Journal of Steroid Biochemistry, Vol. 11, pp. 1567 to 1571
Pergamon Press Ltd 1979. Printed in Great Britain

THE EFFECT OF ANABOLIC ANDROGENS ON GALL-BLADDER BILE ACIDS AND CHOLESTEROL IN MICE

C. M. DICKSON*, MILENA LESNA† and W. TAYLOR‡§
The Animal Unit* and the Departments of Pathology† (Newcastle General Hospital) and Physiology‡, The Medical School, The University, Newcastle upon Tyne NE1 7RU, England

(*Received* 14 *March* 1979)

SUMMARY

Male and female Balb/C mice were treated for 12 weeks with a daily oral dose of 150 μg methyltestosterone, or a weekly s.c. injection of 25 μg of Decadurabolin® for up to 22 weeks. No discernible effects on the types or amounts of the gall-bladder bile acids were observed with either type of treatment. Biliary cholesterol concentration was not affected. After treatment with both types of anabolic androgen diffuse hepatocytic hyperplasia was seen, and male mice were more affected than were female mice. No signs of peliosis hepatis nor focal nodular hyperplasia were observed in any of the livers.

INTRODUCTION

Epidemiological and experimental investigations have clearly shown the natural and synthetic oestrogens influence the metabolism of biliary lipids. Thus women who use oral contraceptives or are treated with synthetic oestrogens exhibit an increased tendency to develop gallstones [1, 2]. Other studies have shown that ingestion of oral contraceptives by women leads to the formation of a lithogenic bile, and bile acid metabolism is affected, but these effects are reversed when use of oral contraceptives is stopped [3]. Work from this laboratory has shown that administration of as little as 30 μg of ethynyl oestradiol for seven days to normal male subjects also causes disturbances of biliary lipid composition [4].

Experimental studies with animals support the results obtained in investigations of human subjects. Ethynyl oestradiol alone or in combination with a progestin has been shown to affect biliary lipid composition in rats [5], baboons [6] and cats [7].

However, no comparable studies of the effects of anabolic androgens on biliary lipid composition and bile acid metabolism appear to have been carried out. In this paper we report attempts to affect biliary lipid composition and to induce hepatic lesions by relatively long-term administration of a 17α-alkyl androgen, methyltestosterone, and a commonly used anabolic androgen, 17β-hydroxy-19-nor-4-androsten-3-one-17β-*n*-decanoate (Decadurobolin®), which lacks a 17α-alkyl substituent, using male and female mice.

§ To whom all correspondence and requests for reprints should be addressed.

The trivial names of the bile acids mentioned in the text (and the abbreviations used in Tables 1 and 2) are: allocholic acid, 3α,7α,12α-trihydroxy-5α-cholanoic acid (5α-3α,7α,12α); α-muricholic acid, 3α,6β,7α-trihydroxy-5β-cholanoic acid (5β-3α,6β,7α); cholic acid, 3α,7α,12α-trihydroxy-5β-cholanoic acid (5β-3α,7α,12α); deoxycholic acid, 3α,12α-dihydroxy-5β-cholanoic acid (5β-3α,12α); 3α,6β-dihydroxy-5β-cholanoic acid (5β-3α,6β); β-muricholic acid, 3α,6β,7β-trihydroxy-5β-cholanoic acid (5β-3α,6β,7β); hyodeoxycholic acid, 3α,6α-dihydroxy-5β-cholanoic acid (5β-3α,6α); chenodeoxycholic acid, 3α,7α-dihydroxy-5β-cholanoic acid; ω-muricholic acid, 3α,6α,7β-trihydroxy-5β-cholanoic acid; lithocholic acid, 3α-hydroxy-5β-cholanoic acid: all are -cholan-24-oic acids.

METHODS

Animals

Age-matched male and female Balb/C mice, 3 months old, were obtained from recognised dealers or reared from the breeding stock of this Animal Unit. They were acclimatised for 3 weeks in a room containing no other animals; temp. 20°C, and with a 12 h light-dark cycle. They had free access to water and a diet of commercial rodent food (Fortified Rodent Food Pellets; British Petroleum Ltd.). The experimental animals receiving steroid were kept in separate cages, since preliminary experiments had shown that the androgens used produced aggressive behaviour in both male and female mice. Control animals receiving arachis oil only were kept in groups of 5 animals per cage. No disease or deaths were encountered.

