CHAPTER 45

Estrogens and Antiestrogens in the Male

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A. Introduction

Estrogens and men – this is a topic which has been neglected for quite some time and it is only relatively recently that discussion of it has intensified remarkably. The discussion has not been restricted to the scientific community. In former times, the role of estrogens in male physiology received little attention since androgens clearly have a dominant role. It is now becoming more and more evident that estrogens are far more important in the male than we had supposed (HABENICHT 1998).

But no area of hormone replacement in elderly males has been discussed so much and is so little known as the usefulness of estrogen therapy. Does a male estrogen deficit, or even an extensive full or partial aromatase defect (with normal testosterone levels and reduced estrogen serum concentrations), exist at all, or do males have lifelong resources (at least of brain estrogen) through the intracerebral conversion of testosterone to estrogen, thus avoiding late-life estrogen deficiency (BIRGE 1996)? Research has been extraordinarily stimulated by the availability of transgenic mice with disruption of the estrogen-receptor gene and by reports of estrogen-receptor deficiencies in males (DIXON et al. 1997). Certain conclusions of this research will be discussed in this chapter.

In fact, men have much higher 17β-estradiol levels than postmenopausal women. In the testicles and by peripheral aromatization of androgens, the human male produces considerable amounts of estrogens whose function has not yet been sufficiently clarified. The fact that the presence of estrogen receptors (ERs) have been demonstrated in many tissues suggests a physiological role of estrogens, even in males. Because the circulating levels of testosterone in the male are similar to the K_m of aromatase (20–30 nmol/l), it is likely that circulating testosterone can be converted efficiently in extragonadal sites to give rise to local concentrations of estradiol sufficient to transactivate both α- and β-ERs (K_D~1 nmol/l; SIMPSON and DAVIS 1998).

Recent clinical reports (SMITH et al. 1994; FOREST et al. 1996; SUDHIR et al. 1997) of disruptive mutations of the genes for ERs or for cytochrome P-450 aromatase have shed new light on the role of estrogen. A mutation in the estrogen-receptor gene in a 28-year-old man led to elevated estradiol and estrone serum concentrations, and serum testosterone concentrations (T) were

normal. Serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels were increased. Glucose tolerance was impaired, and hyperinsulinemia was present. The bone mineral density of the lumbar spine (LS) was 0.745 g/cm^2, 3.1 SD below the mean for age-matched normal women; there was also biochemical evidence of increased bone turnover. The patient was tall (204 cm) and had incomplete epiphyseal closure, with a history of continued linear growth into adulthood despite otherwise normal pubertal development. The patient had no detectable response to estrogen administration despite a tenfold increase in the serum free-estradiol concentration. Contrary to previous view, disruption of the ER in humans need not be lethal (SMITH et al. 1994).

Males with an aromatase defect have an absolute estrogen deficiency, with increased FSH and LH. The skeletal age appears to be retarded, with unclosed epiphyses. The clinical picture essentially corresponds to osteoporosis. Moreover, increased basal insulin levels and a reduced high-density to low-density lipoprotein (HDL–LDL) quotient are observed (BULUN 1996). In females, the lack of estrogen due to aromatase deficiency leads to pseudohermaphroditism and progressive virilization at puberty whereas, in males, pubertal development is normal. In members of both sexes, epiphyseal closure is delayed, resulting in an eunuchoid habitus, and osteopenia is present (MORISHIMA et al. 1995). These findings suggest a crucial role of estrogen in skeletal maturation. CARANI et al. (1997) describe a therapeutic response to estrogen therapy, but not to androgen therapy, in a man with aromatase deficiency. However, the questions of when to initiate the estrogen treatment, at what doses, and for how long remain to be investigated in further studies.

