IN VITRO SYNTHESIS OF ANDROGENS BY TESTICULAR TISSUE OF RAT DEFICIENT IN ESSENTIAL FATTY ACIDS

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Summary. Slices of testes from normal rats and rats fed diets deficient in essential fatty acids (EFA) were incubated with [$1\text{-}^{14}\text{C}$]acetate, [$7\alpha\text{-}^3\text{H}$]cholesterol and [$4\text{-}^{14}\text{C}$]progesterone as substrates. Incorporation of activity in testosterone and androstenedione was measured.

With [$1\text{-}^{14}\text{C}$]acetate and [$7\alpha\text{-}^3\text{H}$]cholesterol as substrates there was an approximate doubling in the yield of testosterone and androstenedione in the testes of EFA-deficient rats, but with [$4\text{-}^{14}\text{C}$]progesterone as substrate the differences between groups were not marked. It is postulated that increased biosynthesis of androgens (from acetate and cholesterol substrates) in EFA-deficient animals is perhaps due to structural changes in the mitochondrial wall causing increased permeability of the substrate to the mitochondrial enzymes. No change in the yield of androgens with [$4\text{-}^{14}\text{C}$]progesterone as precursor is perhaps due to non-involvement of mitochondria for synthesis of progesterone→androgens in the rat testis.

INTRODUCTION

Essential fatty acid (EFA) deficiency has been known to result in reproductive failures in both male and female rats (Burr & Burr, 1929, 1930). In females, delayed implantation, prolonged gestation, foetal maceration and resorption were observed (Evans, Lepkovsky & Murphy, 1934a, b). In males, impaired sexual drive and sterility were striking (Evans, Lepkovsky & Murphy, 1934c). Histological examination of the testes of EFA-deficient animals indicated tubular degeneration and spermatogenic arrest, the spermatogenesis having failed to go beyond the secondary spermatocyte stage (Alfin-Slater & Bernick, 1958; Panos & Finerty, 1954); these changes in the testes were reversed following gonadotrophin therapy (Panos, Klein & Finerty, 1959). Microscopic changes in the cellular morphology of the hypophysis similar to those observed in the hypogonadism suggest that testicular androgens were not secreted (Panos & Finerty, 1954; Panos *et al.*, 1959). Similar data describing the changes in the pituitary morphology, testicular morphology and impaired sex drive are reported in the EFA-deficient rabbits (Ahluwalia, Pincus & Holman, 1967).

However, histochemical studies in the rat (unpublished) and in the rabbit (Ahluwalia *et al.*, 1967) indicated the presence of enzymes (Δ^5 -3 β -hydroxy-steroid dehydrogenase, glucose-6-phosphate dehydrogenase) involved in steroid biosynthesis. These studies, therefore, appear to suggest that gonads are capable of secreting androgens; whether these are in sufficient quantities is not known. Because of the histochemical evidence of the presence of enzyme necessary for testicular androgen production, it seemed pertinent to investigate whether androgen production was decreased or whether the target organs were incapable of responding to the circulating androgens (Southren, Ross, Sharma, Gordon, Weingold & Dorfman, 1965; French, Van Wyk, Baggett, Easterling, Talbert, Johnston, Forchielli & Dey, 1966; Wilkins, 1957). In this report androgen production in the testes of EFA-deficient rats was investigated using various exogenous radio-active precursors.

MATERIAL AND METHODS

Thirty-one weanling, male Sprague-Dawley rats were fed a semi-synthetic, fat-deficient diet for periods of up to 100 days. The percentage composition of the experimental diet was as follows: fat-free casein 18.0; sucrose 70.0; cellulose 4.0, and wesson salt 4.0; in addition, the feed mixture contained (mg per kg) vitamin A, 5.0 (20,000 units/g); calciferol, 0.1; α -tocopherol, 10.0; ascorbic acid, 500; calcium pantothenate, 70.0; inositol, 1.32; 2-methyl-1,4-naphthoquinone, 5.0; niacin, 50; *p*-aminobenzoic acid, 500; pyridoxine hydrochloride, 30.0; riboflavin, 30.0; thiamine hydrochloric acid, 73.0; choline, 13.2; folic acid, 11.0; biotin, 9.5; and vitamin B₁₂, 2.0. An equal number of animals was used as a control and fed a commercial diet (Purina Laboratory Chow). The animals were fed and watered freely. All animals were kept on their diet for a minimum of 70 days; at the end of this period, four animals chosen randomly from each group were killed; the testes were excised and stored in 20 ml of physiological normal saline at -20°C until the time of analysis.