Administration of steroids

Methyltestosterone, in powder form (kindly donated by Organon Laboratories, Ltd.), was dissolved in arachis oil to provide a solution containing 1.5 mg steroid/ml. The experimental animals were given 0.1 ml of the solution daily between 0900–10.00 h by the oral route using a blunt-ended flanged stainless-steel tube (5 cm long) fitted to a 1.0 ml syringe. Control animals received 0.1 ml arachis oil only by the same method.

Table 1. Bile acids and cholesterol in gall-bladder bile of male and female Balb/C mice after treatment with 17β-hydroxy-19-norandrost-4-en-3-one-17n-decanoate (Decadurabolin®) for 22 weeks

	Male								Female							
	Control				Treated				Control				Treated			
Cholanoic acids	(mg/ml)		(% Total)		(mg/ml)		(% Total)		(mg/ml)		(% Total)		(mg/ml)		(% Total)	
Experiment No.	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
5α-3α,7α,12α	2.0	2.2	2.9	3.1	2.4	3.0	3.6	4.3	1.6	2.0	2.3	3.0	2.0	2.3	2.8	3.2
5β-3α,6β,7α	3.2	3.0	4.6	4.3	3.3	3.2	4.9	4.5	2.8	2.3	4.1	3.4	3.8	1.6	5.4	2.4
5β-3α,7α,12α	39.7	41.2	57.2	58.8	39.0	40.6	58.4	57.6	38.4	37.4	55.6	55.3	39.7	36.7	55.9	54.7
5β-3α,12α	5.4	5.0	7.8	7.1	4.9	5.4	7.3	7.7	5.0	5.1	7.2	7.5	5.6	6.0	7.9	8.9
*5β-3α,6β	1.2	0.4	1.7	0.6	2.2	1.1	3.3	1.6	0.7	0.7	1.0	1.0	1.2	1.9	1.7	2.8
*5β-3α,6β,7β	15.4	16.9	22.2	24.1	13.5	15.1	20.2	21.4	18.0	19.1	26.1	28.3	16.9	18.0	23.8	26.8
5β-3α,6α	2.5	1.4	3.6	2.0	1.5	2.1	2.2	3.1	2.5	1.0	3.6	1.5	1.8	0.6	2.5	0.9
Totals	69.4	70.1			66.8	70.5			69.0	67.6			71.0	67.1		
Cholesterol (mg/ml)	3.1	3.2			3.1	3.0			3.0	3.1			3.1	3.1		
Number of animals	7	10			6	10			7	9			7	10		

* Complex peaks: for details see text.

Table 2. Bile acids and cholesterol in gall-bladder bile of male and female Balb/C mice after treatment with 17α-methyl-17β-hydroxyandrost-4-en-3-one (methyltestosterone) for 12 weeks

	Male								Female							
	Control				Treated				Control				Treated			
Cholanoic acids	(mg/ml)		(% Total)		(mg/ml)		(% Total)		(mg/ml)		(% Total)		(mg/ml)		(% Total)	
Experiment No.	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
5α-3α,7α,12α	2.2	2.0	3.4	3.1	1.4	1.0	2.1	1.5	2.1	1.5	3.2	2.2	2.1	1.6	3.1	2.4
5β-3α,6β,7α	2.8	3.2	4.3	5.0	2.6	2.3	4.0	3.5	2.3	2.7	3.6	4.0	2.4	1.4	3.5	2.1
5β-3α,7α,12α	38.3	38.6	58.4	58.8	40.0	40.8	60.8	63.0	39.8	44.9	61.5	66.8	43.0	41.3	62.6	62.5
5β-3α,12α	5.0	5.3	7.6	8.3	4.7	5.0	7.1	7.7	5.0	5.8	7.7	8.7	5.1	5.3	7.4	8.0
*5β-3α,6β	1.2	1.5	1.8	2.4	1.6	1.2	2.4	1.9	1.2	0.9	1.9	1.4	1.7	1.0	2.5	1.5
*5β-3α,6β,7β	13.2	11.8	20.1	18.4	13.5	13.1	20.5	20.2	12.9	10.7	19.9	15.9	12.4	14.5	18.0	22.0
5β-3α,6α	2.9	1.6	4.4	2.5	2.0	1.4	3.0	2.2	1.4	0.7	2.2	1.0	2.0	0.9	2.9	1.4
Totals	65.6	64.0			65.8	64.8			64.7	67.2			68.7	66.0		
Cholesterol (mg/ml)	3.2	3.1			3.0	3.2			3.1	3.0			3.2	3.1		
Number of animals	5	10			7	10			6	9			5	10		

* Complex peaks: for details see text.

