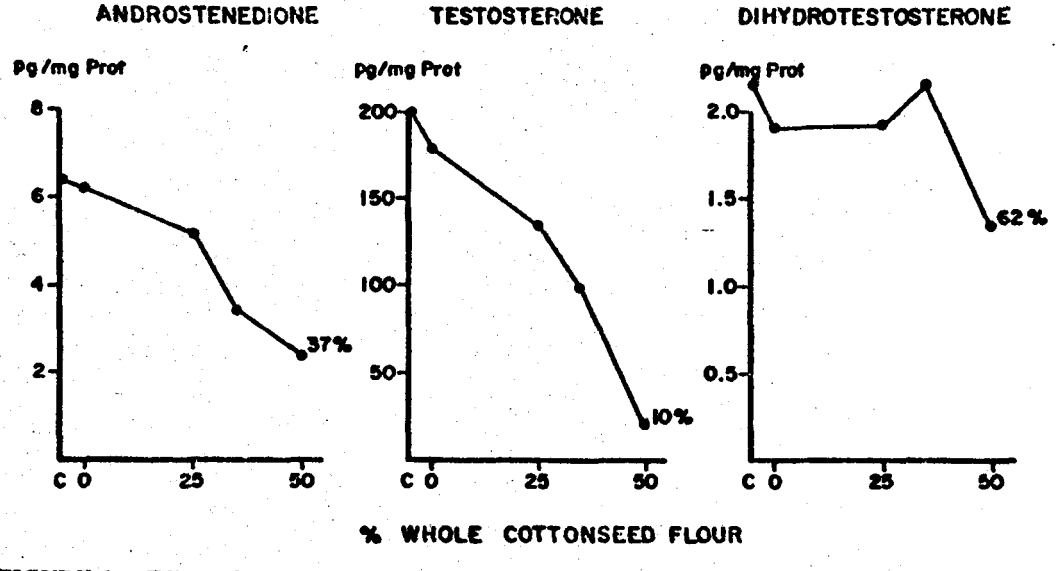

FIGURE 1. Effect of different CSF-supplemented diets on androgens contents in rat testes. Each point represents the mean of four determinations.

Samples and standard curves were incubated at 4°C for 18 hr and the bound and free steroids were separated by centrifugation after adding an activated charcoal–Dextran T-70 suspension (0.625%–0.0625%). The bound fraction was counted in scintillation vials with 5 ml of Instagel in a Packard 3320 liquid scintillation counter. The homogenate protein content was determined [4].

RESULTS

The effect of CSF supplemented diets on the testicular content is shown in Fig. 1. A significantly dropped in the groups III and IV, with respect to the control group, dropped in groups II and IV, and DHT only in group IV. In serum (Fig. 2), the levels were significantly less than controls ($p < 0.05$) for A in group III, T in group II, and DHT in both groups III and IV. In Fig. 3, the androgen testicular content during the recovery period is shown; the three steroids exhibit a tendency to reach the control values. A and DHT were similar to controls at 6 weeks and T was significantly lower at the same time and only in group IV. In the serum, the tendency towards levels similar to those found in the controls is evident but only DHT was significantly equal after 6 weeks. A and T did not regain their initial values (Fig. 4).

DISCUSSION

The diminution of the testicular content and plasma concentrations of the three androgens measured by the effect of the CSF-supplemented diets was evident; the modification was proportional to the quantity of CSF present in diets. Clearly, the main effect was on testosterone, the final product of testicular steroidogenesis, although A

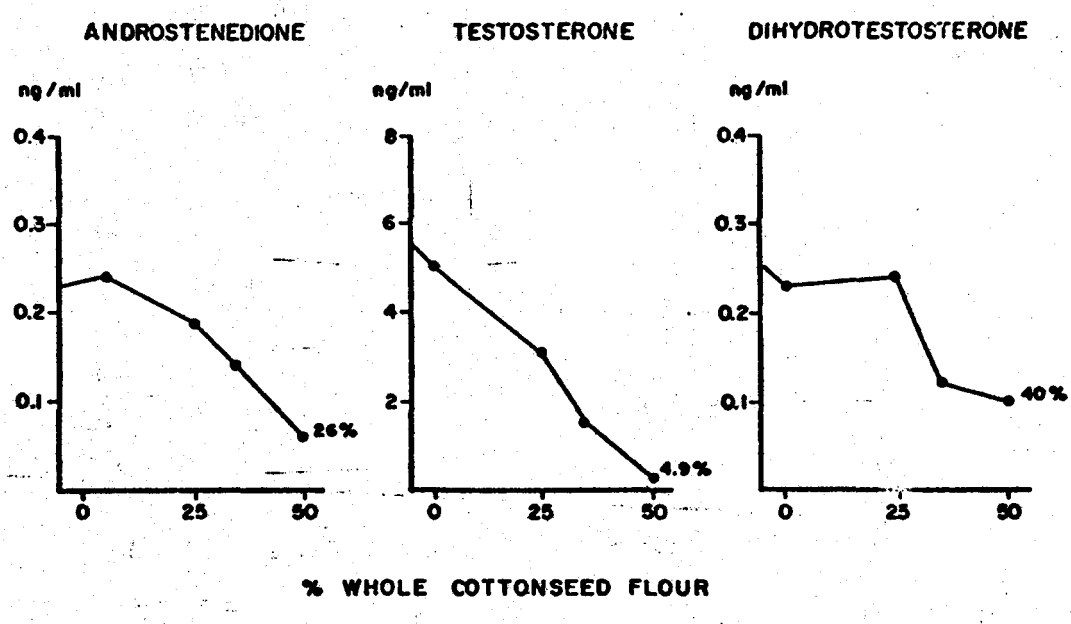

FIGURE 2. Effect of different CSF-supplemented diets on serum levels in rats.

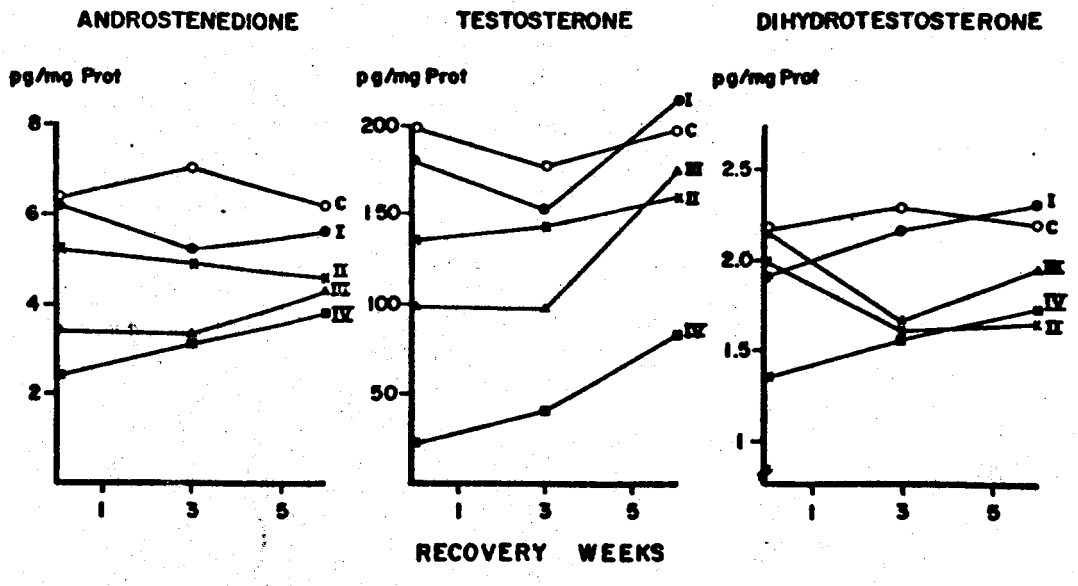

FIGURE 3. Changes on the androgen contents of the different experimental groups (see text) during the recovery period. Each point represents the mean of three determinations.

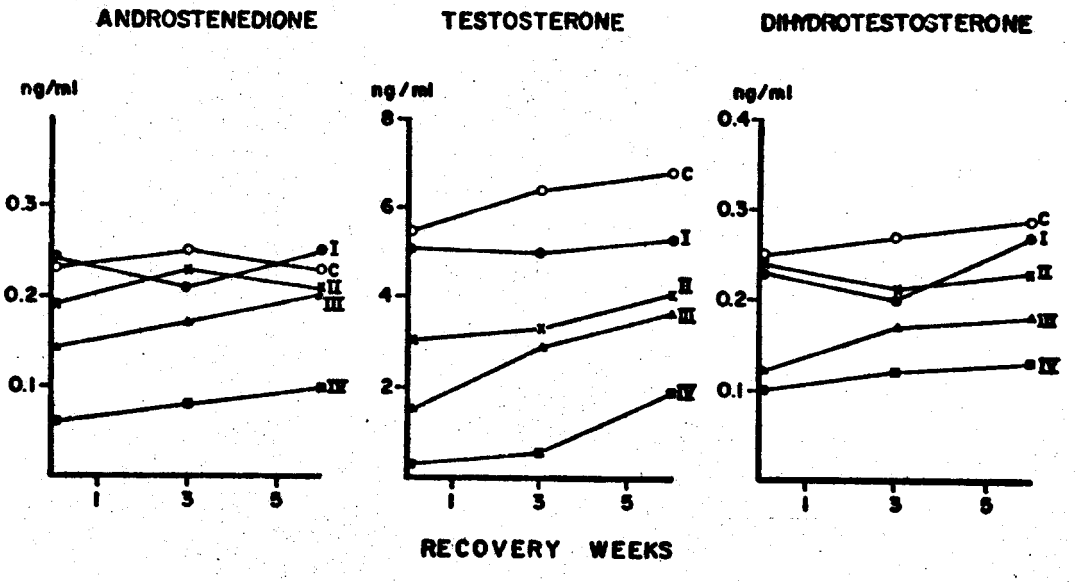

FIGURE 4. Changes on the serum concentrations during the recovery period. Code similar to Fig. 3.

and DHT also decreased; A is an intermediary step of the metabolic pathway and its diminution suggests a blockade of the steroidogenesis before it. On the other hand, DHT is a peripheral metabolic product and it is not affected as much as T is. The testicular effects were reflected on the serum concentrations with a decrease in the T levels to 5% of the control values, A only diminished to 37%, and DHT remained at 62% of their respective controls. As with the testicular content, the serum diminution has a tendency towards the control levels and only DHT reached concentrations similar to controls after the recovery period; these findings verify its peripheric origin.

The results found in this study are in agreement with those previously informed [8], where the testicular incubation with precursors did not produce testosterone, even after stimulation with LH or AMPc, suggesting a blockade of the steroidogenesis. The serum androgen levels decreased in a similar fashion and the recovery after the experimental period was also comparable.

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Hypergonadotrophic hypogonadism in an XX female subject due to 17,20 steroid desmolase deficiency

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Abstract. A 22 year old XX female patient with primary amenorrhoea and sexual infantilism was studied. Persistently elevated serum LH and FSH concentrations and exaggerated LRH pituitary responsiveness indicated deficient ovarian hormonal production. Serum levels of C_{21} and C_{19} steroids measured by specific radioimmunoassays before and after appropriate stimulations demonstrated an impairment of adrenal and ovarian steroid biosynthesis. Baseline levels of androstenedione (Δ^4-A), testosterone (T), and oestradiol-17β (E_2) were persistently below the normal range for healthy women at early follicular phase, whereas progesterone (P) and 17α-OH-progesterone (17-OH-P) serum levels were significantly higher than those observed for normal women. Adrenal and gonadal stimulation with ATCH and hCG, respectively, resulted in a considerable rise in serum P and 17-OH-P without any significant change in circulating levels of Δ^4-A, T, and E_2. These findings were consistent with the diagnosis of 17,20 steroid desmolase deficiency at both adrenal and ovarian levels. This is the first report of a 17,20 desmolase deficiency in an XX individual, and is in line with previous suggestions that familial occurrence of the disorder would fit an autosomal recessive pattern of inheritance.

In 1972 Zachmann et al. (1972) reported a familial form of male pseudohermaphroditism secondary to an inherited deficiency of 17,20 steroid desmolase, the microsomal enzyme responsible for side chain cleavage of C_{21}, 17α-hydroxylated steroids. Since then, Goebelsman et al. (1976) and Forest et al. (1980) have reported a number of cases with testicular impairment of testosterone (T) biosynthesis due to this enzyme deficiency. Based on the study of a family with 3 affected members with this disorder, Forest et al. (1980) suggested an autosomal recessive type of inheritance, although a recessive X linked trait could not be ruled out, particularly since only XY male individuals have been reported to be affected.

The present report describes clinical features and endocrine studies in an XX female individual with hypergonadotrophic hypogonadism secondary to 17,20 steroid desmolase deficiency. The finding of this enzyme deficiency in a woman with normal parents supports the concept that the disorder may be inherited as an autosomal recessive trait.

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Material and Methods

Clinical summary

R.R., a 22 year old phenotype female was referred to the Endocrine Clinic because of primary amenorrhoea and sexual infantilism. The patient was the second child in a family of five, born by normal full-term delivery after an uneventful pregnancy. The family history was unremarkable. Her parents are probably not related and are of normal intelligence and stature. The patient had been

on oestrogen-progestin replacement therapy before referral. Physical examination revealed normal vital signs, a blood pressure of 120/80 mmHg; height 158 cm; weight 47.5 kg; arm span 162 cm; upper segment 78 cm; lower segment 80 cm; discrete cubitus valgus and a high arched palate. Mammary glands were poorly developed; the axillary and pubic hair were scanty and the external genitalia were infantile in type. Routine laboratory analyses including haematogram, blood sugar, urea, and creatinine were normal. Baseline urinary excretion of 17-hydroxycorticosteroids (17-OHCS) was normal, whereas urinary 17-ketosteroids (17-KS) were persistently low or undetectable. Urinary pregnanetriol (P_3) and pregnanediol (P_2) were persistently elevated. A normal 46 XX karyotype was found in peripheral blood cells. Serial vaginal smears revealed anoestrogenism. X-ray pelvic pneumographic examination revealed the presence of bilateral ovarian enlargement and a small uterus.

Administration of chlormadinone acetate (2 mg/day) for 5 consecutive days failed to induce endometrial bleeding, and clomiphene citrate administration (100 mg/day) for 5 consecutive days induced neither endometrial bleeding nor ovulation. Anatomic and functional integrity of the endometrium was implied by normal endometrial bleeding induced by combined oestrogen-progestin treatment.

Methods

Reagents for LH and FSH assays were kindly furnished by NIAMDD NIH (Bethesda MD.). Serum LH and FSH were measured by double antibody radioimmunoassays (RIA) as previously described (Scaglia et al. 1976). Results were expressed as mIU/ml according to the 2nd IRP-HMG. Coefficients of variation (CV) for LH and FSH assays were 9.15% and 8.50%, respectively. Serum steroid hormones were determined by RIA after chromatography. Progesterone (P) 17α-OH-progesterone (17-OH-P) were extracted and purified before assays as previously described (Rios et al. 1974). Androstenedione (Δ^4-A), T and oestradiol-17β (E_2) were extracted with ether and further purified by thin layer chromatography (Rios et al. 1974). Cortisol (F) was measured in serum aliquots as previously described (Ruder et al. 1972). Recoveries in all assays as measured by the addition of 1000 CPM of the respective tracer were always above 80%. CV for serum steroid assays was less than 7%. Serum samples for hormone determinations were assayed in triplicate at two different dilutions to ascertain parallelism with the standard curves. Urinary 17-OHCS, and 17-KS, P_2, and P_3 were measured by conventional procedures.

Dynamic studies

Basal levels of serum gonadotrophins were measured in blood samples drawn at frequent intervals. Pituitary reserve and responsiveness were evaluated by the administration of an iv bolus (100 μg) of synthetic LRH.