B. Age-Related Changes of Estrogen Secretion

Young men have estrogen levels between 10 pg/ml and 100 pg/ml (mean: 40 ± 15 pg/ml; KUHL 1997). In another communication, the serum 17β-estradiol levels (E2) are 51.6 pg/ml on average [standard error (SE): 5.3, range: 20–90; AMBROSI et al. 1981]. They correspond to the levels found in women in the early follicular phase (KUHL 1997) and are sufficient to develop a female phenotype (testicular feminization) in the case of an androgen-receptor defect, e.g., absence of androgenic actions. Elderly men have higher estradiol or estrone levels than postmenopausal women (JANSSEN et al. 1998).

ANDERSSON et al. (1997), in a cross-sectional study, determined the E2 and T in 400 healthy Danish prepubertal, pubertal, and adolescent males aged 6–25 years. While the highest mean concentrations of about 22 ± 5 nmol/l for T were reached at age 19, for 17β-estradiol the highest mean concentrations (±SD) of about 100 ± 25 pmol/l were reached at about age 24 years. This means that the estradiol rise is flatter in young males, with peak values being reached about 6 years after the secretion peak of testosterone. In pubertal boys, the serum estrogen concentrations were established mainly through aromatization of testosterone (MCDONALD et al. 1979).

Comparing the ratio of T to E2 in younger and older men, we can at first assume that relatively and/or absolutely more 17β-estradiol is present in the elderly (Pirke and Doerr 1973; Baker et al. 1976; Murono et al. 1982; Deslypère et al. 1987). Estradiol has been reported to increase or remain unchanged with age (Kley et al. 1974; Stearns et al. 1974; Rubens et al. 1974; Zumoff et al. 1982; Kaiser et al. 1988). In this context, fatty tissue is an important site of aromatization, and obesity is associated with increased estrone concentrations in aged men (Kley et al. 1979; Stanik et al. 1981). Therefore, it is not surprising to find some data suggesting an increase in estrogenic concentration with age, as the percentage of body fat in normal individuals increases as an age-related phenomenon. Additionally, the metabolic clearance of estradiol decreases with age (Baker et al. 1976).

In our own study (Winkelmann 1998), measuring different hormone serum levels of 698 male patients aged 21–88 years with or with suspicion of coronary heart disease, we found the following results:

- A significant increase of FSH and LH (Fig. 1); that means a hypogonadotropic or – at least in some cases – an eugonadotropic state
- A significant decrease of total testosterone (Fig. 2). Of the voluteers, 59.6% are eugonadal, 15.5% hypogonadal (total T less than 10 nmol/l), and 24.9% of the males are in the borderline range. That means partial androgen deficiency of the aging male (PADAM)
- No significant changes of E2 and significant decrease of the T/E2 ratio (Fig. 3)

Of the patients studied, 52.9% showed physiological total T (>14 nmol/l) and E2 (>20 pg/ml); 37.0% were hypogonadal (T < 14 nmol/l) but normo- or hyperestrogenic (E2 > 20 pg/ml). Of the patients studied, 3.4% were simultaneously in a hypoandrogenic and a hypoestrogenic state. Interestingly enough, 6.7% of the men showed at least a partial aromatase defect. That means normal total T over 14 nmol/l but extremely low E2 (<20 pg/ml). This aromatase defect doesn't show any age-related changes (Fig. 4).

However, these data are not at all consistent with other reports of a decrease in E2 with age. In contrast to the above-mentioned studies indicating an age-related increase or constancy in E2 levels in men, more and more papers show an age-related decline in estrogen secretion. In an additional small pilot study with healthy men, we found 31 (27%) of a total of 116 men (aged 43–71 years) in an estrogen-deficient state (E2 < 30 pmol/l). Among the 47 hypogonadal men (T < 14 nmol/l), 32 (68%) showed normal or elevated E2 (hypoandrogenic and normo- or hyperestrogenic state) whereas 15 men (32%) showed E2 decreased below 30 pmol/l (hypoandrogenic and hypoestrogenic states). However, there were 16 (14%) eugonadal men (T > 14 nmol/l) with estrogen deficiency or with an aromatase defect. Despite the presence of enough substrate for the aromatase there was not enough estrogen in the blood (Heinemann 1998). This shows that T and E2 do not correlate, and partial aromatase defect in men should be given greater attention in the future.