Decadurabolin® (Organon Laboratories Ltd.) provided in ampoules of 50 mg/ml in arachis oil–benzyl alcohol (9:1, V/V) was diluted with arachis oil to provide a sterile solution containing 125 μg/ml. The experimental animals were given 0.2 ml of the solution at weekly intervals by s.c. injection. Control animals were injected with arachis oil only.

The animals were weighed before the experiment and at weekly intervals thereafter.

Collection and analysis of gall-bladder bile and livers

On the day preceding killing, the animals were fasted for 14 h but were allowed free access to water. The mice were killed by cervical dislocation. The gall-bladders were removed with fine forceps and pooled in groups according to sex and treatment. The bile was stored at −25°C until analysed. The livers were removed and placed in buffered formol-saline.

Bile acids were determined in duplicate 50 μl volumes of bile, using ethanolic-alkaline hydrolysis and gas–liquid chromatography on columns of 1% HiEff 8BP as described by Taylor[8]. Since mouse gall-bladder bile contains at least 10 bile acids a full assessment of the overall accuracy of the method cannot be given. However, analysis of a pool of mouse gall-bladder bile showed that the reproducibility of the within-batch analysis of the major bile acids was ±3% and ±5% for minor bile acids. The between-batch reproducibility was ±5% for major bile acids and ±7% for minor components. We have calculated that the overall accuracy of the method is approx. ±5% for total bile acids, ±7% for individual components and ±5% for total bile acids.

Cholesterol was determined in duplicate 50 μl bile samples by a g.l.c. method [5] except that 5β-cholestan-3α-ol was used as internal standard, and the trimethylsilyl ethers of the sterols were separated on columns of 1% HiEff 8BP. The overall accuracy of this method was ±5%.

Treatment of animals

Two experiments were carried out with each type of androgen. In Experiment 1, 6–8 animals of each sex were used in each group. In Experiment 2, there were 20 animals in each group. Half of the Decadurabolin-treated group were killed after 11 weeks, and half of the methyltestosterone group were killed after 4 weeks. Since no changes in bile acids nor in liver architecture were observed in these animals, the results are not recorded here.

Liver tissue

Sections of liver for histological examination were prepared by standard methods, and the results will be reported elsewhere [9].

RESULTS

The weight (±SD) of the male control animals was 32 ± 1 g at the start of the experiment, and 32 ± 1 g at the end. The corresponding weights of the female animals were 24 ± 1 g and 24 ± 1 g respectively. The weights of the male mice given methyltestosterone were 32 ± 0.5 g before and 34 ± 1 g after treatment; the corresponding weights of the female animals were 24 ± 0.5 g and 26 ± 0.5 g respectively. The weight-gain occurred over the first 2–3 weeks of treatment and was maintained thereafter. No marked changes in weight occurred in the animals treated with Decadurabolin®. This expected weight increase in methyltestosterone treatment contrasts with the loss in weight of rats treated with ethynyl oestradiol [5].

Gall-bladder bile acids and cholesterol

The bile acid patterns of mouse bile shown in Tables 1 and 2 are very similar to those described by Eyssen *et al.*[10] in that cholic and β-muricholic acids are the predominant bile acids, with smaller amounts of other bile acids. Those authors also found appreciable amounts of chenodeoxycholic and ω-muricholic acids not found in the present investigation, but they used male C3H mice, prevented coprophagy, and maintained the animals under different conditions with a different diet from those used by us. These differences could account for the slightly different patterns of bile acids found in the bile.

No abnormalities were observed in the types or amounts of gall-bladder bile acids in male and female mice treated with Decadurabolin® for 22 weeks (Table 1), nor in animals treated with methyltestosterone for 12 weeks (Table 2).