Lipid extraction and gas-liquid chromatography (GLC)

The tissues were thawed, homogenized (Omni mixer, Servall), and the lipids extracted with 20 vol. of chloroform-methanol (2:1 v/v) according to the procedure described by Folch, Lees & Sloane Stanley (1957). An aliquot of lipids was transesterified with 5% HCl-methanol and the methyl esters were analysed by GLC. A Gas Chromatograph (Research Specialty) with a hydrogen flame detector was used. Throughout the experiment a U-shaped glass column 6 ft $\times$ $\frac{1}{4}$ in. was packed with 20% ethylene glycol succinate with 2% phosphoric acid on Gas-Chrom P, 80 to 100 mesh. Helium was used as carrier gas at a flow rate of 80 to 90 ml/min. Column temperature was set at 190 to 195 $^\circ\text{C}$. The fatty acid esters were identified by calculated equivalent chain length (Hofstetter, Sen & Holman, 1965) and by comparison of retention times with authentic standards obtained from the Hormel Institute, Austin, Minnesota. The fatty acid esters were quantitated by triangulation of the area under appropriate curves obtained from GLC charts, and the data are reported as area of total fatty acids. Amounts less than 0.1% are expressed as trace.

In vitro incubations

After being fed the fat-free diet for 70 days, the animals were divided into three groups of nine each. One of the following radio-active precursors was used in each incubation: $[1-^{14}\text{C}]$ acetate sodium salt, specific activity (s.a.) 20 mc/m-mole; $[4-^{14}\text{C}]$ progesterone, s.a. 56.0 mc/m-mole; $[7\alpha-^3\text{H}]$ cholesterol, s.a. 9.8 c/m-mole. All radio-active chemicals were obtained from New England Nuclear Corporation. The purity of steroid radiochemicals before use was determined by thin-layer chromatography (TLC) prepared from silica gel GF₂₅₄, in the solvent systems used for analysis of products. An analysis in each group (nine rats) was accomplished in 10 days time so that the first group was completed within 70 to 80 days, another group between 80 and 90 days, and the last group between 90 and 100 days after being started on the experimental diet.

Incubation conditions

Three experiments were conducted in each of which eighteen animals were used: nine from the control group and nine from the group which had been maintained on the fatty-acid-deficient diet.

In each experiment, testicular tissue from three rats of each group was incubated with each of the following radio-active precursors: $[1-^{14}\text{C}]$ sodium acetate (10 μC), $[7\alpha-^3\text{H}]$ cholesterol (25 μC) and $[4-^{14}\text{C}]$ progesterone (0.5 μC). The animals were killed by cervical dislocation and duplicate samples of 500 mg of tissue were removed from each animal, and transferred immediately to a petri dish placed on crushed ice. The tissues were minced and distributed into 50-ml Erlenmeyer flasks containing 6.0 ml of sodium bicarbonate buffer at pH 7.4 (Hanks' solution, Difco Laboratories, Detroit, Michigan). Before the addition of buffer and the tissue to the flask, the radio-active substrate was dried under a stream of nitrogen; and approximately five drops of propylene glycol were added to the flasks containing $[4-^{14}\text{C}]$ progesterone and $[7\alpha-^3\text{H}]$ cholesterol precursors: the $[1-^{14}\text{C}]$ acetate precursor was used without solvent. The incubations were carried out in a Dubnoff metabolic incubator at 37° C for 3 hr in an atmosphere of 95.0% O₂ and 5.0% CO₂. At the end of incubation 10 ml of ethyl acetate were added to each flask to stop all enzymic reactions.