Adrenal function was assessed by exogenous ACTH stimulation. Synthetic corticotrophin 1-24 (Synacthen, CIBA) (25 IU) was administered in a 4 h iv infusion. Blood was drawn before (−30, −15 and 0 min) and during ACTH administration (60, 120 and 240 min).

Gonadal stimulation with hCG was performed during adrenal suppression. Adrenal suppression was achieved by the administration of dexamethasone (0.5 mg/6 h) for 6 days. Blood samples were obtained before and on the third day of dexamethasone suppression. Adrenal suppression was continued and a concomitant gonadal stimulation with hCG 5000 IU for 4 days was undertaken. Blood samples were drawn daily during hCG stimulation.

After completion of endocrine studies the patient was submitted to an exploratory laparotomy.

Results

Gonadotrophin dynamics

The serum levels of LH and FSH (mean ± SEM, n = 15) were 24.6 ± 2.28 mIU/ml, and 10.3 ± 0.65 mUI/ml, respectively (Fig. 1 A). These baseline levels were significantly higher than those observed for normal menstruating women in the early follicular phase (3−12 mUI/ml for LH and 0.05−5 mUI/ml for FSH). Acute administration of LRH resulted in a significant increase of the radioimmunoassayable serum levels of both LH and FSH. A significant rise in LH levels (5.6-fold) was already observed 30 min after LRH injection while a slight and delayed though significant increase in serum FSH levels (2.1-fold) was also noted (Fig. 1 B).

Steroid hormones

The serum and urine basal levels of different steroids are shown in Table 1. As can be noticed P and 17-OH-P levels were significantly higher than those observed in healthy women in the early follicular phase. In contrast Δ^4-A and T levels were persistently below the normal range. Serum E_2 values were found within the assay limits of sensitivity.

Adrenal function was found to be normal as assessed by circulating F and urine 17-OHCS levels, but 17-KS were undetectable. Urine P_2 and P_3 excretion was significantly higher than in normal controls.

Adrenal stimulation

The administration of ACTH resulted in a further increase of the already elevated serum levels of P

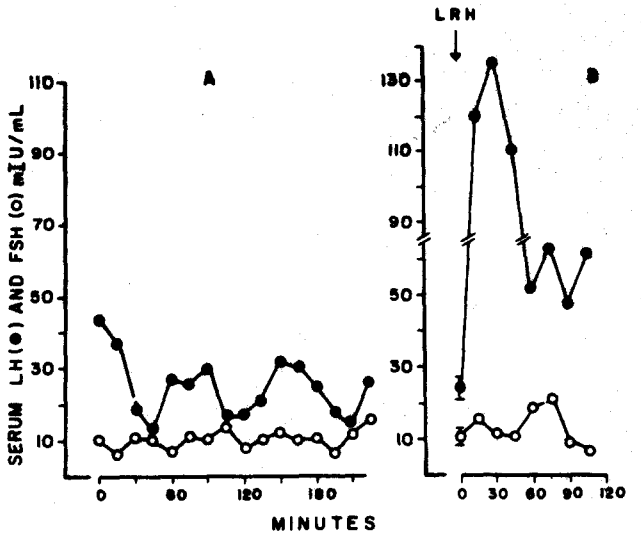

Fig. 1.

Baseline serum levels of LH (●) and FSH (○) and their responsiveness to LRH. A) Gonadotrophin levels were measured in serum samples drawn at 15 min intervals. B) LH and FSH response to an iv bolus of LRH (100 μg). Serum samples were drawn before and after pituitary stimulation.

and 17-OH-P (4- and 1.3-fold, respectively) whereas no significant changes in serum concentrations of Δ^4-A and T were observed (Fig. 2). The urine steroid profile after ACTH infusion was similar to that observed in serum. Indeed P_2 and P_3 exhibited a significant increase while 17-KS remained undetectable (data not shown).

Ovarian stimulation

The results of ovarian hCG stimulation during adrenal suppression are shown in Fig. 3. Serum levels of both P and 17-OH-P measured after 3 days of continuous dexamethasone administration were slightly lower than baseline levels. Dexamethasone administration did not modify in a significant manner the almost undetectable circulating levels of Δ^4-A. Serum F and urine 17-OHCS were significantly diminished during adrenal suppression.

Administration of hCG during sustained adrenal suppression induced a significant increase of serum levels of P and 17-OH-P (6- and 2-fold,

Table 1.

Baseline levels of several steroid hormones.

F*	P	17-OH-P	Δ^4-A	T	E_2	P_2	P_3	17-OHCS	17-KS
Present case									
430	20	11	1	0.3	ND	37 752	5944	4966	1733
Normal values**									
(140–550)	(2–3)	(2–3)	(3–5)	(< 1.7)	(0.055–1.10)	(< 4056)	(< 4458)	(5518–14 898)	(13 868–34 670)

* F, P, 17-OH-P, Δ^4-A, T, and E_2 expressed as nmol/l; P_2, P_3, 17-OHCS and 17-KS as μmol/24 h.

** From Abraham et al. (1972), Kim et al. (1974) and Abraham (1974) and from our own laboratory. The numbers in brackets are the range of normal values for normal menstruating women at the follicular phase.

ND = non detectable.

respectively). In contrast serum Δ^4-A and T and urine 17-KS levels remained unchanged throughout hCG administration.

Anatomical findings

Laparotomy revealed the presence of small normal Mullerian derivatives and moderate bilateral ovarian enlargement. Histological examination of bilateral ovarian biopsies disclosed the presence of primordial follicles, subcapsular cysts, and clusters of luteinized cells forming corpora luteal-like structures as shown in Fig. 4. No corpora albicans were however identified.

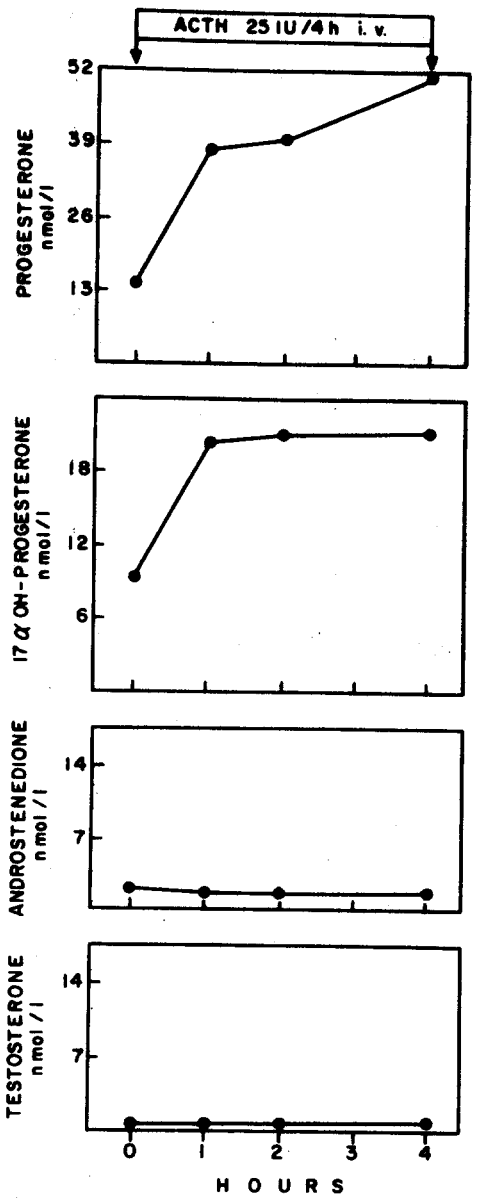

Fig. 2.

ACTH adrenal stimulation. Serum immunoreactive levels of P, 17-OH-P, Δ^4-A, and T before and during an iv infusion of synthetic ACTH, 25 IU.

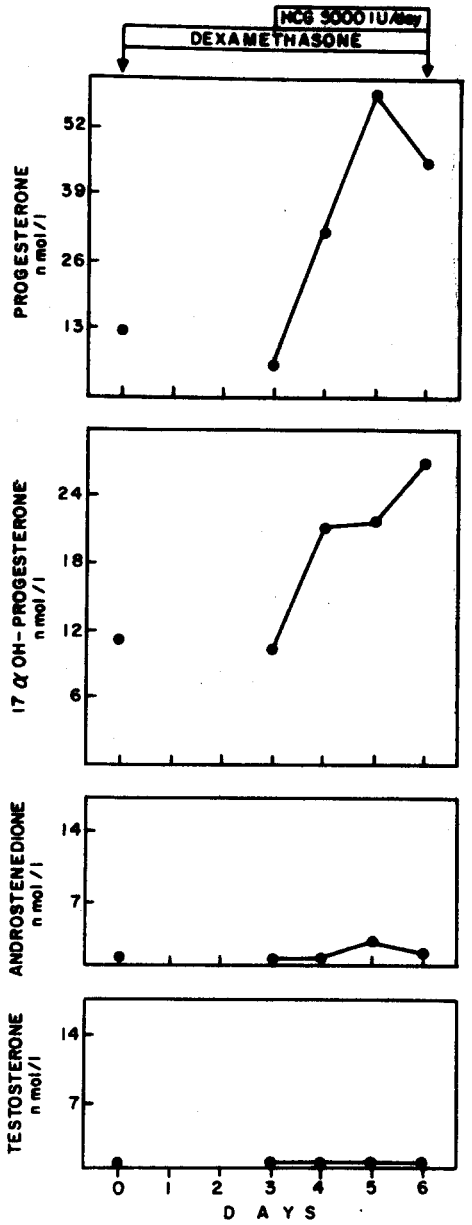

Fig. 3.

Serum steroid profile before and during hCG gonadal stimulation during dexamethasone-induced adrenal suppression.

Fig. 4.
Right ovarian biopsy at the time of laparotomy. Ovarian morphology showed scanty primordial follicles and clusters of luteinized cells forming corpora lutea-like structures, no corpora albicans were found. The left ovary showed identical findings.

Discussion

This paper describes the clinical features and the endocrine function of a non-mosaic 46 XX woman with primary amenorrhoea, sexual infantilism, and anoestrogenism secondary to 17,20 steroid desmolase deficiency.

Persistently elevated circulating levels of pituitary gonadotrophins and an exaggerated pituitary responsiveness to exogenous LRH stimulation were found, indicating normal function of the hypothalamic-pituitary axis as well as a gonadal failure to produce normal amounts of oestrogens. This failure could have been related either to dysgenetic or atrophic gonads (Rios et al. 1974) or to a genetic defect in steroid hormone biosynthesis in an otherwise normally diffentiated ovary (Mallin 1969). The finding of enlarged rather than small ovaries by X-ray pelvic pneumographic studies ruled out the first hypothesis and prompted us to look for an impairment of sex steroid hormone production.

Analysis of the serum and urine steroid hormone profile in this amenorrhoeic woman revealed extremely low levels of Δ^4-A, T, E_2, and undectectable 17-KS with concomitant abnormally high levels of P, 17-OH-P, P_2 and P_3. The unsual C-21/C-19 steroid ratio found in the basal state was clearly indicative of an enzyme blockade at the level of the side-chain cleavage of C_{21}, 17α-hydroxylated steroids. Since the 17,20 desmolase enzyme is normally present in both the adrenals and the ovaries, dynamic studies were then undertaken to assess whether the enzyme deficiency affected both glands.

An adrenal defect was supported by the lack of any increase in serum Δ^4-A and T and urinary

17-KS and an exaggerated rise in serum P, 17-OH-P, and urinary P_2 and P_3 after ACTH stimulation.

As expected, selective 17,20 desmolase deficiency would not lead to an adrenal impairment of glucocorticoid biosynthesis. Indeed normal baseline serum F and urinary 17-OHCS levels were found in this patient, but a subnormal adrenal cortisol response to ACTH was noted, as previously reported in males bearing this enzyme deficiency (Forest et al. 1980). Whether the low F adrenal reserve might be the result of the high serum levels of progesterone as reported in women on synthetic progestins therapy remains to be ascertained (Hellman et al. 1976).

A slight decreased of the serum basal levels of progestagens was observed after dexamethasone administration, thus indicating that the ovaries were the major source of these C_{21} steroids. Furthermore, evidence that the ovaries shared the enzyme deficiency with the adrenals was derived from the significant rise in serum P and 17-OH-P without any change in the circulating levels of Δ^4-A, and T observed following hCG gonadal stimulation during dexamethasone-induced adrenal suppression.

The morphological findings from bilateral ovarian biopsies, particularly the large amount of luteinizing cells, are in line with the results of endocrine studies and therefore support the diagnosis of 17,20 steroid desmolase deficiency in an XX female individual.

Although a limited number of families affected with 17,20 desmolase deficiency has been reported, it has been suggested that this rare entity is inherited as an autosomal recessive trait (Zachmann et al. 1972; Goebelsman et al. 1976; Forest et al. 1980) like most of the enzyme defects resulting in abnormal steroid biosynthesis in humans (Finkelstein & Shaefer 1979). Nevertheless the possibility of an X-linked recessive type of inheritance could not have been ruled out particularly since only XY male individuals have been recognized to be affected so far.

Although definite identification of the mode of inheritance awaits more extensive families studies, this report of an XX individual affected with 17,20 steroid desmolase deficiency strongly supports an autosomal recessive pattern. While this case represents an almost complete enzyme deficiency in a woman, an occurrence of the disorder with different degrees of severity might be expected, as reported in males (Forest et al. 1980).

Acknowledgments

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MORPHOPHYSIOLOGICAL CORRELATION OF BOAR LEYDIG CELL DEVELOPMENT DURING POSTNATAL STAGE

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ABSTRACT To analyze the possible correlation between steroidogenic activity of the Leydig cells and their morphology and ultrastructure in the boar testes at different ages, testes from six Yorkshire boars were obtained by orchidectomy at 8,14,28,40,70,78,86,94 and 100 days of age. Using representative sections of testicular parenchyma, light as well as transmission electron microscopic studies were performed. Homogenized, albuginea-free tissue was further used for quantitative estimation of total proteins and metabolic pools of androstendione (A), testosterone (T) and 5 α-dihydrotestosterone (DHT). Under light microscopy, abundant hyperplastic Leydig cells were observed from 14 to 40 days, thereafter was a decrease in their size and number. Spermatogenesis remained in the spermatogonia stage while the tubules increased in diameter until age 80 days; from 90 days on, pachytene primary spermatocytes could be found. A well developed smooth endoplasmic reticulum was observed in Leydig cells from the 8th postnatal day. Metabolic pools from age 8 to 28 days showed a decrease for T and A from 4.33 to 1.78 and 2.51 to 0.48 μg/mg protein respectively, maintaining thereafter the same levels throughout the age-span studied. However, DHT was found at low levels from the beginning of neonatal period (from 0.07 to 0.38 μg/mg protein).

KEY WORDS: BOAR, LEYDIG CELL, ULTRASTRUCTURE, TESTOSTERONE SECRETION

(Cornell Vet. 1983, 73:67-75)

INTRODUCTION

THE testis has two different functional compartments: the gametogenic compartment composed of the seminiferous tubules, and the endocrine portion or interstitial tissue (6). The main product of this last compartment is testosterone (T), synthesized by the Leydig or interstitial cells (6,8) which have specific structural features when they reach their complete development, namely: rounded nucleus, numerous mitochondria, prominent Golgi complex and abundant smooth endoplasmic reticulum. It is known that testosterone promotes and maintains sexual secondary characteristics typical of each species and it is presumably synthesized from puberty throughout the rest of life (4). However, during the embryologic stage (13,15,17) other factors besides testosterone, participate in the differentiation and development processes of the male genital tract. The appearance of androgens in early life suggests the existence of an initial differentiation and maturation of the Leydig cells (previously shown to occur in various mammal species) which is followed by a postnatal regression both from a functional and morphological view point (9,10). According to previous reports the boar testes are different because the Leydig cells maintain adult ultrastructural characteristics from prepubertal stages with no observable cellular regression (1,2,7,16). The aim of this study was to correlate the potential steroidogenic activities of the porcine Leydig cells with their microscopic and ultrastructural features during the postnatal stage.