The identity of the bile acids listed in Tables 1 and 2 was established by retention time relative to that of the derivative of cholic acid, and by fragment-ion gas chromatography-mass spectrometry with the cooperation of Professor J. Sjövall. The substances marked with an asterisk in the Tables did not give clear mass spectra; the peak corresponding to 3α,6β-dihydroxy-5β-cholanoic acid contained a small amount of chenodeoxycholic acid. The β-muricholic acid, a major component of mouse bile, contained small amounts of closely related bile acids, probably with a double bond in the side chain and/or the ring system (cf. Kern *et al.* [5]).

Morphological changes in the liver

After a few weeks animals treated with either steroid showed nuclear pleimorphism with enlarged hepatocytes. After 22 weeks treatment with Decadurabolin® and 12 weeks with methyltestosterone, most livers showed diffuse hepatocytic hyperplasia. These changes were more marked in male than in female animals. No changes were observed in the control groups.

DISCUSSION

Apart from the present investigation no systematic studies of the effects of anabolic androgens on biliary bile acid composition, and of cholesterol concen-

tration, related to changes in liver architecture have been carried out. It is apparent from Table 1 that administration of Decadurabolin®, a steroid with no 17α-alkyl substituent, at a dose level approximating to that used in man, has no effect on the bile acid and cholesterol concentrations of gall-bladder bile of mice treated for up to 22 weeks. More surprisingly, treatment of mice for up to 12 weeks with relatively large doses of the 17α-alkylated steroid, methyltestosterone, also had no effect on gall-bladder bile acid and cholesterol concentrations (Table 2). These findings are in marked contrast to the effects on biliary lipid composition induced by female sex hormones in man and animals. Such effects occur within a few days of administration of the steroids [4, 5, 7].

A further point of contrast between the actions of these anabolic androgens and female sex hormones is that no histopathological changes were observed in the livers of rats treated with relatively large doses of ethynyl oestradiol for a few days, even though intrahepatic cholestasis and disturbance of biliary lipid occurred [5]. Even though the types of experimental techniques and doses of steroids used have been different, the tentative conclusion may be drawn that the female and male sex hormones act at different levels in the hepatobiliary system. Kern *et al.*[5] have suggested that the action of oestrogens is on the bile salt-independent bile flow.

Oestrogens and androgens differ in their action on the hepatic microsomal enzymes involved in drug metabolism. Oestrogens cause a reduction in microsomal enzyme activity, whereas testosterone greatly increases this enzyme activity [11]. Since cholesterol and bile acids are hydroxylated by these microsomal enzymes [12], an effect on the amounts of hydroxylated bile acids present in mouse bile could have been expected after long-term androgen administration. However, no such effect was observed in the present work.

There were differences in the response of mice to the androgens in terms of changes in liver architecture. After 11 weeks all mice treated with Decadurabolin® showed nuclear pleimorphism and enlarged hepatocytes, but after 22 weeks treatment all male mouse livers showed diffuse hepatocytic hyperplasia, whereas female mice were only mildly affected. Similar changes were also seen in the methyltestosterone treated group, and again the effects were more marked in the male mice. No reason for this sex difference in response to androgen treatment can be given at this stage.

It is frequently stated that synthetic sex hormones, particularly those with a 17α-alkyl or -allyl substituent, have deleterious effects on the liver. These effects range from an impaired ability of the liver to clear bromosulphophthalein to severe intrahepatic cholestatic jaundice, and are usually reversible when steroid hormone treatment is withdrawn [13, 14]. Many authors have assumed that, because of their similarity in structure, the anabolic androgens and the steroidal components of oral contraceptives might be expected to produce the same types of liver dysfunction or histopathological lesions. Thus, Ockner and Davidson[15] suggested that in some women oral contraceptives produced reversible, non-inflammatory intrahepatic cholestasis resembling that produced by certain anabolic androgens. Groos *et al.*[16] put forward the hypothesis that the side effects of oral contraceptives could be due to the 17α-alkylated-19-norandrostane component rather than to the oestrogen component. However, this hypothesis is not tenable in view of epidemiological and experimental evidence from human [2, 4] and animal [5] studies. Kalra *et al.*[17] also suggested that the close chemical relationship between anabolic androgens and oral contraceptive steroids might account for the liver lesions observed in patients receiving these steroids. These statements are quoted at length to emphasize the confusion which can arise by supposing that steroids might necessarily have the same effects on target organs or on the liver.