Extraction

At the conclusion of incubation, 50 μg each of carrier testosterone and androstenedione were added to each flask. Also added were trace amounts (15,000 counts/min) of $[1,2-^3\text{H}_2]$ testosterone (s.a. 46.5 c/m-mole) and $[1,2-^3\text{H}_2]$ androstenedione (s.a. 10.0 c/m-mole) to flasks with $[1-^{14}\text{C}]$ acetate and $[4-^{14}\text{C}]$ progesterone precursor, and to flasks with $[7\alpha-^3\text{H}]$ cholesterol as precursor; trace amounts of $[4-^{14}\text{C}]$ testosterone (s.a. 3.7 mc/m-mole) and $[4-^{14}\text{C}]$ -androstenedione (s.a. 45.2 mc/m-mole) were added to calculate the recovery. The contents of the flask were homogenized and extracted repeatedly with 5 $\times$ 10 ml of ethyl acetate. The combined extracts were evaporated to dryness under reduced pressure and the residue dissolved in 30 ml of light petroleum (b.p. 45° C). The latter was extracted four times with 10 ml of 70% aqueous methanol. The pooled 70% aqueous methanol phases were evaporated to an aqueous

residue which was then extracted five times with 10 ml of dichloromethane (Zander, 1962). The pooled dichloromethane extract was evaporated to a small volume and then chromatographed on TLC in a solvent system of diethyl ether and ethyl acetate (2:5 v/v) (Zander, Forbes, Von Münstermann & Neher, 1958). In this system testosterone and 17-hydroxyprogesterone had the same mobility. The latter were clearly separated from androstenedione.

In order to isolate testosterone from 17 α -hydroxyprogesterone, the area containing these compounds was eluted and acetylated for 24 hr at room temperature (Hall, Sozer & Eik-Nes, 1964). The acetylated compound was dried and extracted repeatedly with 2 ml of 50% dichloromethane and with methanol four times; the extract was reduced to a small volume and rechromatographed on TLC in the solvent system containing benzene and dichloromethane (3:2 v/v) (Hall, 1963). The area corresponding to testosterone acetate was determined by reference to a standard run in parallel and was eluted with 50% dichloromethane and methanol. An aliquot of eluates was used to measure the radio-activity by liquid scintillation spectrometry (Hall & Eik-Nes, 1962). The solution used for counting contained 5 g PPO (2,5-diphenyloxazole) and 0.1 g POPOP (1,4-bis-2-(5-phenyloxazolyl)-benzene)/l of toluene. Recovery of testosterone and androstenedione by this method was 65 to 75%. The counting efficiency disintegrations/min for ^{14}C and ^3H were 62.5% and 32.3% respectively.

Histological studies

Pituitary glands from six rats selected randomly (two from each group of those used in *in vitro* incubation studies) were removed, cleaned and immediately fixed in chrome alum fixative (Elftman, 1957) for 24 hr for routine tissue sectioning. The sections (6 μ) were stained by Loxol-fast-blue-periodic-acid-Schiff-haematoxylin staining technique (McManus & Mowry, 1965).

RESULTS

At the end of 60 days of the dietary regimen, the animals on EFA-deficient diets showed typical deficiency symptoms, e.g. scaly tail, necrosis in the hind legs and loss of hair in the back. These symptoms grew more severe with prolonged feeding of the diets. These observations are consistent with the well-known symptoms of EFA deficiency (Holman, 1954). In order to ensure that these animals, in addition to their gross EFA deficiency symptoms, also showed typical biochemical changes in the testicular lipids before incubation studies, the fatty acid composition of testes was analysed (Table 1).

These data show a marked increase in 5,8,11-eicosatrienoic acid (20:3 ω 9) and a decrease in the linoleic acids (18:2 ω 6) and their major metabolites (arachidonic acid, 20:4 ω 6, docosapentenoic acid, 22:5 ω 6). These data extend the results of numerous reports in the literature describing the typical lipid changes in the EFA deficiency (Holman, 1960). In EFA-deficient tissue, an accumulation of 20:3 ω 9 fatty acids and decrease of linoleic acid (18:2 ω 6) and its metabolites have been shown to be the major biochemical changes (Fulco & Mead, 1959). In Table 2, data on body weights, weights of testes and