MATERIALS AND METHODS

Biological material. The testes of six Yorkshire boars were removed by orchidectomy at 8,14,28,40,70,78,86,96 and 100 days of age. For the morphological studies representative fragments of testicular parenchyma were fixed in Bouin's solution for light microscopy and 3% glutaraldehyde for electron microscopy; the remaining tissue was freed of tunica albuginea and homogenized at 10% w/v with Tris-HCl-0.05 M buffer, pH 7.0 with a teflon glass homogenizer.

Microscopy. Tissue samples were embedded in paraffin and stained with hematoxylin and eosin (H&E) for light microscopy and for electron microscopy, they were fixed with 3% glutaraldehyde, post-fixed with 1% osmium tetroxide and embedded in Epon. A Philips EM-300 electron microscope was used.

Steroidogenic activity. The steroidogenic activity was studied by measuring tissue metabolic pools, of androstendione* (A), testosterone* (T), and

*Abbreviations and trivial names:
RIA = radioinmunoassay
Androsterone = Androst-4-en-3,17-dione
Testosterone - 17 β hydroxy-androst-4-en-3-one
5 α-dihydrotestosterone = 17 β hydroxy-5α-androstan-3-one

5α-dihydrotestosterone* (DHT) by means of specific RIA previously published (3,14). In brief, the method was: 1000 cpm of steroid tracers for each tritiated hormone were added to the samples and then extracted with 10 ml of ethyl-ether using a Vortex mechanical shaker during one minute; the organic phase was then separated and evaporated until dry and the extract applied to silica gel chromatoplates to be developed with the adequate solvent systems, in order to separate the three steroids. The purified steroid aliquots were analyzed using highly specific antibodies to measure their concentration by specific RIA; the separation of fractions was made with carbon-dextran and the bound fraction was counted in a Tri Carb Liquid Scintillation B Counter. The percentage of bound radioactivity was interpolated in a standard curve and tracer recovery was corrected for each sample. Intrassay coefficient of variation was less than 10% for all samples. Protein concentrations on each sample were measured according to Groves (11). The statistical analysis was performed by the Student's t test.

RESULTS

The light microscopic analysis showed abundant hyperplastic Leydig cells in samples from pigs 8 to 28 days of age. These cells decreased in size and number from 40 to 80 days with a final increase by age 100 days (Fig. 1). The spermatogenesis in the seminiferous tubules was maintained at the spermatogonia stage until day 78 and pachytene primary spermatocytes were found later on (Fig. 2). The Leydig cells appeared from early postnatal life to have highly differentiated ultrastructural features, eg: rounded nucleus, mitochondrial abundance, several Golgi bodies and smooth endoplasmic reticulum (Figs. 3 and 4).

Figure 5 shows the steroidogenic activity, for each hormone and age group. A was 2.51 and 2.46 μg/mg protein at age 8 and 14 days, respectively, with a subsequent significant decrease to 0.40 μg/mg at days 28, 40 and 70 ($p < 0.01$), a small increase in day 78 ($p < 0.05$) and a final and gradual decrease to approximately 0.10 μg/mg between days 86 to 100. T had a similar pattern with a higher concentration of 4.32 μg/mg protein at day 14, a decrease ($p < 0.001$) to 1.59 μg/mg at day 28, an increase to 2.14 μg/mg ($p < 0.05$) at day 70 with a gradual decrease reaching 1.30 μg/mg at age 86 days. DHT had lower concentrations when compared with the former two steroids: 0.38 μg/mg protein at day 28, a decrease to 0.07 μg/mg until day 78 ($p < 0.05$), an increase to 0.27 μg/mg at day 96, with a final decrease by age 100 days ($p < 0.05$).

DISCUSSION

The comparative analysis of light microscopy and androgen biosynthesis showed a good correlation between the number and size of Leydig cells and the decrease in steroid production; when the development of this cellular line was

Fig. 1. Micrograph of a testis with numerous Leydig cells (L) and small diameter seminiferous tubules. Note cell population composed by spermatogonia (arrow) and Sertoli cells. Age 28 days. H&E, X800.

Fig. 2. Micrograph of a testis showing numerous Leydig cells with a decreased cytoplasm (L). Seminiferous tubules are wider and showed spermatogenesis up to pachytene primary spermatocytes. (arrow) Age 78 days. H&E, X800.

Fig. 3. Electronmicrograph of a binucleated Leydig cell. Ovoid mitochondria with a dense matrix and tubular crests (arrows) and abundant smooth endoplasmic reticulum (ER) can be seen in the cell cytoplasm. Age 70 days. X11.900

Fig. 4. Electronmicrograph in a higher magnification of a Leydig cell showing the nucleus (N), smooth endoplasmic reticulum (ER) and mitochondria (M). Age 70 days. X32.000

reassumed after the age of 78 days, androgen tissue levels increased again, despite their general tendency to a gradual decrease.

Electron microscope analysis showed that even when cellularity was diminished, no regression or autophagy could be demonstrated and Leydig cells maintained their characteristic steroidogenic appearance along with an important biosynthetic activity. Indeed, androstendione and testosterone

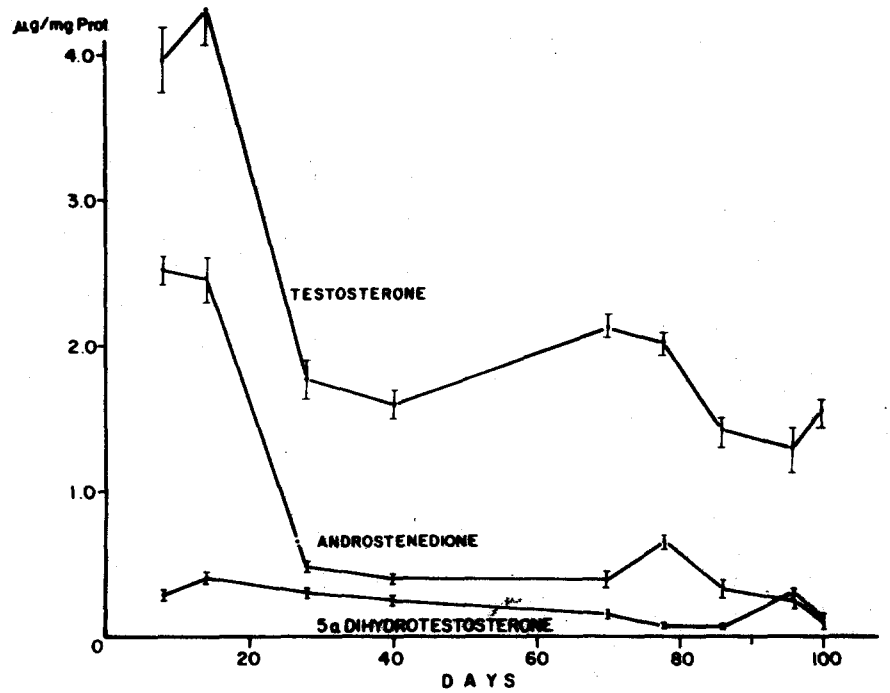

Fig. 5. Testosterone, androstenedione and 5 α-dihydrotestosterone tissular levels in boar testes during postnatal stage. Mean and standard error, six animals for each group.

levels were higher than those reported to occur in the rat adrenal gland (0.21 µg/mg protein after adrenocorticotrophic stimulation) (12) and even higher than in human adult testes, (0.5 ng of testosterone/mg protein) (5).

These results are merely descriptive but point out two main aspects: Firstly, the need to investigate the role of these high androgen levels in the boar testis during neonatal life, and secondly, the reason for the absence of tissue response to these high androgen concentrations.

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Modifications in the Testis Steroidogenic Pathways in Rats Fed With Cottonseed Flour

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Suppression of the testicular T production by effect of the CSF has been previously reported. Changes in the testicular content of intermediates and precursors of T biosynthesis were determined in rats fed during 28 days with diets containing 25, 35 and 50% of CSF and compared with a defated cottonseed flour diet group and a control Purina-chow diet group. Also described were modifications during a six wk post CSF diet period. The results showed a diminution in pregnenolone and progesterone testicular content to a third and a half of the control values (21.5 and 19.3 pg/mg of protein respectively). Other compounds in the Δ^5 pathway, 17 hidroxypregnenolone and dehydroepiandrosterone were undetectable since the 35% CSF diet, the 17 hidroxyprogesterone diminished to undetectable values only with the maximal (50%) CSF content diet. During the post CSF diet period all the steroids showed a tendency toward the control values. The 25% CSF content diet group reached the initial contents; other groups presented different recovery degrees. The most affected was the 50% diet group with a direct effect of the CSF on the testicular androgen steroidogenesis affecting both the Δ^4 and the Δ^5 pathways.

Key Words: Gossypol, testis, steroidogenic pathways.

INTRODUCTION

The gossypol effects on the morphology and motility of spermatozoa are well documented [4, 9, 11], however, its effects on the testicular endocrine function is controversial, since initial reports as well as other papers have found no changes in the plasmatic hormones [1, 11]. Lin et al. showed a significant decrease of plasmatic testosterone (T) levels with a possible blockade of testicular steroidogenesis [10].

The antifertility effect, similar to that of gossypol, was demonstrated in rats fed with whole cottonseed flour (CSF) supplemented diets [12]. A decrease was found in the testicular content of three active androgens as well as a decrease in T serum levels [8].

To correlate those results with a blockade in the androgen steroidogenic pathways the testicular content of Δ^5 precursors pregnenolone (Preg), 17 hidroxypregnenolone (17OHPreg), dehydroepiandrosterone (DHEA); Δ^4 intermediates-progesterone (Prog), 17 hidroxyprogesterone (17OHPrbg), and the active androgens-androstenedione (A), testosterone (T) and 5 α dihydrotestosterone (DHT) were measured by specific radioimmunoassays (RIAs) in rats fed with CSF additioned diets.

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MATERIALS AND METHODS

The CSF diets were prepared and evaluated as reported [12], the experimental procedure was as follows: Diets containing 100% defated CSF or 25, 35 and 50% whole CSF and a control Purina chow diet were used to feed 5 groups with 10 adult male Sprague-Dawley rats each during 4 wk. At the end of the exposure period 4 animals from each group were sacrificed by decapitation; the remaining animals were fed a purina chow diet during 3 to 6 wks to allow their recovery prior to their sacrifice. The testes were separated, decapsulated, and homogenized in buffered saline solution to a final volume of 5 ml, aliquots of homogenates were kept frozen until assays were performed. Radioimmunoassay methods were similar to those previously described [2, 7]. 1000 cpm aliquots of each steroid (S.A.-40-60Ci/mM) were added to the homogenate as tracer. Steroids were extracted with 10 ml ethylic ether and the aqueous phase was frozen in acetone with dry ice in order to completely separate the organic phase. Solvent evaporation residues were applied to silica thin layer chromatographic plates and developed in each one of the following systems: 1) for Δ^5 precursors a) bencene (100%), b) bencene:ethylic acetate (8:2) and c) bencene: methanol (95:5). 2) For Δ^4 intermediates a) bencene (100%) and b) ethylic ether: bencene (2:1). All steroids were eluted and divided into aliquots to count and calculate the tracer recoveries or to proceed with the specific RIA. The antibodies used were prepared in our laboratory and evaluated for specificity and titer [3]. All the antibodies were used 1:10.000 as the final dilution. Samples and standard curves were incubated at 4°C during 18–20 hours. Bound and free steroid fractions were separated by centrifugation after adding an activated charcoal-Dextran T-70 suspension (0.625– 0.625%), and the bound fractions were counted in a scintillation spectrometer. The homogenate protein content was determined by the Groves method [5].

RESULTS

The testicular steroid contents expressed in pg/mg of protein found in the control group are plotted in Figure 1. The higher concentration obtained was for T, the final product of the testicular steroidogenesis. The second higher concentration corresponded to Preg, the first precursor of all the steroidogenic pathways. The tissular content of steroids which form the $^5\Delta$ metabolic pathway were lower than those from the $^4\Delta$ series, with the exception of Preg.

The effect of feeding with different CSF additional diets for each steroid and its corresponding recovery period are plotted in composed graphs. Expressed on the left side of the graph are the effects of the different CSF additional diets and on the right side, the recovery of each diet group with the steroids measured (Figs. 2 and 3). For Preg a decrease of its content was observed, and it was proportional to the CSF present in diets with statistical significance for 35 and 50% CSF additional diets. The reduction in the Preg content was one third of its control value. During the post treatment period, a tendency towards control values was observed; the recovery was reached after 3 wk for the 35% CSF group and after 6 wk for the 50% CSF diet group. For Prog, the changes in both periods were similar. The diminution by effect of CSF diets was significant in 35% and 50% groups; the lower value was half of the control. During the post diet period the 35% CSF diet group recovered its control level, while the 50% CSF diet group did not reach it after six wks.

For the 17 hydroxylated compounds, even with similar changes, some differences were observed. For 17OHPreg a significative diminution occurred with concentration

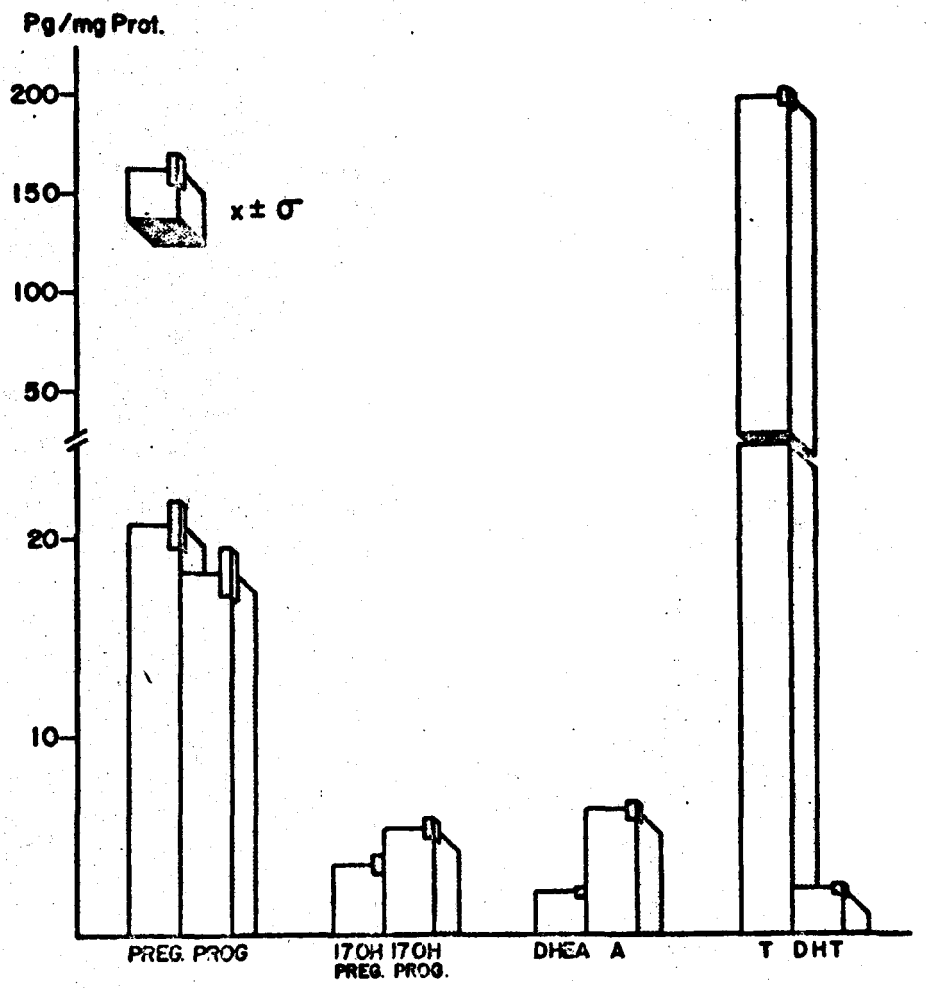

FIGURE 1. Rat testes steroid content in the control group (n = 4).

of 25% CSF or higher; in 35% CSF diet the content was undetectable. During the post diet period the 25% CSF group reached the control values in 3 wk, the 35% and 50% groups had a tendency to the control values; the 50% CSF remained with undetectable levels after 3 wk of post treatment period. For 17OHProg a significant diminution was observed beginning with the 25% CSF additional diet group and the content was undetectable only for the 50% diet group. In the post CSF diet period a tendency towards recovery was observed. The statistical differences disappeared at 3 wk for the 35% diet group, although the 50% diet group did not reach the control levels just a 70% recovery was observed. DHEA had a significant decrease beginning with 25% CSF diet group. The levels in this steroid were undetectable for both 35% and 50% CSF diet groups and remained in the same values after 3 wk of the post diet period.