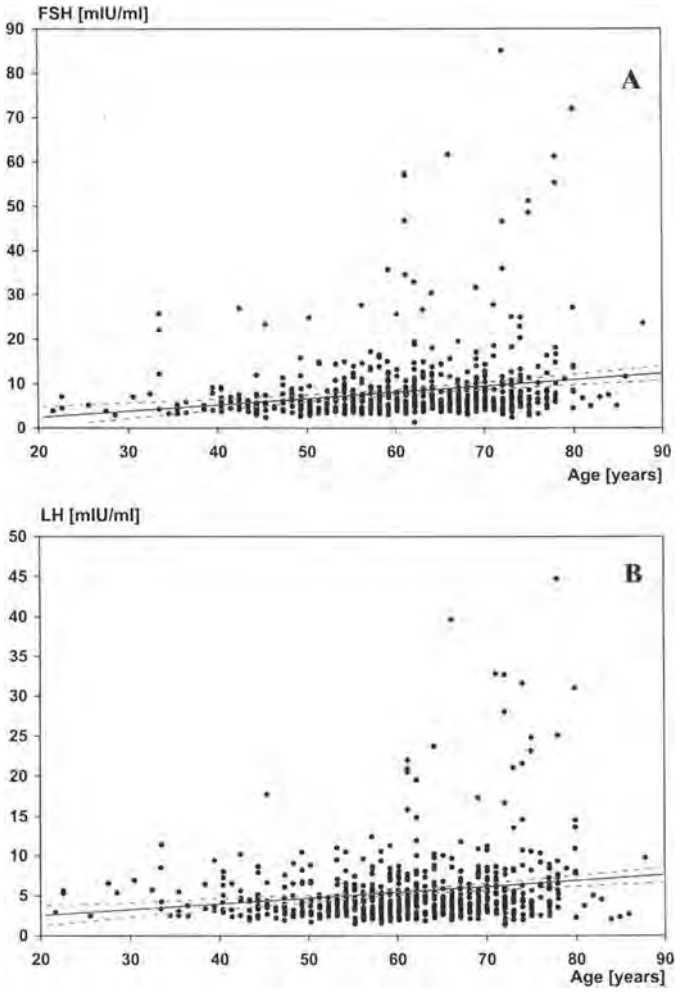

Fig. 1A,B. Serum follicle-stimulating hormone (*FSH*; **A**) and luteinizing hormone (*LH*; **B**) concentrations in 698 men aged 21–88 years. The age-related increase of mean gonadotropin concentrations is significantly ($P > 0.01$) (Winkelmann 1998)

Ferrini and Barrett-Connor (1998) found that testosterone and bioavailable estradiol levels in plasma samples obtained from 810 men aged 24–90 years (Rancho Bernardo Study) decreased significantly with age, independently of covariates. In contrast to the weaker association between age and total estradiol, there was a strong association of bioavailable estradiol with age. The age-associated decrease in bioavailable estradiol among these men may be partially explained by decreasing levels of testosterone, the primary substrate for male estradiol production, coupled with the higher levels of sex-hormone-binding globulin (SHBG) in older adults (Field et al. 1994).