TABLE 1
FATTY ACID COMPOSITION OF TESTES FROM CONTROL AND EFA-DEFICIENT RATS

	Animal no.	12:0	14:0	16:0	16:1 $\omega 7^*$	18:0	18:1 $\omega 9^*$	18:2 $\omega 6$	18:3 $\omega 3$	20:3 $\omega 9$	20:3 $\omega 6$	20:4 $\omega 6$	22:4 $\omega 9$	22:4 $\omega 6$	22:5 $\omega 6$	22:6 $\omega 3$
Control	4		1.8† ± 0.5	32.0 ± 1.3	4.9 ± 1.2	6.4 ± 1.0	18.6 ± 0.6	6.2 ± 0.2	0.8 ± 0.1	Trace	1.2 ± 0.5	11.0 ± 1.1	Trace	1.5 ± 0.5	13.1 ± 1.2	1.2 ± 0.1
Fat free	4		1.0 ± 0.1	33.9 ± 1.0	7.6 ± 0.8	6.3 ± 0.5	27.2 ± 1.5	0.6 ± 0.1	0.4 ± 0.1	8.4 ± 1.0	Absent	3.8 ± 0.5	3.2 ± 0.8	1.7 ± 0.1	5.4 ± 0.5	Trace

* The abbreviated nomenclature used indicates chain length, number of double bonds and position of the first double bond from the terminal methyl group. In polyunsaturated fatty acids all double bonds are assumed to be *cis* and methylene interrupted; thus 18:1 $\omega 9$ is oleate, 18:2 $\omega 6$ is linoleate and 18:3 $\omega 3$ is linolenate.

† Mean ± S.D.

TR = Less than 0.1%

male accessory glands are presented. There is a significant reduction ($P < 0.01$) in the weight of male accessory glands in the deficient animal. The differences in the testes weights and body weights were not significant.

TABLE 2
BODY WEIGHTS AND WEIGHTS OF TESTES AND MALE ACCESSORY GLANDS IN THE CONTROL AND EFA-DEFICIENT ANIMALS

Group	No. of animals	Age (days)	Body (g)	Testes (g)	Seminal vesicles (mg)	Ventral prostate (mg)
Control	12	110 to 130	291 $\pm$ 24*	2.8 $\pm$ 1.0	473 $\pm$ 34	344 $\pm$ 37
Deficient	12	110 to 130	205 $\pm$ 54	2.0 $\pm$ 0.5	172 $\pm$ 24†	197 $\pm$ 14†

* Mean $\pm$ S.D.

† $P < 0.01$ (differences between control and deficient). Others non-significant.

TABLE 3
RADIOCHEMICAL PURITY OF RADIO-ACTIVE TESTOSTERONE ACETATE FROM POOLED SAMPLES IN CONTROL AND DEFICIENT ANIMALS*

Cryst. no.	Group†						
	1	2	3	4	5	6	7
1	1113	2340	1410	1880	3120	4170	4110
2	1140	2190	1380	1320	3840	4050	5160
3	1140	2100	1440	1350	3930	4110	4800

* Specific activity disintegrations/min/mg.

† Groups 1 to 7 were selected randomly (from either control or deficient) from groups listed in Table 5.

TABLE 4
RADIOCHEMICAL PURITY OF RADIO-ACTIVE ANDROSTENEDIONE FROM POOLED SAMPLES IN CONTROL AND DEFICIENT ANIMALS*

Cryst. no.	Group†		
	1	2	3
1	1458	1104	3690
2	1290	801	2864
3	1260	837	2850

* Specific activity disintegrations/min/mg.

† The groups 1 to 3 (from either control or deficient) were chosen randomly from the groups described in Table 5.

Tables 3 and 4 demonstrate the radiochemical purity of testosterone acetate and androstenedione isolated by the method used in this study. The testosterone acetate and androstenedione from the duplicate incubations were pooled and seven of the nine samples for testosterone acetate and three samples of

TABLE 5
TESTOSTERONE* SYNTHESIS IN CONTROL AND EFA-DEFICIENT RAT TESTES†

Group†	Precursors			
	[1- ¹⁴ C]Acetate		[7 α - ³ H]Cholesterol	
	Control	Deficient	Control	Deficient
1	5,573 $\pm$ 1,570§ ⁽ⁱ⁾	11,333 $\pm$ 3,483	14,510 $\pm$ 2,130 ^b	26,826 $\pm$ 1,740
2	5,695 $\pm$ 1,579 ^b	11,175 $\pm$ 1,225	15,655 $\pm$ 1,726 ^b	24,391 $\pm$ 1,104
3	6,535 $\pm$ 1,958 ^{b(iii)}	9,581 $\pm$ 882	14,589 $\pm$ 8,279 ^b	21,787 $\pm$ 2,120

* Testosterone disintegrations/min determined as acetate.

† Disintegrations/min/500 mg tissue.