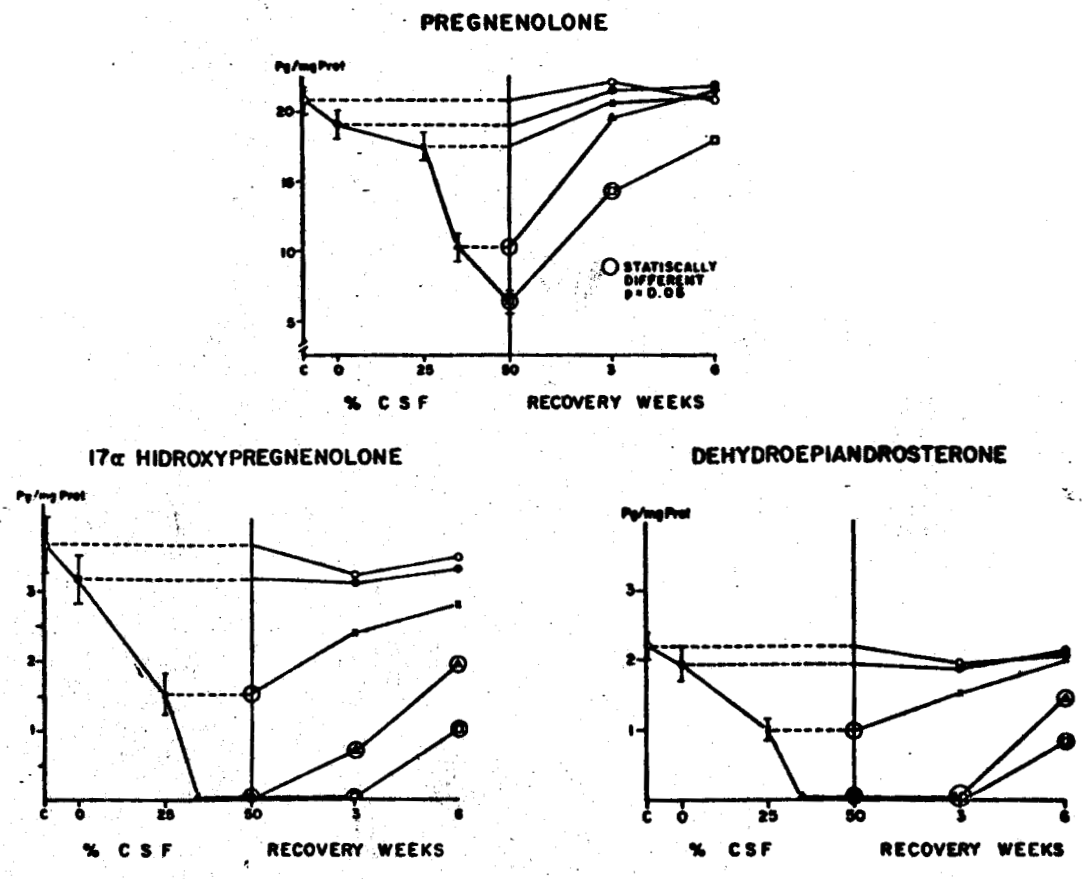

FIGURE 2. Δ^5 precursors content changes during CSF diets and recovery period in rat testes. O = Control Purina chow diet, ● = 100% defated CSF diet, X = 25% whole CSF diet, ▲ = 35% whole CSF diet, □ = 50% whole CSF diet.

DISCUSSION

The active androgens A and T had similar changes to the preceding steroids. The CSF additional diet for A diminished the testicular content to one third of the control and at the end of the post-diet period only the 50% CSF diet group remained lower and different from the control. The T changes showed a significant decrease to one tenth of the control with the 50% CSF diet group and a 40% recovery after the 6 wk post CSF diet. For DHT no differences were observed with values similar to the controls although there were significative differences with the 50% CSF diet group in the CSF period and at the third week of the post diet period.

The Δ^4 steroids were predominant in the testicular contents of the control groups with an important exception in Preg with values around 20 pg/mg. The most affected compounds were 17OHPreg and DHEA which were undetectable with 35 and 50% CSF

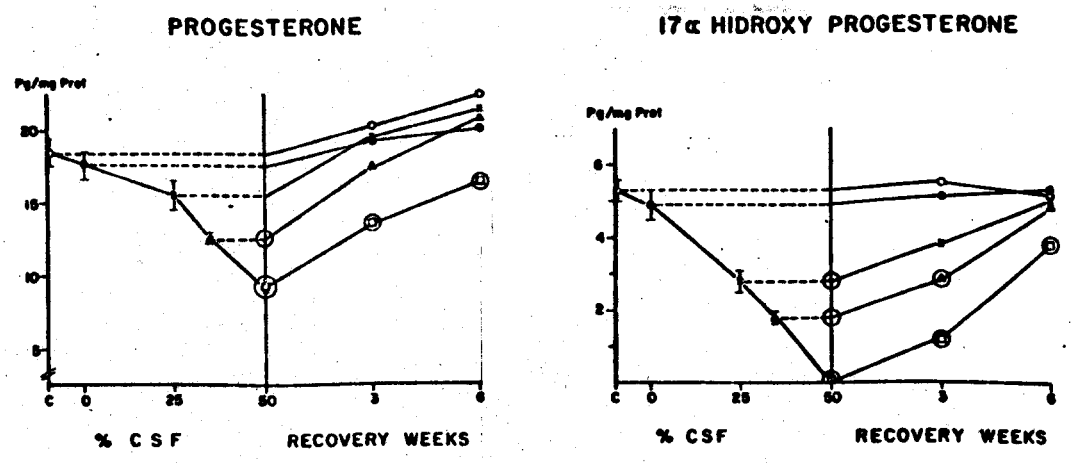

FIGURE 3. Δ^4 **intermediates content changes during CSF diets and recovery period in rat testes. Code as Figure 2.**

diet and for DHEA which remained in low values even after 3 wk of the post diet period. The Δ^4 steroids were less affected and the evidences allows establishment of a preferential steroidogenic pathway formed by preg-prog-17OHProg-A and T.

The total steroidogenic pathway was reduced by effect of the CSF additioned to the Purina chow diets, in according with Lin [10] and Hadley et al. [6] which results showed an inhibition of the testicular steroidogenesis. The data found in this study could not establish the site of action of gossypol of the CSF components; however, its effect began since the initial precursor Preg with the consequences on the final products.

In conclusion the CSF inhibits the testicular steroidogenesis before the Preg step, the inhibition follows a dose-effect pattern, and the Δ^5 alternative pathway was more affected than the Δ^4 one.

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four concentrations of furazolidone. The mixture was pelleted again and given to the animals for two months at the longest period, as follows: I Furazolidone 0.011% + Roma, Mexico, II Furazolidone 0.22% + Roma, Mexico, III Furazolidone 0.033% + Roma, Mexico, IV Furazolidone 0.066% + Roma, Mexico and V Roma, Mexico without furazolidone. Before starting medication in the feed, all animals were housed for two weeks in a new location with the same light and dark conditions aforementioned. During this period and along the experiment, the weight of each animal was recorded every 15 days.

It was decided to evaluate the fertility of five mice of the 0.066% after a month of a daily ingestion of furazolidone. They were mated with normal females which were observed for pregnancy during 15 days. These males failed to fertilize and were killed for morphological studies. In the remaining animals of the 0.066% group, furazolidone was discontinued for a month and they were tested for fertility. Animals from the other groups were also tested for fertility after two and one-half months and finally killed for morphological studies. The animals were anesthetized with Droperidol (1 mg/kg) and Ketalar (50 mg/kg), the heart was exposed and a Karnowsky solution (9) was injected by the left ventricle. Once fixation was completed the testes were removed. The right one was weighed, sectioned and post fixed in 10% buffered formaline for paraffin embedding and light microscopic studies were performed on section of 6 micra thick, stained with hematoxylin and eosin. The left testicle was finely trimmed into small fragments, post fixed in 1% Osmium tetroxide solution and embedded in araldite for electron microscopic studies. Ultrathin sections were obtained in a Reichert Ultracut 4 ultramicrotome, mounted on copper grids and stained with heavy metals (18, 20) for observation with a Philips 300 EM electron microscope.

Hormone Values Correlation

Groups of ten mice matched with those killed for morphological studies were also sacrificed after a month of medication, the testes were freed of the tunica albuginea and homogenized at 10% w/v with Tris-HCl-0.05 M buffer, pH 7.0 in a teflon glass homogenizer. The steroidogenic activity was evaluated by means of tissue metabolic pools measuring by specific RIA, androstenedione (A), testosterone (T) and 5-dihydrotestosterone (DHT) as previously described (2,6).

Fertility Test

After a month of furazolidone treatment the fertility test was performed in five males of each group, by mating them with young females in which fertility was proven before, they were observed for 25 days. Pregnant animals were allowed to deliver and their offspring evaluated as to their number and phenotipic characteristics. Animals without pregnancy were observed for the same period and later mated with normal males.

RESULTS

There were no significant differences in the weights of control and medicated groups for 1,2 and 5 months with 0.011%, 0.022% and for the group with 0.033% of furazolidone during both periods. Group with 0.066% concentration showed a slight diminution in the first month. In the same table, the testes with 0.033% and 0.066% of furazolidone disclosed a diminution of the weight after one month of treatment. A statistical significance of $p < 0.05$ was found using the non parametric Mann Whitney test, when the control was compared to the 0.066% of furazolidone treated group.

Effect of Furazolidone on Spermatogenisis in Mice: A Fertility and Morpho-physiological Correlation

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ABSTRACT

The testes of mice consuming four concentrations of furazolidone for two months were analyzed by means of androgen content, morphological and fertility studies. Furazolidone at concentrations of 0.011% and 0.022% did not induce modifications in these parameters. The 0.033% concentrations showed no significant diminution of testes weight but slight modifications in the nuclei of pachytene primary spermatocytes. Androstenedione (A) decreased and dihydrotestosterone (DHT) increased. The 0.66% group disclosed a significant diminution of the testes weight, decreased spermatogenesis with severe changes in 20% of the seminiferous tubules, and with spermatogenesis arrest at primary spermatocytes. In this group, A was found with significantly lower values and DHT was slightly increased. Mice of this group were infertile after a month of furazolidone ingestion and were fertile again after withdrawal of the drug for a month. The medicated groups with lower doses all were able to fertilize. The Leydig cells and testosterone levels were unaltered in all groups.

INTRODUCTION

Furazolidone, N- (5 nitro-2 furfurilidine)-3 amino-2-oxazolidone is one of the furans commonly used in animals for antibacterial, protozoolizide purposes and recently used as a food additive in human meals. Early knowledge on nitrofurazone pharmacology, a similar furan, points out to its toxic effect on germinal epithelium of mice testes. In this regard, hyperplasia and hypertrophy of the Leydig cells were noted when a dose of 0.066% was given orally for a month. A lower dose (0.044%) induced atrophy of the seminal vesicle without any damage to the semiferous tubules. An antiandrogenic effect was then proposed related with an overactivity of the pituitary producing an excess of gonadotropins due to withdrawal of an inhibitory substance produced by the seminiferous tubules (14). There was an interruption of the spermatogenesis in roosters consuming two concentrations of furazolidone (0.011%, prophylactic dose, 0.022%, therapeutical dose). Ten percent of the seminiferous tubules were affected with the first done in a two month experiment and when the therapeutical dose was used for 15 days, 70% of the tubules were affected (5).

With the aim to search the mammalian reproductive physiological changes due to furazolidone, we decided to correlate the fertility, steroidogenic activity and testicular morphology of mice, after the ingestion of low and high concentrations of furazolidone.

MATERIALS AND METHODS

Morphological Studies

One hundred male mice two months old from the NIH strain obtained under barrier conditions were allotted in five groups of twenty animals each in individual cages, allowing 12 hours of light and 12 hours of darkness. A mice balanced diet (Roma, Mexico) pellets were pulverized and micromixed with

Light Microscopic Studies

No modifications in the seminiferous tubules and Leydig cells cellularity were found in furazolidone treated groups at 0.011%, 0.022% and 0.033%. The spermatogenesis process was found to be complete up to mature spermatids in a similar form than controls (Figure 1). Treated groups with 0.066% of furazolidone showed a decreased spermatogenesis within seminiferous tubules with a loss of spermatids and arrest of the spermatogenesis process at primary pachytene spermatocytes. Severe affected tubules (20%) showed abscence of spermatids and several round spaces interpreted as areas where spermatids were located. The remaining germinal epithelium showed only primary spermatocytes with a vacuolated cytoplasm and densely stained nuclei. Both the spermatogonia and Sertoli cells appeared unaltered. Less severe affected seminiferous tubules (60%) disclosed immature spermatids with degenerative changes. Twenty percent showed maturation up to mature spermatids. The intertubular space was filled with Leydig cells, which were normal in number and in appearance.

Electron Microscopy

Control and treated groups with 0.011% and 0.022% of furazolidone showed a normal appearance of the germinal epithelium which can be seen in Figure 1b. With the previous knowledge related with induction of lesions (5), special attention was given to the pachytene primary spermatocytes of 0.033% treated animals. Thirty out of 100 examined pachytene spermatocytes, showed nuclei changes consisting in chromatin condensation irregularities and cytoplasmic vacuoles (Figure 1c). In the control group 15% of all the pachytene spermatocytes studied, showed irregularities at the nuclear membrane. No modifications were found on spermatids, spermatogonia or on Leydig and Sertoli cells (Figure 1d). The group of mice receiving 0.066% of furazolidone, showed drastic changes on the germinal epithelium. Seminiferous tubules in which only primary spermatocytes were present showed cells with dark condensed nuclei and cytoplasm, its identification was difficult (Figure 2b). Primary spermatocytes were found with cytoplasmic membrane separation between them and those of the Sertoli cells. Vacuoles formation and disgregation of nuclei membrane with elongation of the outer nuclear cisterna were frequent cell changes.

Hormone Profile

In all cases androstenedione levels were low and in relation with the furazolidone concentration (Table 1). However, statistical significance using the Student's "t" test was found only in the 0.066% concentration group when compared with controls. An inverse effect was found with dihydrotestosterone increasing its concentration in accordance with the drug dose, but with no significance. Testosterone disclosed no modifications.

Fertility Test

All males of groups 0.011%, 0.022% and 0.033% were able to fertilize females. Offspring were normal in number and in their phenotipics features. Males of group 0.066% were infertile after a month of furazolidone treatment. The females mated with infertile males were fertilized by normal males. After a month of discontinuation of furazolidone recuperated males were fertile again and offspring were also normal.