Strong evidence has been provided for a direct relationship between long-term treatment of human patients with anabolic androgens and a characteristic liver lesion, peliosis hepatis [18]. The cases of androgen-induced peliosis hepatis reported between 1952 and 1976 have been recorded by Paradinas *et al.*[19]. Those authors have suggested that methyltestosterone exerts its hepatotoxic effects by an action on the hepatic veins, whereas oestrogens are thought to act on bile-salt independent bile flow. Peliosis hepatis is rarely, if ever, found in patients treated with oral contraceptives.

We observed only minor histopathological changes in the livers of the treated mice, and no lesions resembling peliosis hepatis were seen. It may be that the steroid treatment was not continued for a sufficiently long period, but 12 and 22 weeks represent a considerable proportion of the 1.5–3 y life-span of the mouse.

The lack of effect of the two anabolic androgens on the cholesterol concentration may appear to be somewhat unexpected. Administration of similar steroids to human subjects causes marked changes in endocrine secretions. For example, Zumoff and his collaborators found that Norethandrolone® (19-nor-17α-ethyltestosterone) produced abnormal changes in cortisol [20], and that Calusterone® (7β,17α-dimethyltestosterone) has a pronounced effect on testosterone metabolism [21]. Also, the non-steroidal antiandrogen Flutamide® (4′-nitro-3′-trifluoromethylbutyranilide) caused a definite increase in the conversion of testosterone to androsterone [22]. The latter steroid produces a significant decrease in plasma cholesterol [23], and chronic administration of synthetic androgens and oestrogens produces changes in gonadotrophin secretion in man [24]. Such effects on the endocrine system could have a profound effect on the metabolism of cholesterol and other sterols in man and animals. The absence of any change in biliary

cholesterol in the present study may be due to different effects on the various cholesterol pools in the body. We have observed that treatment of normal men with 30 μg of ethynyl oestradiol for 7 days causes a rise in biliary cholesterol, and a decrease in plasma testosterone, but plasma cholesterol concentrations remain unchanged [4]. The relationship between the plasma and biliary cholesterol pools is not well understood, and no explanation can be given at this time for the failure of the two anabolic androgens to alter the biliary cholesterol concentration in mice.

It is concluded from our results that the actions of anabolic androgens on the hepatobiliary system differ in many respects from the effects of female sex hormones. The androgens produced detectable hepatocytic hyperplasia with no discernible changes in biliary lipids. However female sex hormones, at least in the rat, produced no detectable liver lesions but caused marked changes in the metabolism of biliary lipids [5].

Acknowledgements—We gratefully acknowledge the assistance of Mr. B. Errington and Mrs. E. Moody in caring for the animals. Mrs. V. Nicholson provided skilled technical assistance in the preparation of the liver sections. W. T. is grateful to Professor J. Sjövall, Karolinska Institute, Stockholm, for providing the mass spectrometry facilities (Swedish Medical Research Council Grant No. 13X-219), and to The Wellcome Trust for a Travel Grant. The Smith Kline and French Foundation provided financial support for the purchase and feeding of animals. Organon Laboratories Ltd. made generous gifts of methyltestosterone.