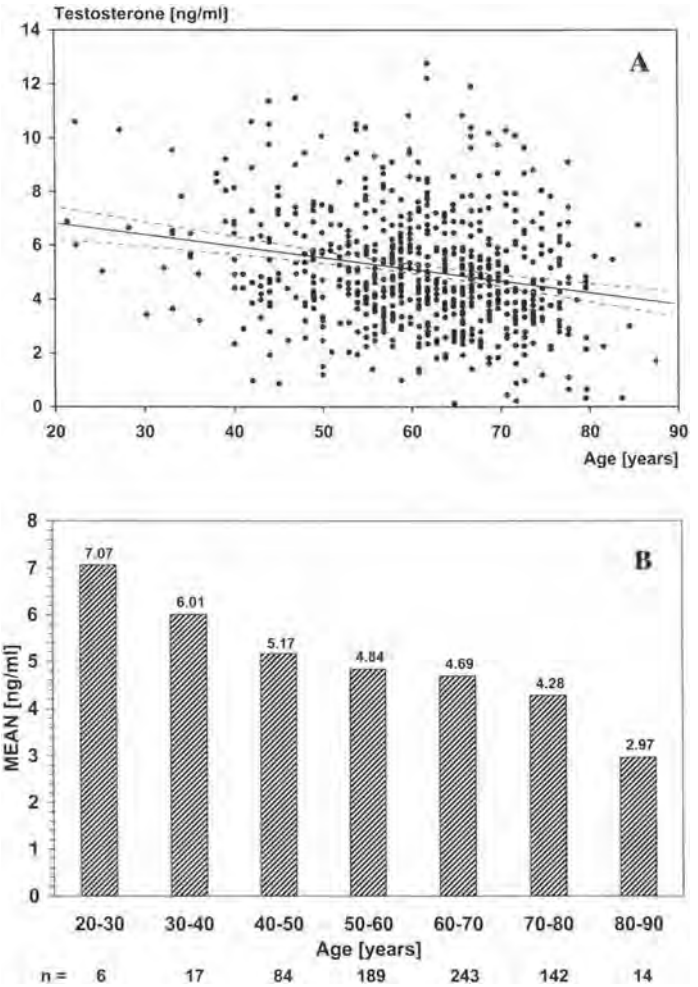

Fig. 2A,B. Total testosterone serum concentrations (**A**) in 698 men aged 21–88 years including the mean levels. The mean testosterone concentrations (**B**) are significantly age-related decreased ($P > 0.001$) (WINKELMANN 1998)

In 466 male subjects, ranging in age from 2 years to 101 years, BAKER et al. (1976) found that free testosterone levels, as well as estradiol levels, fell in old age. The metabolic clearance rates (MCRs) of testosterone and estradiol also fell in old age, while the conversion of testosterone to estradiol was increased. SIMON et al. (1992) found that both total testosterone and estradiol levels showed a significant stepwise decrease with age ($P < 0.001$) starting in the early adult years, while estrone levels did not vary. This decline in testosterone and estradiol with age remained significant after adjustment for body-mass index, subscapular skinfold, and tobacco and alcohol consumptions, and they were