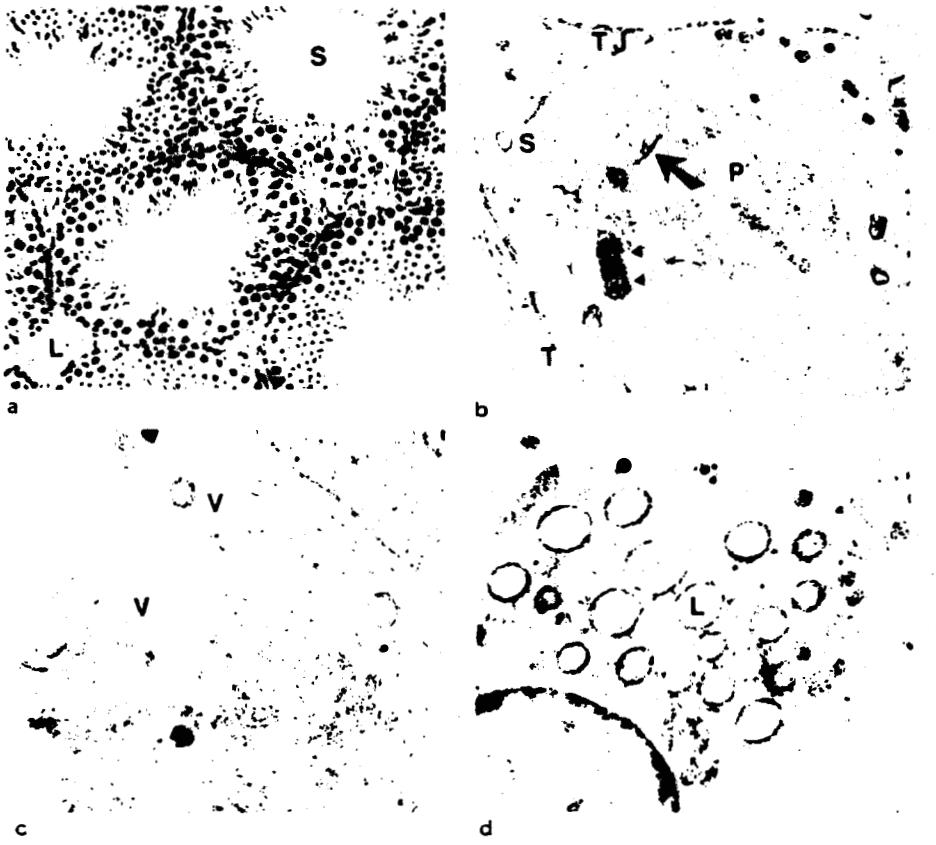

Figure 1 a) Microphotography of 0.033% one month furazolidone treated mice testes. The spermatogenesis is completed in all seminiferous tubules. (S) The Leydig cells formed compact groups in the interstitium (L) Hematoxylin and eosin. 400X b) Electronmicrography of a control not treated mice testes. A pachytene primary spermatocyte (P) is shown close to the seminiferous tubular wall (T) and surrounded by Sertoli cell processes (S). Part of tight junctions (TJ) of the blood testicular barrier can be seen. The nucleus shows synaptonemal complexes and typical chromatin aggregation of pachytenes(arrows) and of the sexual vesicle (double arrows) 8.085X c) Electronmicrograph of a pachytene of a 0.033% furazolidone treated group. The nucleus and the cytoplasm showed multiple vesicles (V) 14.850X d) Electronmicrograph of part of Leydig cells of 0.033% furazolidone treated group. The nucleus shows euchromatin closely associated to the nuclear membrane and a nucleoli. The cytoplasm is filled with smooth and endoplasmic reticulum and within it, lipidi droplets (L) and mitochondria can be seen 14,850X.

Table 1. Steroid metabolic pools after one month of furazolidone ingestion.

Dose	Androstenedione	Testosterone	Dihydrotestosterone
0.011%	7.48 ± 0.87[b]	322.2 ± 19.1	4.25 ± 0.36
0.022%	6.23 ± 0.68	284.6 ± 22.1	3.83 ± 0.10
0.033%	5.16 ± 0.62	336.3 ± 47.8	5.65 ± 1.03
0.066%	3.14 ± 1.04[c]	382.6 ± 82.5	6.85 ± 2.28
Control	8.12 ± 0.99	334.0 ± 30.5	4.92 ± 0.28

a. Picograms/mg of protein b. Mean ± Standard deviation c. p 0.05 Student "t" test

Figure 2. a) Microphotography of 0.066% one month furazolidone treated mice testes severely by affected seminiferous tubules. (A) showed to be atrophic remaining only damaged primary spermatocytes (arrow) and Sertoli cells with cytoplasmic processes, demarcating spaces of lost germinal cells. (double arrows). Other seminiferous tubules (B) are less affected. Leydig cells appear normal in a wide spaced instersitium. Hematoxylin and eosin. 400X b) Electronmicrograph of a presumably primary severe affected spermatocyte of 0.066% furazolidone treated group. Note unclear chromatin condensation, the formation of a large vesicle in the nuclear membrane (arrow). No cytoplasmic organelles are discernible, and not any more identifiable cytoplasmic organelles. Sertoli cell processes (S) surround the affected cell. 8.085X

DISCUSSION

Although, furazolidone is poorly absorbed by the digestive tract (16) the small proportion absorbed is rapidly metabolized by several organs (17). Damage to the testes due to furazolidone ingestion has been well documented in Guinea pigs (11) in which decreased spermatogenesis was reported after two months of 0.066% in the feed. Similar testicular changes were demonstrated in the rat with 0.044% of furazolidone in the diet (12). All mice with the highest dose of furazolidone showed the majority of the seminiferous tubules (60%) with decreased mature spermatids, having as a consequence infertile males after a month of the drug ingestion. This effect was reversible since animals allowed to recuperation for one month, all were fertile again. Except for a minimal decrease in body weight, no side effects were detected nor were there any gross alterations.

The modifications of pachytene primary spermatocytes in group 0.033% may well represent early changes in the nuclei due to furazolidone medication. Nevertheless, controls showed irregularities of the nuclei membrane in a 15% of the studied pachytene spermatocytes. Pachytene stage of the meiotic differentiation process is a labile step in which structural changes may be detected. The mechanism of action of furans in the testes is the inhibition of acetil coenzyme A (19) which renders the primary spermatocytes with no significant energy for completing the meiotic process of differentiation. Other proposed mechanisms are those concerned with inhibition of the synthesis of DNA (8, 15) which have the primary spermatocytes as target cells.

Testosterone hormone values showed no changes in any group. This is in accordance with morphological studies in which Leydig cells appear normal in number and in their ultrastructural features. Decreased testicular size in group 0.066% is only produced by the loss of mature germinal cells remaining a certain number of primary spermatocytes with the capacity to continue their normal maturation steps. The androstenedione decrease could be related with its small metabolic pool size and since the testosterone pool is more important than the androstenedione one, the metabolism addressed the intermediary metabolic pools in order to maintain the final product concentration, as it was observed previously in rats on diets containing cottonseed flour as a source of gossypol (7).

The toxic effects of furazolidone treated guinea pigs and rats with up to 1.0% induces cellular damage in the gastric mucosa and cortical adrenal cells. At present morphological studies are in process in order to determine whether the 0.066% dose induces in these and other organ changes in mice. The possibility to regulate male fertility using furazolidone as a selective drug that induces a reversible decrease in spermatogenesis seems to be promissory. Nevertheless, the Food and Drug Administration of the United States of America limited the use of furazolidone in hens, probably due to its carcinogenic potential of some related furans. Furazolidone in concentrations of 15 mg per day for 4 months increases with no statistical significance, the induction of fibroadenoma in rats (3, 13). However, other authors using lower concentrations failed to produce such changes (1,4,10).

Furazolidone decreased the spermatogenesis since 0.066% supplemented diet and in lower doses produced mild damage in the germinal cells. The testicular endocrine function only with the high dose presented some alterations. Its potential use as a male contraceptive deserve further research in animals as well as in man.

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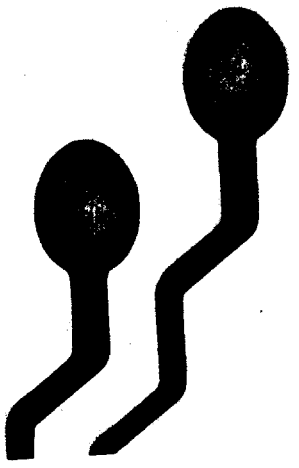

LOW PLASMA ZINC AND ANDROGEN IN INSULIN-DEPENDENT DIABETES MELLITUS

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Plasma zinc and pituitary and testicular hormone concentrations were measured in two groups of male adolescents. One group comprised insulin-dependent diabetes mellitus patients, aged 14–19 years; the other, as control, included 12 healthy youngsters aged 13–19 years. Plasma concentration of zinc, prolactin, testosterone, and dihydrotestosterone were lower in diabetics than in controls, whereas the ratios of androstenedione and androsterone to testosterone + dihydrogestosterone were higher. Plasma FSH and LH were normal. These results suggest a diminished conversion of androstenedione to testosterone and relate zinc with the 17-β-hydroxysteroid dehydrogenase enzyme activity.

Key Words: Testicular androgens; Type I diabetes mellitus.

INTRODUCTION

Abnormal androgen levels have been reported in patients with insulin-dependent diabetes mellitus (IDDM), namely diminished plasmatic concentration of testosterone (T) and dihydrotestosterone (DHT). Nevertheless, normal plasma luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were found combined with normal or abnormal pituitary reserve [3, 4, 8, 17]. Others have reported that plasma zinc concentrations are frequently low in diabetics [6, 15]. Recently, a correlation between plasma Zn and androgen levels in uremic patients has been described [13]. The aim of this study was to correlate the plasma Zn, pertinent pituitary hormones, and testicular androgen concentrations in a group of adolescents with IDDM.

MATERIALS AND METHODS

Nine young males aged 14–19 years suffering IDDM without evidence of renal damage with 5.4 ± 3.3 years of duration whose sexual development was rated Tanner V. The control group consisted of 12 healthy adolescents with comparable age and sexual development. Heparinized blood samples were

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taken in fasting conditions; the plasma was separated and stored at −20°C until assay. On each plasma sample the following determinations were carried out: Zn [7]; FSH, LH, and prolactin (PRL) using commercially available kits (International CIS-SORIN, France). The results were expressed in ng/ml LEH 907 for FSH and LH and in ng/ml MRC 75/504 for PRL. A, T, and DHT were measured by radioimmunoassays previously described [10, 11]. The results are plotted as mean ± SEM. Differences between groups were determined by the Student's T-test for unrelated samples, and correlation coefficients were determined by the least-squares approach [16].

RESULTS

The individual values and the mean ± SEM from control and IDDM patients are shown in Fig. 1. Patients with IDDM had lower plasma ZN concentrations than controls (73.49 ± 19.31 and 112.46 ± 24.74 μg/ml, respectively; $p < 0.001$). No differences were found in FSH and LH. PRL concentrations were lower in IDDM than in controls (2.75 ± 0.97 and 4.67 ± 2.11 ng/ml, respectively; $p < 0.025$). Testosterone (3.32 ± 1.48 ng/ml) and DHT (0.24 ± 0.13 ng/ml) levels were also lower in IDDM patients than in controls (8.56 ± 1.28 ng/ml, $p < 0.001$ for T, and 0.38 ± 0.15 ng/ml, $p < 0.05$ for DHT). On the other hand, androstenedione in the IDDM group was slightly greater than in controls, but

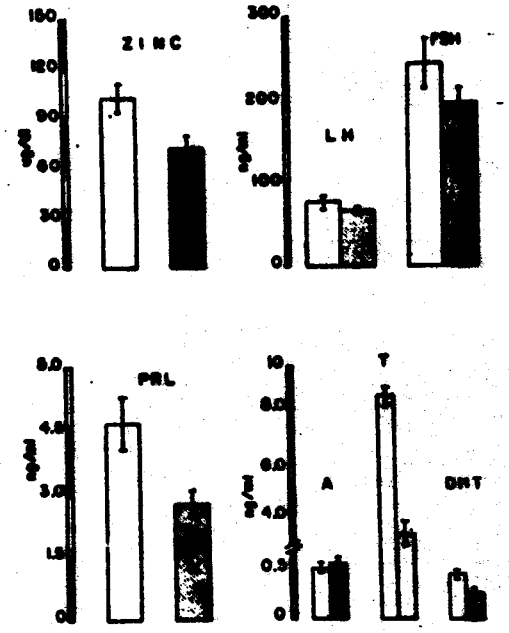

FIGURE 1 Zinc, pituitary hormones, and testicular androgens in diabetics (hatched bars) and controls (open bars). The bars represent the mean ± SEM.

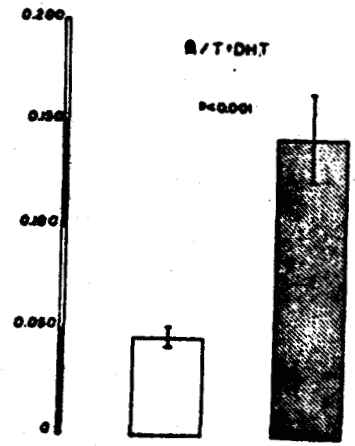

FIGURE 2 Comparison between controls (open bar) and diabetics (hatched bar) in the A/T + DHT ratio.

the difference was not significant (0.551 + 0.123 and 0.448 + 0.138 ng/ml, respectively). The difference in plasma A was evident when expressed as the A/T+DHT ratio, being higher in the diabetic group than in controls (0.144 ± 0.073 and 0.046 ± 0.017 ng/ml, respectively; $p < 0.001$) (Fig. 2).

DISCUSSION

Decreased plasma testosterone has been correlated with diminished libido and impotence in diabetic patients [1, 9]. Some reports in adult rats have suggested a primary testicular disorder as the cause of these alterations [5]. In patients suffering chronic renal failure, an impaired conversion of A to T secondary to low plasma Zn has been reported [13].

The present results indicate low plasma Zn and PRL values in young diabetics associated with a hormonal pattern characterized by normal plasma A and diminished T and DHT, suggesting a reduced conversion of A to T. Although absolute values of A were not significantly different, the A/T+DHT ratio was far higher in the diabetic group, supporting such steroid biosynthesis alteration. Low Zn concentrations in plasma have been previously associated with 17α-hydroxysteroid dehydrogenase (17α-OHSD) enzyme activity [13, 14]. It may be postulated with these results that Zn plays an important role in androgen biosynthesis in diabetic young males, as a metalloenzyme, activator, or protective agent giving support to the membranal enzymes, which is the case for the 17α-OHSD [2].

In addition, it was demonstrated in dwarf mice that PRL administered SC increases the 17α-OHSD enzyme activity [12]. In this regard the low PRL values could be associated with the low T concentration and consequently with the DHT diminution.