‡ Each group comprised three animals, and the figure in each animal is average of duplicate analysis. Groups 1 to 3 represent the time of analysis completed within 10 days in each group (between 70 and 100 days).

§ Mean $\pm$ S.D.

^b Differences between control and deficient are significant ([1-¹⁴C]acetate and [7 α -³H]cholesterol precursor). *P* value < 0.01 with the exception of (i) and (ii) where *P* value is < 0.05.

^c Differences between control and deficient are not significant ([4-¹⁴C]progesterone precursor).

TABLE 6
ANDROSTENEDIONE SYNTHESIS IN CONTROL AND EFA-DEFICIENT RAT TESTES*

Group†	Precursors			
	[1- ¹⁴ C]Acetate		[7 α - ³ H]Cholesterol	
	Control	Deficient	Control	Deficient
1	13,405 $\pm$ 2,307 ^{†b}	24,983 $\pm$ 3,635	26,538 $\pm$ 2,031 ^b	47,590 $\pm$ 1,671
2	13,858 $\pm$ 1,988 ^b	21,433 $\pm$ 836	25,093 $\pm$ 2,485 ^b	54,122 $\pm$ 5,423
3	11,202 $\pm$ 294 ^b	21,660 $\pm$ 1,646	24,841 $\pm$ 1,199 ^b	50,500 $\pm$ 1,494

* Disintegrations/min/500 mg tissue.

† Groups shown here are similar to those described in Table 5.

‡ Mean $\pm$ S.D.

^b Differences between control and deficient are significant (*P* < 0.01) ([1-¹⁴C]acetate and [7 α -³H]cholesterol precursor).

^c Differences between control and deficient are not significant ([4-¹⁴C]progesterone precursor).

androstenedione were chosen at random and after addition of carrier were recrystallized to constant specific activity from three different solvent systems (Zander *et al.*, 1958). The specific activity upon recrystallization did not change appreciably, thereby demonstrating radiochemical homogeneity of the isolated steroids.

Tables 5 and 6 show the data on testosterone and androstenedione synthesis. There was a significant increase in the yield of testosterone and androstenedione in the deficient tissue when $[1-^{14}\text{C}]$ acetate and $[7\alpha-^3\text{H}]$ cholesterol were used as precursors; however, with $[4-^{14}\text{C}]$ progesterone incubations, the differences were not marked.

The histological examination of the pituitary, stained by Luxol-fast-blue-PAS (Pl. 1, Figs. A and B), revealed that, in the deficient animal, PAS-positive basophil cells were hypertrophied and the cytoplasm filled with colloid particles. The microscopic appearance of these cells (Signet-ring cells) resembles those described in the spayed animal (Purves & Griesbach, 1955). Panos *et al.* (1959) have described similar changes in the pituitary of fat-deficient rats. First appearance of hypertrophied or spaying cells were observed between 70 and 80 days of deficient diet, and thereafter there was a progressive increase in the number of these cells. With the staining technique used in this study, it is not possible to make a positive differentiation between two types of basophils (FSH and LH type).

DISCUSSION

The data presented here extend the results of several studies in the past describing male infertility in EFA-deficient animals (Panos *et al.*, 1959; Greenberg & Ershoff, 1951). In the study reported here, there was a significant reduction in the weights of male accessory glands (Table 2) in the deficient animals which suggests insufficiency of androgen secretion; however, our previous histochemical studies in the deficient animal have indicated the presence of enzymes (Δ^5 -3 β -hydroxydehydrogenase, glucose-6-phosphatase) involved in steroid biosynthesis in the testes (unpublished observations). The adequacy of testicular androgen biosynthesis was confirmed in the present study (Tables 5 and 6). But the most intriguing result arising from these studies is that there is a significant increase in the yield of testosterone and androstenedione in the deficient testes. The reason for increased yield of androgen in the deficient animal cannot be explained on the basis of this data, but the following explanation has been presented from the results of several studies.