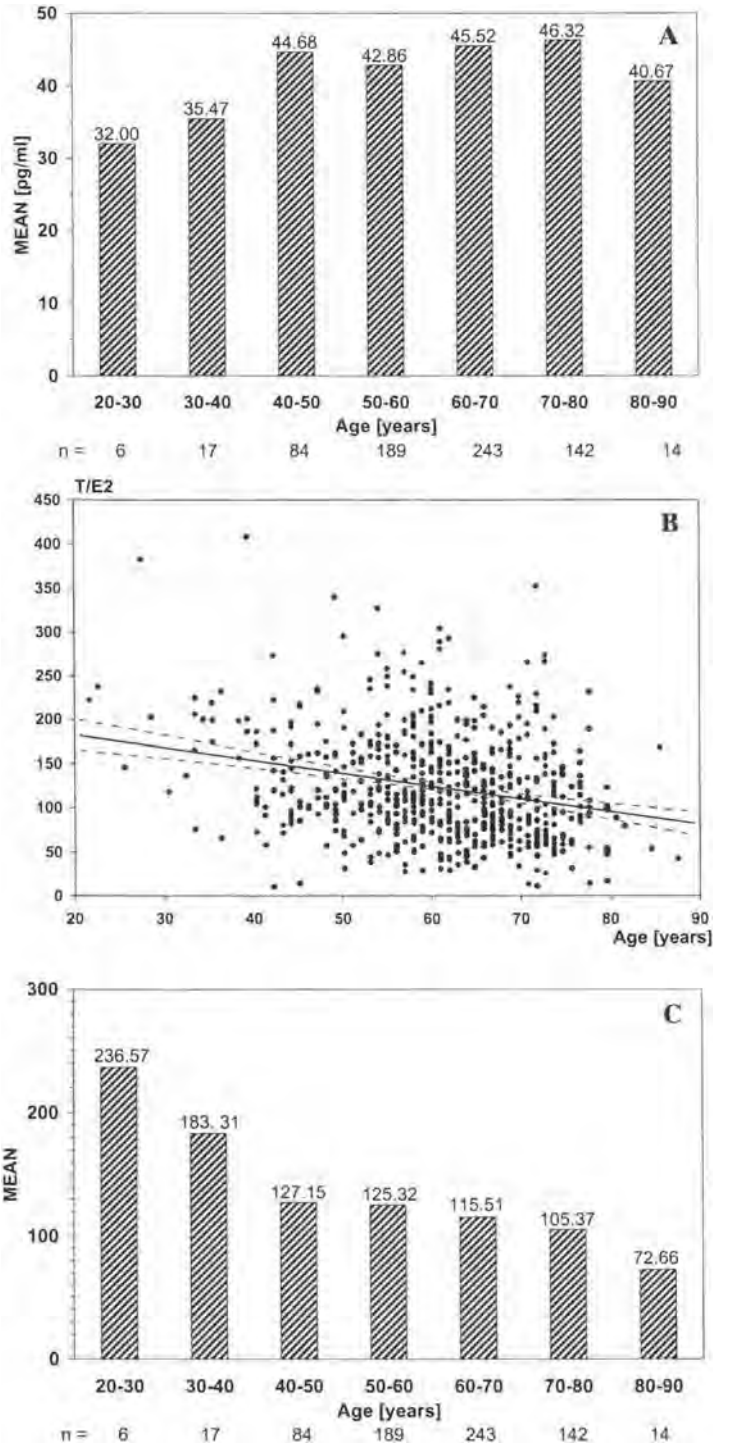

Fig. 3A–C. Mean serum-17β estradiol levels (**A**) in 698 men aged 21–88 years. There are no age-related changes. Additionally, the serum testosterone/estradiol-ratio (**B**) and the mean of the testosterone/estradiol-ratio (**C**) is shown. The mean testosterone/estradiol-ratio exhibits a significant age-related decrease ($P < 0.001$) (WINKELMANN 1998)