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A New Inherited Variant of the 3β-Hydroxysteroid Dehydrogenase-Isomerase Deficiency Syndrome: Evidence for the Existence of Two Isoenzymes*

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ABSTRACT. The clinical and endocrine features of a unique form of adrenal insufficiency secondary to an inherited deficiency of 3β-hydroxysteroid dehydrogenase-isomerase (3-HSD) were studied. The propositus was a 19-yr-old man with a history of repeated episodes of acute adrenal crisis. Family study disclosed that a 6-yr-old female sibling also was affected, and a third sibling had died during the course of an adrenal crisis. The diagnosis of adrenal insufficiency was established on the basis of extremely low serum cortisol levels and urinary 17-hydroxycorticosteroid excretion with concomitantly elevated serum ACTH levels and lack of cortisol response to ACTH administration. Impairment of C-21 steroid 3-HSD activity was strongly suggested by persistenty elevated serum 17-hydroxypregnenolone to 17-hydroxyprogesterone and pregnenolone to progesterone ratios, their significant increase after ACTH administration, and their return to normal during cortisol therapy in both patients. Nevertheless, the serum dehydroepiandrosterone to androstenedione ratio, both basally and after ACTH and/or hCG stimulation, was normal. These findings coupled with the normal phenotypic development and onset of puberty in the two patients indicated intact C-19 steroid 3-HSD activity. The overall results indicate an inherited impairment of 3-HSD activity confined only to C-21 steroid substrates and, thus, suggest the existence of at least two 3-HSD isoenzymes under independent genetic regulation. (*J Clin Endocrinol Metab* 63: 360, 1986)

SINCE its original description in 1961 (1), deficiency of 3β-hydroxysteroid dehydrogenase-isomerase (3-HSD) has been recognized as the underlying abnormality of one form of congenital adrenal hyperplasia (2–8). The classical form of this entity included the association of salt-losing adrenal insufficiency and ambiguity of external genitalia in both sexes (1–3, 5, 6, 9–11). However, a number of clinical variants have been reported, indicating wide clinical heterogeneity. Indeed, both nonsalt-losing (2, 7, 8) and mild salt-losing (5, 12) varieties have been described in kindreds with well documented 3-HSD impairment.

Abnormal development of external genitalia has been reported in all affected males (1–3, 5–8, 10, 11), and inadequate endocrine and gametogenic testicular function was found in those patients who survived until puberty (6, 10, 11, 13–15). Congenital or postnatal virilization of external genitalia has occurred in affected females (1, 2, 5, 7, 9, 15), with the exception of the patient reported by Zachmann *et al.* (4, 16), in whom normal genitalia development coexisted with the 3-HSD defect.

This report describes the clinical and endocrine features of two siblings with a unique type of congenital adrenal hyperplasia secondary to 3-HSD deficiency. The study of adrenal and gonadal steroid dynamics disclosed that 3-HSD activity for C-21 steroids was impaired, while 3-HSD activity for C-19 steroids was not, implying the existence of at least two substrate-specific 3-HSD isoenzymes with different genetic control in humans.

Case Reports

Patient A

The propositus (Fig. 1, III-3), a 19-yr-old phenotypic man, was referred to our Metabolic Research Unit (MRU) because

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† Postdoctoral Fellow in Reproductive Biology, supported by a PUIC-UNAM fellowship award.

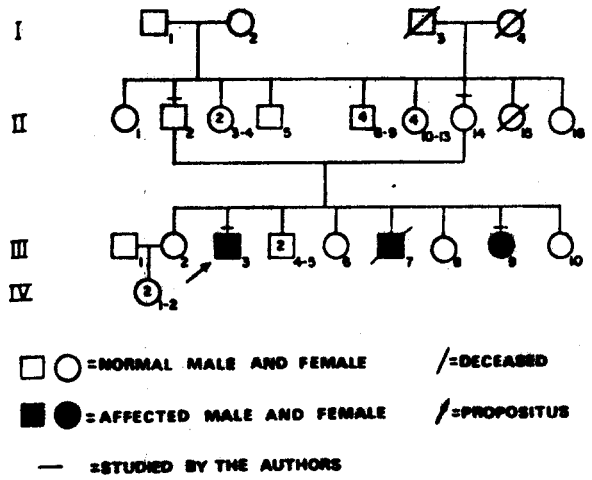

FIG. 1. Pedigree of the family with the 3-HSD deficiency syndrome. The inheritance pattern is compatible with an autosomal recessive trait.

of repeated episodes of acute adrenal crisis. He was born by normal delivery after a full-term uneventful pregnancy. At the age of 5 yr, the diagnosis of adrenal insufficiency was made on the basis of severe dehydratation, persistent hyponatremia, and hyperkalemia, as well as undetectable urinary 17-hydroxycorticosteroid (17-OHCS) excretion both basally and after im administration of ACTH (40 U/day for 4 days). Urinary 17-ketosteroid (17-KS) excretion was normal. Thereafter, he was treated with gluco- and mineralocorticoid replacement therapy until age 13 yr, when medication was withdrawn for unknown reasons. Afterward, his physical and intellectual capabilities decreased, but, nevertheless, signs of pubertal development were noticed when he was 15 yr old. At the age of 19 yr, he was admitted to the MRU. Physical examination revealed a normal upper to lower segment ratio, height of 171 cm, and body weight of 54 kg. Blood pressure was persistently low (80/50 mm Hg). External genitalia were normal. Nonerectile penis length was 7.5 cm. Testicular volume was normal (15 cm^3), calculated according to an ellipsoid model (volume = $\pi/6 \times$ length $\times$ width^2). Pubic and axillary hair also were normal, and there was no gynecomastia. Routine laboratory studies were normal, except that his serum Na^+ level was persistently low (130–134 meq/liter), and serum K^+ was elevated(5.8–6.8 meq/liter). Semen analysis was normal (18.6×10^7 spermatozoa/ml). After completion of the study, therapy with hydrocortisone (30 mg/day) was begun. Normal body weight and blood pressure as well as a marked improvement in his general condition were evident 6 months later.

Patient B (Fig. 1, III-9)

A 6-yr-old female sibling of the propositus was admitted for endocrine evaluation. She was delivered at term after a normal pregnancy. The external genitalia were normal at birth, as was her psychomotor development. At age 3 yr, signs and symptoms suggestive of mild adrenal insufficiency as well as mucocutaneous hyperpigmentation appeared, and poor adrenal reserve was documented by exogenous ACTH stimulation. However, she was advised to take glucocorticoid therapy only during acute illnesses. When hospitalized at age 6 yr, physical examination revealed normal body proportions. Her height was 111 cm, weight was 19 kg (10th percentile), and vital signs were normal. Her skin and mucous membranes were hyperpigmented. No breast development or axillary or pubic hair were present. Gynecological examination revealed normal prepubertal external genitalia. Routine laboratory studies were normal. Impairment of cortisol production was confirmed. She was treated with glucocorticoid replacement therapy (20 mg cortisol/day). During this treatment she continued to grow along the 10th percentile, as shown in Fig. 2. At the age of 12 yr, her endocrine function was reassessed (see *Results*). At this time, physical examination was normal, and x-ray films revealed a normal bone age. Recently, early signs of pubertal development appeared (13 yr old).

Family history

There was no family history of consanguinity. The parents (Fig. 1, II-2 and II-14) are normal; neither has clinical or

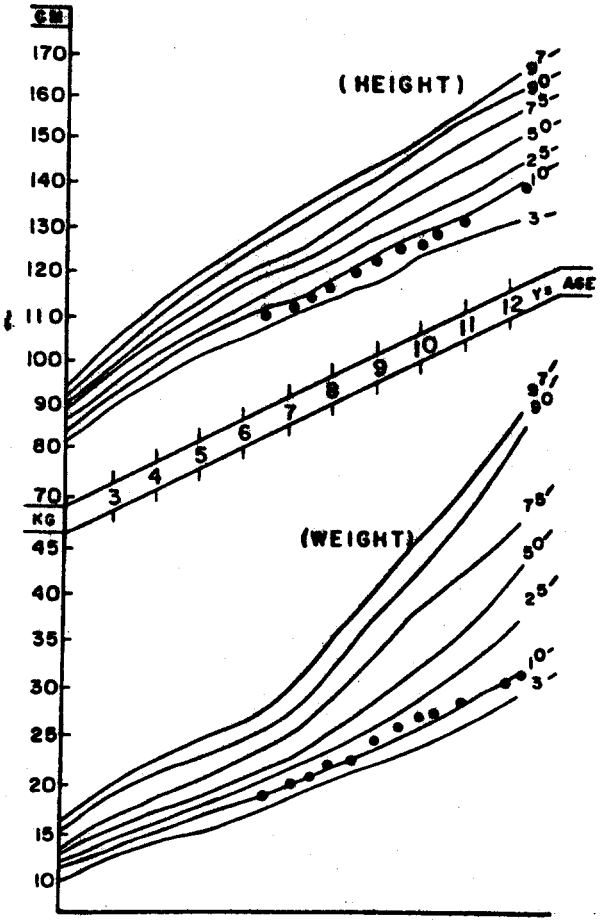

FIG. 2. Height and weight curves for patient B during a 6-yr period.

biochemical features suggestive of late onset or cryptogenic forms of adrenal enzyme deficiency. A third member of the sibship (Fig. 1, III-7) died of adrenal crisis before the family was studied, but he is believed to have been affected with a similar disorder on the basis of history of adrenal insufficiency and normal male phenotypic development.

Materials and Methods

Serum LH and FSH levels were measured by specific RIAs (17) using reagents kindly provided by the WHO Matched Reagent Program. The results were expressed as milliinternational units per ml Second International Reference Preparation of human menopausal gonadotropin. Serum ACTH was measured by RIA using a commercial kit (Diagnostic Products Corp., Los Angeles, CA). Serum pregnenolone (Δ^5-Preg), progesterone (Δ^4-P), 17-hydroxyprogesterone (17-OHP), 17-hydroxypregnenolone (Δ^5-17-Preg), dehydroepiandrosterone (DHA), androstenedione (Δ^4-A), testosterone (T), and 17β-estradiol (E_2) were determined by RIA after chromatographic purification steps using either silica gel thin layer plates or Celite columns, as previously described (18–20). Serum cortisol was measured by direct RIA without chromatographic purification (21). The high specificity of these steroid assays was reported previously (20–22). Urinary aldosterone was measured using a commercial RIA kit (Sorin Biomedica, Milan, Italy). Inter- and intraassay coefficients of variation for all serum and urinary steroid RIAs were less that 15% and 10%, respectively. Urinary 17-OHCS, 17-KS, pregnanediol (P_2), and pregnanetriol (P_3) excretion was measured by colorimetric methods. PRA was measured by the method of Sealey and Laragh (23). Serum and urinary Na^+ and K^+ levels were determined with a flame photometer.

Adrenal and gonadal steroid dynamics

The protocol for endocrine studies was approved by the Institute Ethical Committee, and informed consent was obtained from the patients' parents.

ACTH stimulation tests were conducted in the two siblings by the iv infusion of 0.25 mg synthetic ACTH (Synacthen, Ciba-Geigy, Basel, Switzerland) for 480 min and by daily im administration of 40 U synthetic ACTH (Acthar-Gel, Grossman; Mexico City, Mexico) for 4 consecutive days. Blood samples were drawn before, during, and after ACTH administration at frequent or daily intervals. Serum samples were kept frozen at −20 C until assayed. Baseline and ACTH-stimulated 24-h urine samples were collected. Δ^5-Steroid precursors and their corresponding Δ^4-steroid products were measured in all serum samples, and the urinary steroid metabolite concentrations also were determined. A gonadal stimulation test during cortisol therapy was performed in patient A by daily im administration of 5000 IU hCG (Pregnyl, Organon Mexicana, Mexico City, Mexico) for 4 consecutive days. PRA and urinary aldosterone and electrolytes were measured while the patients consumed a normal Na^+-containing diet (100 meq/day). Samples for PRA determination were obtained after 4 h of walking.

Results

Adrenal studies

Patient A. Baseline serum cortisol levels (normal, 70–220 ng/ml) and urinary 17-OHCS excretion (normal, 1.7–5.1 mg/24 h) were below the sensitivity limits of the assays (cortisol, 10 ng/ml; 17-OHCS, 0.7 mg/24 h), whereas serum ACTH was 250 pg/ml (normal, 10–100 pg/ml). The serum electrolyte profile revealed severe hyponatremia with concomitant hyperkalemia. Serum cortisol and urinary 17-OHCS did not become measurable after exogenous iv and im ACTH administration.

The basal serum Δ^5-17-Preg concentration was elevated (15.3 ng/ml), whereas 17-OHP, its Δ^4-3-keto corresponding product, was undetectable (Fig. 3). Administration of im ACTH resulted in a significant increase in serum Δ^5-17-Preg, but serum 17-OHP did not increase. Cortisol treatment induced a dramatic decrease in serum Δ^5-17-Preg to below 0.2 ng/ml. The basal level of another C-21 steroid possessing the Δ^5-3β-OH configuration, Δ^5-Preg, was within the normal male range (2.6 ng/ml), whereas the concentration of its corresponding Δ^4-3-keto product (Δ^4-P) was low (0.3 ng/ml), as shown in Fig. 3. The abnormally high Δ^5-Preg to Δ^4-P ratio further increased after iv and im administration of ACTH, as shown in Table 1. Serum Δ^5-Preg decreased significantly (62%) during cortisol treatment. Analysis of baseline and ACTH-stimulated urine samples revealed that both P_2 and P_3 were below 1 mg/24 h (normal P_2 and P_3, <1 mg/24 h).

Basal serum DHA and Δ^4-A levels were less than 1 ng/ml and did not increase after either iv or im ACTH (Fig. 3). Furthermore, urinary 17-KS excretion was within the normal range (6 mg/24 h), but did not increase after ACTH treatment. The finding of elevated serum Δ^5-17-Preg with concomitantly low serum DHA could be explained on the basis of 17–20 desmolase inhibition induced by the high Δ^5-17-Preg concentrations, as previously demonstrated in *in vitro* studies (24).

During a normal Na^+ intake diet, urinary sodium excretion was 200 meq/24 h, and PRA was above 30 ng/ml·h (normal, 0.3–3.3 ng/ml·h). Urinary aldosterone was undetectable (normal, 2–16 pg/24 h).

Patient B. At the time of her first admission (age 6 yr), the basal serum cortisol level (48 ng/ml) and urinary 17-OHCS excretion (<1 mg/24 h) were both subnormal, while serum ACTH was markedly elevated (249 pg/ml). Exogenous im ACTH administration did not induce any significant increase in serum cortisol or 24-h urinary 17-OHCS excretion. The basal serum Δ^5-17-Preg levels were slightly elevated, whereas its corresponding Δ^4-3-keto compound (17-OHP) was undetectable (Fig. 4A). The im ACTH administration increased serum Δ^5-17-Preg to 4.2

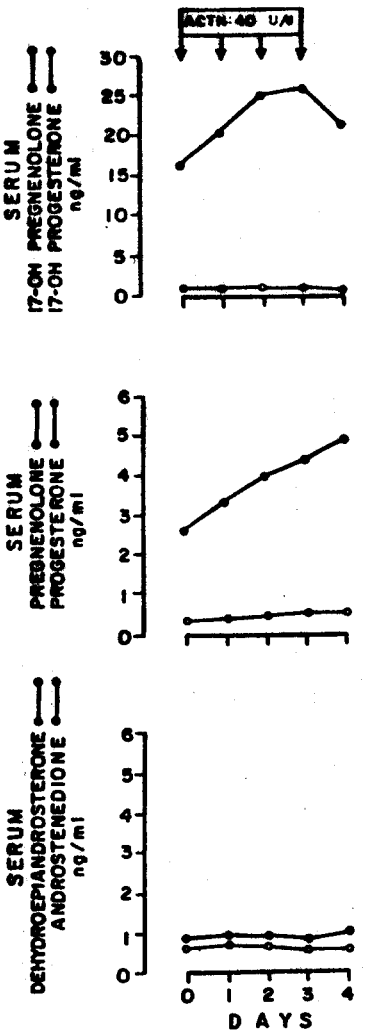

FIG. 3. Steroid concentrations before and during im ACTH stimulation for 4 consecutive days in a postpubertal man with 3-HSD deficiency (patient A). Normal basal values (mean ± SD) for men (n = 10), expressed in nanograms per ml, are: Δ^5-17-Preg, 5.5 ± 2; Δ^5-Preg, 2.6 ± 0.7; DHA, 3.7 ± 0.5; 17-OHP, 1.2 ± 0.5; Δ^4-P, 1.18 ± 0.4; and Δ^4-A, 0.6 ± 0.2.

ng/ml, but 17-OHP did not increase, resulting in a further increase in the Δ^5-17-Preg to 17-OHP serum ratio. Basal serum Δ^5-Preg and Δ^4-P concentrations were within the normal limits for the subject's age; however, the Δ^5-Preg to Δ^4-P ratio was elevated in both basal and ACTH-stimulated samples (Fig. 4A and Table 1). Basal urinary P_2 and P_3 excretion was below 1 mg/24 h and did not increase during ACTH stimulation. Serum DHA and Δ^4-A were both normal (<1 ng/ml) for age. ACTH stimulation resulted in a slight increase in DHA to 2.5 ng/ml, but no change in Δ^4-A, as has been reported in prepubertal girls (7, 25) (Fig. 4A). Her adrenal status was reassessed 6 yr later (age 13 yr). After glucocorticoid withdrawal, basal and ACTH-stimulated serum cortisol levels and urinary 17-OHCS excretion were low, as at the time of her first admission. On this occasion, the serum Δ^5-17-Preg level was higher (12.7 ng/ml) than previously encountered, whereas the levels of its corresponding Δ^4-3-keto steroid (17-OHP) was very low (0.4 ng/ml), leading to a Δ^5-17-Preg to 17-OHP ratio of 31.7 (Fig. 4B and Table 1). Furthermore, iv ACTH infusion resulted in a tremendous increase in the serum Δ^5-17-Preg to 17-OHP and Δ^5-Preg to Δ^4-P ratios (86.8 and 9.0, respectively), as shown in Fig. 4B and Table 1. Baseline 24-h urinary excretion of P_2 and P_3 was extremely low.