REFERENCES

1. Boston Collaborative Drug Surveillance Programme.: Oral contraceptives and venous thromboembolic disease, surgically confirmed gall-bladder disease, and breast tumours. *Lancet* **i** (1973) 1399–1404.
2. Boston Collaborative Drug Surveillance Program.: Surgically confirmed gall-bladder disease, venous thromboembolism, and breast tumours in relation to postmenopausal estrogen therapy. *New Engl. J. Med.* **290** (1974) 15–18.
3. Bennion L. J., Ginsberg R. L., Garnick M. B. and Bennett P. H.: Effects of oral contraceptives on the gall-bladder bile of normal women. *New Engl. J. Med.* **294** (1976) 189–192.
4. James O. F. M., Anderson A., Brooks A. and Taylor W.: The effect of ethynyl oestradiol on biliary lipid composition in human males. Proc. Internat. Assocn. for Study of the Liver. Fuengirola. Spain. 1978 (Abstract).
5. Kern F. Jr, Eriksson H., Curstedt T. and Sjövall J.: Effect of ethynylestradiol on biliary excretion of bile acids, phosphatidylcholines and cholesterol in the bile fistula rat. *J. Lipid Res.* **18** (1977) 623–634.
6. Morrissey K., Panveliwalla D., McSherry C., Dietrick J., Niemann W. and Gupta G.: Effects of contraceptive steroids and pregnancy on bile composition and kinetics in the baboon. *J. surg. Res.* **22** (1977) 598–604.
7. Hirst B. H., Lund P. K., Reed J. D., Sanders D. J., Shaw B. and Taylor W.: Effects of a combined oestrogen-progestin preparation on gastric acid and pepsin secretion, serum gastrin concentration and biliary secretion of bile acids, phospholipids, and cholesterol in the cat. *Br. J. Pharmacol.* **65** (1978) 87–95.
8. Taylor W.: The bile acid composition of rabbit and cat gall-bladder bile. *J. steroid Biochem.* **8** (1977) 1081–1084.
9. Lesna M., Rodriguez M. S. and Taylor W.: Androgen induced hepatocytic hyperplasia. *Beitrg. Path. Bd.* (submitted for publication).
10. Eyssen H. J., Parmentier G. G. and Mertens J. A.: Sulfated bile acids in germ-free and conventional mice. *Eur. J. Biochem.* **66** (1976) 507–514.
11. Gram T. E. and Gillette J. R.: The role of sex hormones in the metabolism of drugs and other foreign compounds by hepatic microsomal enzymes. In *Metabolic Effects of Gonadal Hormones and Contraceptive Steroids* (Edited by H. A. Salhanick, H. A. Kipnis and R. L. Vande Wiele). Plenum Press, New York (1969) pp. 86–94.
12. Gustafsson J.: Effect of biliary obstruction on 26-hydroxylation of C_{27}-steroids in bile acid synthesis. *J. Lipid Res.* **19** (1978) 237–243.
13. Sherlock S.: Biliary secretory failure in man: the problem of cholestasis. In *The Biliary System* (Edited by W. Taylor). Blackwells Scientific Publications, Oxford (1965) pp. 585–589.
14. Davidson C. S.: In *Liver pathophysiology: its relevance to human disease*. Little, Brown and Company, Boston (1970) pp. 20–21.
15. Ockner R. K. and Davidson C. S.: Hepatic effects of oral contraceptives. *New Engl. J. Med.* **276** (1967) 331–334.
16. Groos G., Arnold O. H. and Brittinger G.: Peliosis hepatis after long-term administration of oxymethylone. *Lancet* **i** (1974) 874.
17. Kalra T. M. S., Mangla J. C. and dePapp E. W.: Benign hepatic tumours and oral contraceptives. *Am. J. Med.* **61** (1976) 871–877.
18. Bagheri S. A. and Boyer J. L.: Peliosis hepatis associated with androgenic-anabolic steroid therapy: a severe form of hepatic injury. *Ann. Int. Med.* **81** (1974) 610–618.
19. Paradinas F. J., Bull T. B., Westaby D. and Murray-Lyon I. M.: Hyperplasia and prolapse of hepatocytes into hepatic veins during long term methyltestosterone therapy: possible relationships of these changes to the development of peliosis hepatis and liver tumours. *Histopathology* **1** (1977) 225–246.
20. Zumoff B., Bradlow H. L., Cassouto J., Gallagher T. F. and Hellman L.: Reversible reproduction of the abnormal cortisol metabolite pattern of cirrhosis by administration of Norethandrolone. *J. clin. Endocr. Metab.* **28** (1968) 92–99.
21. Zumoff B., Levin J., Bradlow H. L. and Hellman L.: The effect of 7β,17α-dimethyltestosterone (Calusterone®) on testosterone metabolism in women with advanced breast cancer. *J. clin. Endocr. Metab.* **44** (1977) 1203–1205.
22. Hellman L., Bradlow H. L., Freed S., Levin J., Rosenfeld R. S. and Zumoff B.: The effect of Flutamide on testosterone metabolism and the plasma levels of androgens and gonadotropins. *J. clin. Endocr. Metab.* **45** (1977) 1224–1229.
23. Hellman L., Bradlow H. L., Zumoff B., Fukushima D. K. and Gallagher T. F.: Thyroid-androgen interrelations and the hypocholesterolemic effect of androsterone. *J. clin. Endocr. Metab.* **19** (1959) 936–948.
24. Sawin C. T., Ryan R. J., Longcope C. and Fisher L. E.: Effect of chronic administration of estrogen, androgen, or both, on serum levels of gonadotropins in adult men. *J. clin. Endocr. Metab.* **46** (1978) 911–915.