Changes in the composition of the dietary fat influence the composition of subcellular particles (Rahm & Holman, 1964; Bartley, 1964) and, therefore, may affect the rate of cellular metabolism (Rahm & Holman, 1964). Other studies have shown that essential fatty acids may satisfy a structural requirement in normal cell membrane (Aaes-Jørgensen, 1961), and they may be essential components in the lipid enzyme complexes of subcellular particles (Klein & Johnson, 1954). In EFA deficiency, increased metabolic rate (Burr & Beber, 1937), increased membrane permeability (Hayashida & Portman, 1963) and increased swelling (Hayashida & Portman, 1960; Johnson, 1963;

PLATE 1

A. (Anterior Pituitary) Sex zone of normal rat stained with PAS photographed through a green filter. The normal distribution and granulation of basophils is depicted. $\times 1125$. Inset: High magnification of normal basophils; the clear space corresponds to the nucleus. $\times 3600$.

B. Sex zone of EFA-deficient rat stained with PAS and photographed through a green filter. The basophil cells are increased in size and number. $\times 1125$. Inset: Many basophils are transformed into castration cells with a larger colloid vacuole adjacent to the nucleus. $\times 3600$.

(Facing p. 270)

Levin, Johnson & Albert, 1957), perhaps due to structural changes in the mitochondrial membranes (Stein & Stein, 1964), have been reported previously. These and other related studies suggest a relationship between the swelling tendency and electron transfer and/or uncoupled oxidative phosphorylation in the mitochondria of EFA-deficient rats. The literature cited above concerns liver mitochondria; however, our current investigation on the swelling studies of mitochondria of EFA-deficient testes revealed similar results (Ahluwalia, Shima & Pincus, 1968). Further studies on the structural and enzymatic changes in the mitochondria in EFA-deficient animals are under investigation and will be presented elsewhere. In isolated liver mitochondria *in vitro* studies, certain agents, for example oleic acid (C 18:1 ω 9), increased the swelling, and the degree of swelling is proportional to the concentration of oleates in the medium (Utsumi, Ohara, Yamamoto, Inaba, Urkami & Yamamoto, 1962). This fatty acid is known to increase significantly in EFA deficiency (Table 1); whether this would also increase mitochondrial swelling *in vivo* is speculative.

On the basis of this information, therefore, it is possible that an increased yield of androgens from [1- 14 C]acetate and [7 α - 3 H]cholesterol as precursors in *in vitro* incubations is perhaps due to structural alterations in the mitochondria wall, causing increased permeability of exogenous precursors to the enzymes located on the mitochondria.

When [4- 14 C]progesterone was used as precursor, there was no difference in the yield of testosterone and androstenedione in either group. It is difficult to postulate the reason for these data; however, on the basis of several studies, there is evidence that the steps for conversion of cholesterol $\rightarrow$ 2 α -hydroxycholesterol $\rightarrow$ 20 α ,22-dihydroxycholesterol $\rightarrow$ pregnenolone take place in the mitochondrial particles in adrenals (Halkerston, Eichhorn & Hechter, 1961), corpus luteum (Hall & Koritz, 1964) and testes (Menon, Drosowsky, Dorfman & Forchielli, 1965), and the conversion of pregnenolone $\rightarrow$ androgens occurs in the microsomal particles (Samuels, 1960). It would, therefore, appear that since there is no change in amounts of androgen production from [4- 14 C]-progesterone in EFA-deficient rat testes, the steps for conversion from pregnenolone to androgens outside the mitochondria remained essentially intact in this deficiency. Meanwhile we do not exclude the possibility that increased pituitary gonadotrophins (LH) in EFA deficiency (Panos, 1963) could also bring about increased biosynthesis of androgens (Major, Armstrong & Greep, 1967). Hypertrophied basophils (spayed-type cells) as shown in our study (Pl. 1, Figs. A and B) in the deficient animal suggest increased gonadotrophins in the pituitary of deficient animals (Rennels, 1957). Studies are currently planned to determine the LH contents of pituitary and plasma in the deficient animals.

The increased capacity of EFA-deficient testes to synthesize androgens from exogenous precursors seems to contradict the significant decrease in the weights of male accessory glands (Table 2) in the deficient animal. However, an explanation that the sensitivity of targets (male accessory glands) to circulating androgens is decreased in EFA deficiency could be applicable to this discrepancy (unpublished observations). Southren *et al.* (1965) have postulated that the absence of virilization in the human female due to presence of feminizing

testes is due to insensitivity of the end organs to normal levels of circulating androgens.

The studies are planned in our laboratory and the data obtained so far confirm some of the postulations offered in the study presented here. Whether a single factor or a combination of various factors results in increased biosynthesis of androgens in EFA deficiency needs to be confirmed.

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