Serum DHA levels were slightly higher than at the time of the subject's first admission (2.8 ng/ml), but normal for her age (25), whereas Δ^4-A remained low, thus resulting in a normal to high serum DHA to Δ^4-A ratio (11.2). This ratio increased (39.5) during the iv ACTH infusion, as shown in Fig. 4B and Table 1.

During a normal Na^- diet, urinary sodium excretion was 72 meq/24 h, and urinary aldosterone was 3.2 pg/24 h (normal, 10–27 pg/24 h). PRA was 28 ng/ml·h (normal, 1.5–5.7 ng/ml·h).

Gonadal studies

Patient A. During glucocorticoid therapy, the serum concentrations of adrenal steroids declined considerably (Fig. 5), and serum LH, FSH, and T levels were normal. Serum T (4 ng/ml) increased 2.5-fold during hCG administration (Fig. 5). Serum Δ^5-Preg and Δ^5-17-Preg also increased during hCG stimulation, whereas Δ^4-P and 17-OHP did not, leading to a Δ^5-Preg to Δ^4-P ratio of 1.7 and a Δ^5-17-Preg to 17-OHP ratio of 6.6. The hCG-induced rises in serum DHA and Δ^4-A were normal (as indicated in Fig. 5), and the DHA to Δ^4-A ratio was 1.

Patient B. Serum LH, FSH, and E_2 were within the normal prepubertal range when the subject was 6 and 12 yr, before signs of puberty appeared. Recently, detectable levels of E_2 (35 pg/ml) were found.

Discussion

This report describes the clinical and endocrine features of a new familial form of congenital adrenal hyperplasia secondary to 3-HSD deficiency. The diagnosis of adrenal insufficiency in the two siblings was unequivocally established on the basis of extremely low or undetectable serum cortisol levels and urinary 17-OHCS excretion, concomitantly elevated serum ACTH, and unresponsiveness of cortisol to exogenous ACTH stimula-

TABLE 1. Baseline and ACTH-stimulated serum Δ^5-17-Preg to 17-OHP, Δ^5-Preg to Δ^4-P, and DHA to Δ^4-A ratios in two patients with inherited 3-HSD deficiency and in normal subjects

	Δ^5-17-Preg:17-OHP		Δ^5-Preg:Δ^4-P		DHA:Δ^4-A	
	Baseline	ACTH	Baseline	ACTH	Baseline	ACTH
Patient A	—*	—*	8.6	9.6	0.9	0.6
Normal men (n = 5)						
Mean	2.09	5.2	3.33	3.0	6.2	6.4
Range	0.57–3.19	1.4–7.0	0.77–5.37	1.8–6.3	3.2–12	4.0–12
Patient B						
6 yr old	—*	—*	5.0	4.8	—*	—*
12 yr old	31.7	86.8	2.0	9.0	11.2	39.5
Normal prepubertal children[b]						
Mean	1.75	2.9	0.67	1.68	2.9	2.7
Range	0.33–7.7	0.78–6.7	0.3–1.6	0.7–4.6	0.6–13	0.35–7.3

* Noncalculable ratio because Δ^4-steroid concentrations were undetectable.
[b] From Pang *et al.* (7).

tion. In addition, both patients had episodes of adrenal failure with clinical and biochemical signs of salt losing, including low urinary aldosterone excretion and elevated PRA levels. These data indicated an inherited adrenal defect in the biosynthetic pathways of gluco- and mineralocorticoid production.

The finding of significantly elevated basal serum Δ^5-17-Preg levels with undetectable 17-OHP levels, leading to an abnormal serum Δ^5- to Δ^4-steroid ratio, and its striking increase after ACTH administration in both patients strongly suggested 3-HSD impairment, at least for C-21 steroids. This observation was in line with the finding of persistently low urinary P_3 excretion. Further evidence for the 3-HSD defect was provided by the finding of an abnormally high serum Δ^5-Preg to Δ^4-P ratio, both basally and after ACTH treatment, with a concomitant low urinary P_2 excretion. The demonstration that the 3-HSD defect was more evident in the 17-hydroxysteroid as compared with the 17-deoxysteroid pathway in this family confirms previous observations (6, 7, 16) which indicated that increased serum Δ^5-17-Preg was the most reliable index for diagnosing 3-HSD deficiency, even in the late-onset form of the syndrome (15, 26, 27). This situation could be the result of either a variable degree of enzyme impairment or different enzyme affinity for Δ^5-steroid substrates (8). The possibility that the high serum Δ^5-17-Preg levels may inhibit the activity of the 17–20 desmolase, as documented under *in vitro* conditions (24), has been proposed as an alternate explanation (7).

Since excretion of the urinary reduced metabolites of 17-OHP (P_3 glucuronide) has been found to be either normal or elevated in a number of patients with the 3-HSD defect, the existence of a peripheral enzyme with different genetic regulation has been suggested (1, 2, 4–7, 10, 11, 15). However very little, if any, urinary P_3 excretion was found in the two siblings studied. Whether this finding indicates enzyme impairment at both endocrine and nonendocrine organ levels is not known.

The most striking clinical finding in these patients was the occurrence of normal external genital development. Indeed, a normal male phenotype was found in the two affected boys, and the affected girl had a female phenotype and no signs of postnatal virilization. These patients differ from most patients with 3-HSD deficiency (15), because the enzyme impairment is usually, if not always, associated with external genital ambiguity in males and prenatal or postnatal virilization in females. Since abnormal development of the male embryo is the result of either inadequate androgen synthesis or defective androgen action (28), it would be expected that individuals affected with 3-HSD deficiency with concomitant impaired fetal T production would have a variable degree of genital ambiguity. Furthermore, administration of 3β-HSD inhibitors to pregnant rats causes adrenal cortical hyperplasia in the fetus, incomplete masculinization of external genitalia in the male offspring, and clitoral hypertrophy in the females (29, 30). The slight degree of genital virilization found in affected girls has been attributed to the accumulation of Δ^5-3β-OH C-19 steroids with weak androgenic potency, particularly DHA, as the result of the enzyme defect (28, 29).

The normal phenotypic development in our patients with well documented 3-HSD deficiency, at least for C-21 steroids, raises the intriguing question as to whether the enzyme defect is confined to the adrenal gland with a normally preserved gonadal enzyme, or if it is the reflection of a gene mutation affecting solely 3-HSD activity for C-21 steroid substrates, with normally intact 3-HSD activity for C-19 steroids. To delineate the nature

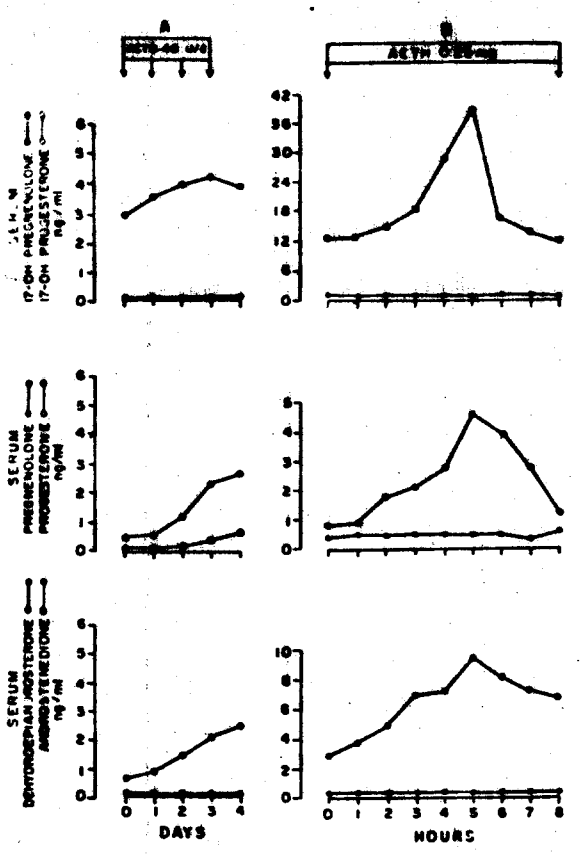

FIG. 4. Steroid levels before and during ACTH stimulation in patient B at age 6 yr (A) and 12 yr (B). Normal baseline values (mean ± SD) from prepubertal children, aged 6–12 yr, expressed in nanograms per ml [from Pang *et al.* (7)] are: Δ^5-17-Preg, 0.81 ± 0.81; Δ^5-Preg, 0.25 ± 0.26; DHA, 0.64 ± 0.69; 17-OHP, 0.63 ± 0.33; Δ^4-P, 0.21 ± 0.15; and Δ^4-A, 0.27 ± 0.31.

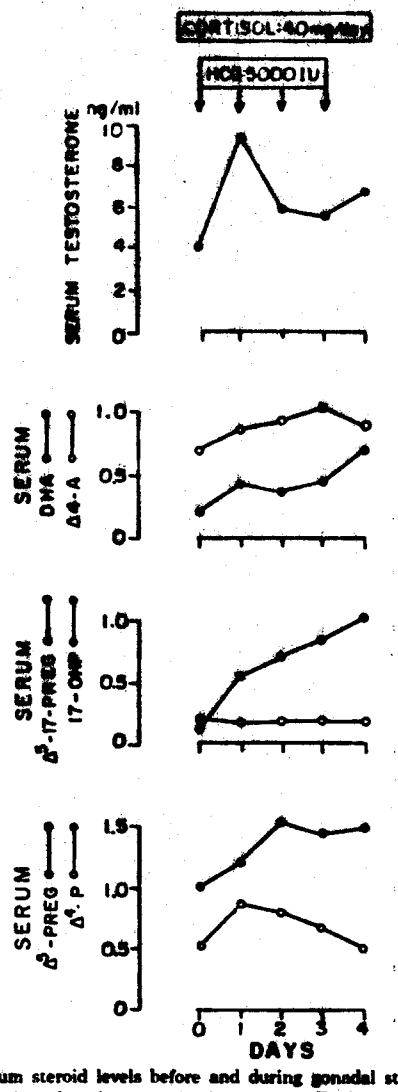

FIG. 5. Serum steroid levels before and during gonadal stimulation with hCG during adrenal suppression in patient A. For baseline normal values, see Fig. 3 and the text.

of the underlying abnormality in this familial disorder, we examined C-19 steroid dynamics.

Basal serum DHA levels were not elevated in either patient, and ACTH stimulation induced little rise in serum DHA in patient A. The lack of accumulation of this C-19 steroid with the Δ^5-3β-OH structure suggests the integrity of the enzyme-mediated conversion of Δ^5-3β-OH to Δ^4-3-keto configuration for this steroid series in the adrenal gland. In patient B, serum DHA dynamics were slightly different at the two ages studied. Thus, at 6 yr, both basal and stimulated DHA levels were normal. At age 12 yr, the basal DHA level was normal, but it increased (3.3-fold) after ACTH stimulation, leading to a DHA to Δ^4-A ratio that exceeded reference values from prepubertal children (6–12 yr) (7). This observation could suggest a late-onset mild 3-HSD defect for C-19 steroids. However, since a similar enhanced adrenal DHA response has been reported to occur in normal girls throughout early puberty (25), this possibility seems unlikely. In summary, it appears that the adrenal 3-HSD deficiency for C-21 steroids was more severe in patient A and, therefore, resulted in higher circulating Δ^5-17-Preg levels and a concomitantly higher degree of 17–20 desmolase inhibition (24) compared with those in patient B.

Further support for the concept that the 3-HSD deficiency is selective for C-21 steroids was furnished by the finding of normal circulating T levels and a normal testicular response to hCG in terms of Δ^4-A and T in

patient A. Even during adrenal suppression, hCG administration caused abnormally high serum Δ^5-17-Preg to 17-OHP and Δ^5-Preg to Δ^4-P ratios, evidence of the existence of a similar enzyme defect at the gonadal level. Although gonadal function was not assessed in patient B, the early signs of pubertal development (Tanner stage II) and the finding of detectable serum E_2 levels add evidence for an operative pathway for C-19,Δ^4-3-keto steroid formation. Taken together, these data indicate that these patients have adrenal and gonadal 3-HSD deficiency confined to C-21 steroids and excluding C-19 steroids, therefore implying the existence of more than one 3-HSD enzyme in humans.

The occurrence of several 3-HSD variants in other mammalian species has been demonstrated; thus, the nonidentity of the 3β-OH-Δ^5-steroid dehydrogenase (1.1.1.145) and the Δ^5-Δ^4-isomerase (5.3.3.1.), active components of the 3-HSD enzyme system, has been well documented in the bovine adrenal and corpus luteum (31). Furthermore, enzyme purification studies have disclosed the existence of three different substrate-specific Δ^5-Δ^4-isomerases in the bovine adrenal (32–34). Although substrate-specific Δ^5-Δ^4-isomerases have not been identified in the human adrenal gland, a number of studies, particularly in the developing human embryo, provided information suggesting enzyme heterogeneity. Indeed, Serra *et al.* (35) demonstrated deficiency of a Δ^5-Preg-specific 3β-HSD system in microsomal preparations of human fetal adrenals at midgestation, an observation that confirmed that adrenal (36, 37) and testicular (38) 3-HSD activities for various Δ^5-3β-OH steroids do not appear at once during fetal life. Interestingly, inability to convert Δ^5-Preg to Δ^4-P was reported in hyperplastic adrenal tissue (39), which was able to convert Δ^5-17-Preg to 17-OHP, thus giving additional support for the existence of several 3-HSD variants in humans.

Overall, the results presented herein provide evidence of familial deficiency of the 3-HSD system confined to C-21 steroids with preserved 3-HSD activity for C-19 steroids. The abnormality resulted in adrenal insufficiency and normal phenotypic development in affected individuals. These findings suggest independent genetic control for the two enzyme activities.

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Normal ovarian function in a mild form of late-onset 3β-hydroxysteroid dehydrogenase deficiency

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Late-onset 3β-hydroxysteroid dehydrogenase (HSD) deficiency was diagnosed in a 30-year-old woman with hirsutism and normal menstrual cycles. No genital abnormalities were present. Elevated basal serum levels of Δ^5-3β-hydroxysteroids were demonstrated. Serum pregnenolone (P_5) was 3.0 ng/ml, and dehydroepiandrosterone sulphate (DHEA-S) 3245 ng/ml. Basal serum levels of Δ^4 steroids were low or within normal limits. Serum progesterone (P) was 0.5 ng/ml, 17α-hydroxyprogesterone (17-OHP) 0.2 ng/ml, androstenedione (Δ^4A) 0.4 ng/ml, and testosterone (T) 0.1 ng/ml. All Δ^5/Δ^4 steroid ratios were elevated. Dexamethasone (DEX) administration normalized the elevated levels of Δ^5-3β-hydroxysteroids, whereas Δ^4-3-ketometabolites exhibited only minor modifications. The DHEA-S/Δ^4A ratio increased more than five times over the basal ratio, and P^5/P and 17α-hydroxypregnenolone (17-OHP_5)/17-OHP ratios did not increase after adrenocorticotrophic hormone ACTH stimulation. Studies of basal ovarian function revealed 17β-estradiol (E_2) and gonadotropins within normal limits according to the menstrual cycle. In the follicular phase, follicle-stimulating hormone (FSH) was 101.3 ng/ml, luteinizing hormone (LH) 46.0 ng/ml, and E_2 49.7 pg/ml; in the luteal phase, FSH was 180.0 ng/ml, LH 69.3 ng/ml, and E_2 50.1 pg/ml. The presence of ovulatory cycles was documented on the basis of the biphasic pattern of the basal body temperature cycles and the increment in P levels. This case demonstrates the existence of normal ovulatory function in a woman with late-onset of a mild form of HSD. Fertil Steril 46:000, 1986

Several studies have demonstrated that 3β-hydroxysteroid dehydrogenase (HSD) deficiency can be manifested during the intrauterine life,[1, 2] childhood,[2] or in the peripubertal stage.[3, 4] When the deficiency is expressed during the intrauterine life, congenital adrenal hyperplasia (salt-losing or not) and ambiguous genitalia are present.[5, 6] In childhood, the deficiency may be the cause of early pubic hair appearance.[2] Late-onset HSD deficiency has been recognized as a cause of hirsutism in women with irregular menses.[4] Affected women have been described with abnormal ovarian function manifested either as irregular menses or primary amenorrhea,[3] and it is frequently associated with polycystic ovaries.[4-7] Axelrod et al.[8] demonstrated the concurrent pres-

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ence of HSD deficiency in both adrenal and ovarian tissues. Rosenfield et al.[3] studied an amenorrheic woman with this enzyme deficiency who failed to ovulate even with glucocorticoid therapy; however, the endocrine abnormalities were normalized when the patient was administered a combination of dexamethasone (DEX) and estrogen-progestin medication, suggesting the coexistence of both adrenal and ovarian enzyme deficiency. This study was undertaken to demonstrate a normal basal ovarian steroid production as well as ovulation in the presence of adrenal HSD deficiency.

CASE REPORT

A 29-year-old woman was referred to the endocrine research unit for clinical evaluation of moderate hirsutism that began 2 years after menarche. At the time of the first interview she had to shave once or twice every two weeks. The parents of this patient were not consanguineous. One sister had moderate hirsutism of pubertal onset, but she already had had two normal pregnancies. The rest of the family history was unremarkable. Breast development appeared at age 12 and progressed normally. Pubic and axillary hair appeared some months later. Subsequently, moderate hirsutism was noticed in the legs, chest, periareolar areas, and face. Her menarche was at 12 years of age, with subsequent regular menstrual cycles. She had never received hormone therapy. Physical examination revealed a height 1.51 m, a weight of 55 kg, and a blood pressure of 120/85. There was neither acne nor hyperpigmented skin. The hirsutism was moderate in the face, periareolar, and midline areas; hair growth was severe on the upper legs. Breast development and external genitalia were considered normal. Pelvic ultrasound examination revealed a normal uterus and mildly enlarged polycystic ovaries. Studies of adrenal and ovarian functions were performed with the consent of the patient and after approval of the human research committee.

MATERIALS AND METHODS

ENDOCRINE STUDIES

Adrenal corticotropic hormone (ACTH) stimulation test and DEX suppression test were performed during the midfollicular phase of a menstrual cycle. The day before ACTH stimulation, 1 mg of DEX was administered orally at 11:00 P.M. Blood samples for hormone measurements were obtained before DEX administration and 9 hours after. At 8:00 A.M., 0.25 mg of ACTH (Cortrosyn, Lab. Organon, West Orange, NJ) was given by intravenous infusion over 180 minutes, and venous blood samples for hormone measurements were obtained every 15 minutes during the time of infusion. Basal serum determinations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), 17β-estradiol (E_2), progesterone (P), and prolactin (PRL) were measured in three blood samples drawn at 15-minute intervals, in both the midfollicular and luteal phase.[5]

HORMONE ASSAYS

Steroid assays included measurements of pregnenolone (P_5), 17α-hydroxypregnenolone (17-OHP_5), dehydroepiandrosterone sulfate (DHEA-S), P, 17α-hydroxyprogesterone (17-OHP), androstenedione (Δ^4A), testosterone (T), E_2, and cortisol. All steroids were determined with the use of radioimmunoassay (RIA) with specific antiserums. Steroid serum extraction and thin-layer chromatography (TLC) studies were done to increase the specificity and accuracy of the assays. Steroid serum extraction were performed with ethylic ether. TLC for isolation of Δ^5-ketometabolites was performed using a double system: (1) benzene/ethyl acetate, 8/2; and (2) benzene/methylic alcohol, 9/1. DHEA-S was measured in its free form after acid hydrolysis; the recoveries obtained were approximately 60%. TLC to purify P and 17-OHP was done with an ethylic ether/benzene, 2/1, system. Δ^4A, T, and E_2 were purified with the use of the following TLC systems: (1) benzene/ethylic acetate, 7/3; and (2) benzene/methylic alcohol, 9/1, with 50% recoveries of the tracer. Cortisol was measured after methylene chloride extraction. Specific antiserums were used for each steroid in a 1/10,000 dilution or higher. Free/bound fractions were separated with dextran-coated charcoal. Serum LH, FSH, and PRL were measured with the use of RIA commercial kits 0.15-v (Sorin, Paris, France). The coefficients of variation interassay and intraassay were < 8% and 5%, respectively, for both steroids and proteic hormones. The results were expressed as ng/ml. Urinary 17-hydroxycorticosteroids (17-OH) and 17-ketosteroids (17-O) were measured with the use of standard colorimetric methods.

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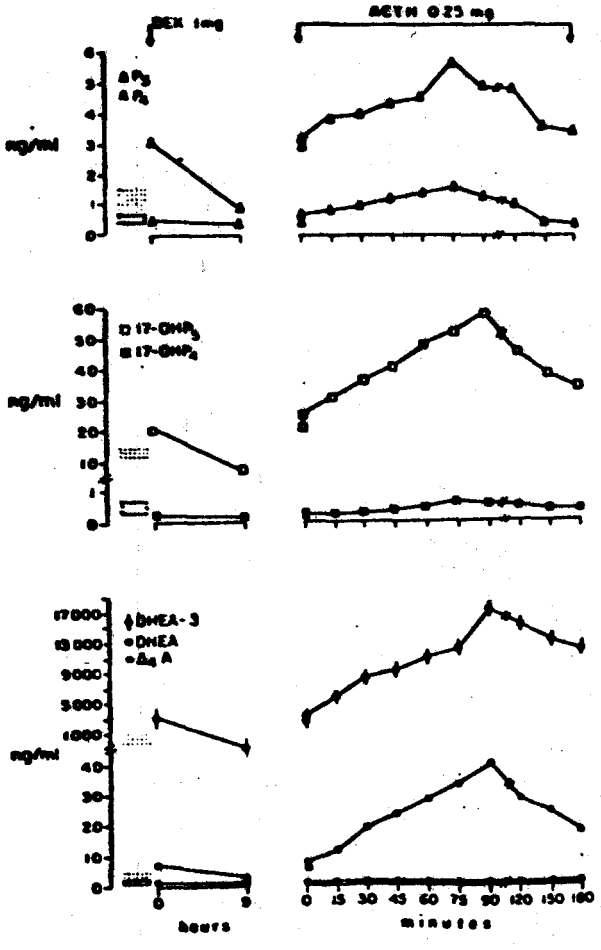

Figure 1
Effect of administration of DEX and ACTH on serum levels of Δ^5 (*open symbols*) and Δ^4 (*closed symbols*) steroids. *Shaded areas* represent normal range of baseline levels of Δ^5 ( ) and Δ^4 ( ) steroids.

TLC plates of silica gel (60^F254 20 × 20 cm and layer thickness of 0.25 mm) were purchased from E. Merck, Darmstadt, FRG. The solvents were analytic reagent (Merck, México City, Mexico) distilled to obtain the highest purity. All other reagents were analytic grade or of the highest purity available. ^{3}H-labeled hormones were purchased from New England Nuclear Corp., Boston, MA. The radiochemicals were assessed with the use of TLC. Nonradioactive steroids were purchased from Steraloids Inc., Pauling, NY and recrystallized before use. Specific antibodies were obtained as previously described[9] with dilutions of 1/10,000 or higher. Dextran T-70 was purchased from Pharmacia Chemicals, Stockholm, Sweden and charcoal from Norit-A, New York, NY.

Table 1. *Serum Basal Levels of Steroids During the Midfollicular Phase*

	Our patient	Normal value
	ng/ml	ng/ml
Δ^5 steroids		
P_5	3.0	1.05 ± 0.3
17-OHP_5	22.6	13.30 ± 5.0
DHEA	7.2	2.75 ± 0.7
DHEA-S	3245.0	953.00 ± 175.0
Δ^4 steroids		
P	0.5	0.57 ± 0.1
17-OHP	0.2	0.48 ± 0.2
Δ^4A	0.4	0.92 ± 0.3
T	0.1	0.21 ± 0.1

RESULTS

Midfollicular phase, mean serum basal levels of Δ^5-3β-hydroxysteroids were elevated as shown in Figure 1 and Table 1. Mean serum basal levels of Δ^4-3-ketosteroids were below normal or in the low normal limits (Table 1). The basal ratios of Δ^5 to Δ^4 steroids, according to Student's *t*-test, were significantly higher ($P < 0.001$) than those observed in normal women in either the follicular or luteal phase (Table 2). DEX administration reduced to normal the levels of P_5 (from 3.01 to 0.85 ng/ml), 17-OHP_5 (from 20.2 to 6.11 ng/ml), DHEA (from 6.92 to 1.34 ng/ml), and DHEA-S (from 3026 to 512 ng/ml) (Fig. 1). P and 17-OHP exhibited a minimum reduction in their levels, whereas Δ^4A values remained unchanged (Fig. 1). ACTH stimulation produced a further increase over the high basal levels of Δ^5-ketometabolites. The maximum increase was observed between 75 and 90 minutes after the infusion was started (Fig. 1). P_5 was raised to 5.7 ng/ml, 17-OHP_5 to 58.1 ng/ml, and DHEA to 39.6 ng/ml. DHEA-S was raised to 17,212 ng/ml and its respective Δ^4-ketometabolites (Δ^4A) remain unchanged, increasing the DHEA-S/Δ^4A ratio five times over the basal ratio. ACTH stimulation raised P to 1.54 ng/ml, and 17-OHP to 0.50 ng/ml. P_5/P and

Table 2. *Ratio of Δ^5 to Δ^4 Steroids in a Patient with Late-Onset HSD Deficiency Studied During the Follicular Phase of a Menstrual Cycle*

Ratio	Our patient	Normal values[10]	
		Midfollicular phase	Luteal phase
	ng/ml	ng/ml	ng/ml
P_5/P	6.08	2.06	0.11
17-OHP_5/17-OHP	113.60	26.12	7.50
DHEA/Δ^4A	18.46	2.41	2.97

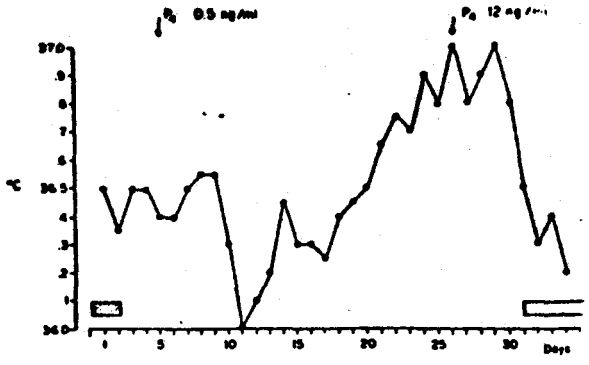

Figure 2
Basal body temperature chart shows the typical biphasic pattern. Serum P level is indicated in this particular menstrual cycle. *Shaded areas* represent menstrual bleeding.

17-OHP$_5$/17-OHP ratios did not increase over their respective basal values. Basal serum cortisol was 164.79 ng/ml (normal, 60 to 250 ng/ml) and showed an increment to 321 ng/ml at 75 minutes of ACTH infusion. Urinary excretion of 17-OH and 17-O were within normal limits.

Ovarian studies in the midfollicular phase showed mean basal serum levels of FSH, LH, PRL, and E_2 of 101.3 ng/ml, 46.0 ng/ml, 10.0 ng/ml, and 49.7 pg/ml, respectively. In the luteal phase, FSH was 180 ng/ml, LH was 69.3 ng/ml, PRL was 12 ng/ml, and E_2 was 50.1 pg/ml. Serum P was 0.5 ng/ml in the midfollicular phase and augmented in the luteal phase to 12 ng/ml. The basal body temperature was biphasic in the five menstrual cycles studied (Fig. 2).

DISCUSSION

In this patient, late-onset adrenal enzyme deficiency was clinically suspected. The diagnosis of HSD deficiency was based on the presence of elevated serum basal levels of Δ^5-3β-hydroxysteroids, low serum basal levels of Δ^4-ketosteroids, and basal Δ^5/Δ^4 steroid ratios significantly higher than those found in normal menstruating women in both the midfollicular and luteal phases. In addition, DEX administration diminished the Δ^5-ketometabolites to normal, suggesting that the adrenal gland was the only source of the abnormality. ACTH stimulation elicited a maximum increase of Δ^5-ketometabolites, which was much higher than that observed in normal and hirsute women[10] as well as in normal men.[11, 12] Despite a slight elevation in basal levels, ACTH stimulation induced the greatest increase in DHEA and DHEA-S levels, without modifications in metabolites Δ^4A values. Therefore, there was an increase over five times that of the basal ratios of DHEA/Δ^4A. On the other hand, P_5/P and 17-OHP$_5$/17-OHP ratios did not increase. These results are similar to those reported by Lobo and Goebelsmann,[10] who demonstrated that the ACTH stimulation produced abnormal values of all Δ^5/Δ^4 steroid ratios in only 33% of patients, who had DHEA-S/Δ^A abnormal ratios. The menstrual regularity in our patient suggested the presence of ovulatory cycles, which was confirmed by typical changes on serum P levels. We conclude that the enzyme deficiency was limited to the adrenal gland and that ovarian function was unaffected. Nevertheless, because ovarian stimulation was not performed, it is not possible to rule out a minor steroidogenic abnormality in ovarian tissue. The HSD deficiency has been reported generally associated with bilateral polycystic ovaries, hirsutism, and oligomenorrhea[3, 4]; however, Pang et al.[4] reported two patients with late-onset HSD deficiency and regular menstrual cycles associated with polycystic ovaries. These latter findings could suggest that the adrenal enzyme deficiency was important enough to produce indirectly some abnormality in gonadotropin secretion, which in turn induced the polycystic ovaries, but some ovulatory function was preserved.[4, 13] In our patient the polycystic ovaries may be the indirect expression of the elevated circulating androgens, which does not necessarily affect the ovarian function. All of these observations support the concept of genetic heterogeneity in congenital HSD deficiency, because such heterogeneity may involve various degrees of enzyme deficiency either in the ovaries or in the adrenal glands.

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