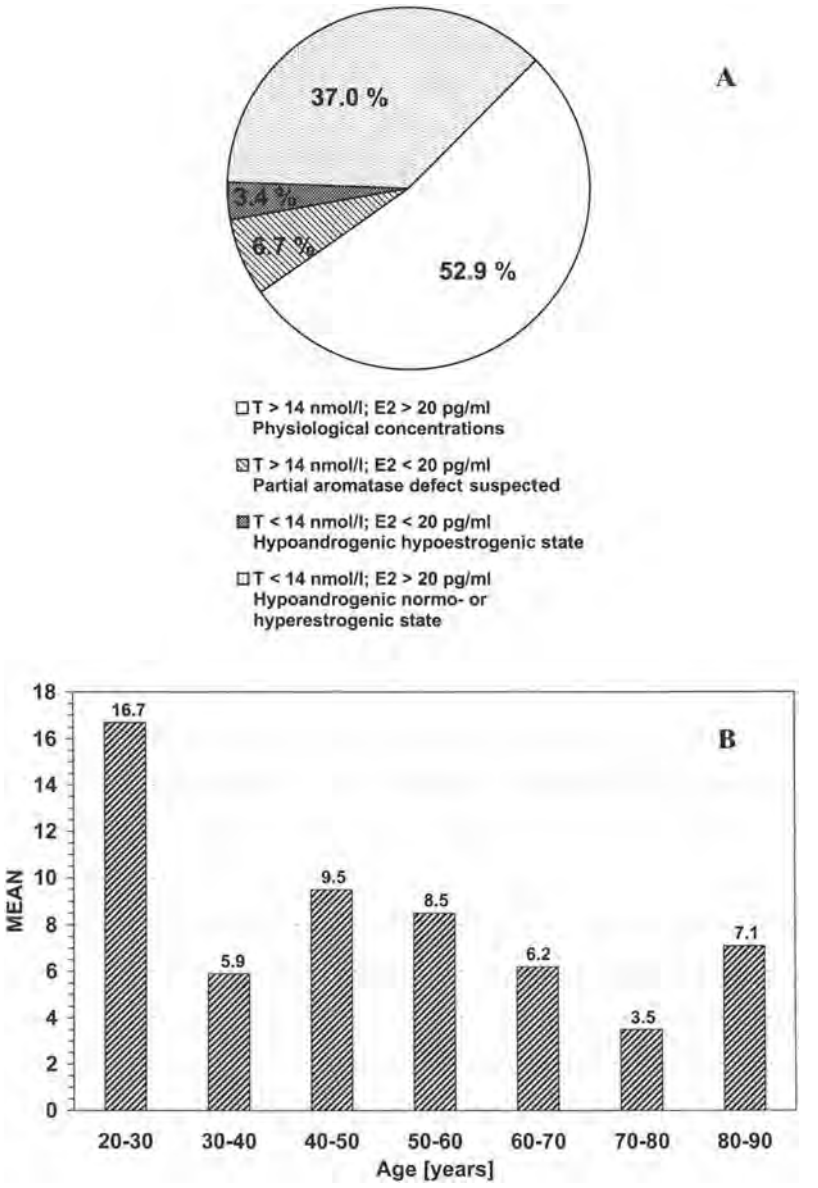

Fig. 4A,B. Testosterone and 17β-estradiol serum concentrations in 698 men aged 21–88 years (**A**). Note the assumed partial aromatase defect in 6.7% of the volunteers. There are no significant age-related changes in the portion of the assumed partial aromatase defect (**B**) (WINKELMANN 1998)

not modified by exclusion of the men who reported chronic disease. Also, VAN DEN BELD et al. (1998) found that estradiol significantly decreased with age in elderly men. In contrast to testosterone, estradiol seems to be an independent determinant of quality of life, physical performance, and proximal femur bone-mineral density (BMD). In a population-based, age-stratified sample of 346 men, aged 23–90 years, serum total T and E (17β-estradiol plus estrone) levels decreased over the life span by 30% and 12%, respectively, but bioavailable (non-SHBG bound) T and E levels decreased by 64% and 47%, respectively (KHOSLA et al. 1998).

As testosterone levels decrease and estradiol levels increase, the ratio of free testosterone to estradiol reaches a critical point and the estrogenic gonadotropin-suppressive effects predominate. The suppression of LH inhibits endogenous testosterone biosynthesis in the Leydig cell. This ratio may signal the biological point of no return and could become one of the criteria for defining the separation of the transitional hypogonadal state from the final "end stage" hypogonadotropic hypogonadal state (COHEN 1998).

However, a hypogonadotropic hypogonadal state represents the relatively infrequent form of hypogonadism in elderly men. The other form observed more often is a hypergonadotropic hypogonadal state (TENNEKOON and KARUNANAYAKE 1993). According to DAVIDSON et al. (1983), the age-related decrease in free testosterone is greater than that in total testosterone. Prolactin and 17β-estradiol did not change with age. There is a simultaneous age-related increase in the gonadotropins FSH and LH.

One could therefore speculate about the two forms of male hypogonadism:

- Hypergonadotropic hypogonadism: T $\downarrow$ and E2 $\rightarrow$ or $\downarrow$
- Hypogonadotropic hypogonadism: T $\downarrow$ and E2 $\uparrow$

LH-pulse frequency is significantly lower in elderly than in young men. The pulse amplitudes are similar. Similarly, T-pulse frequency is lower in elderly than in the young men and the hypothalamo-pituitary complex is more sensitive to dihydrotestosterone (DHT) feedback, as determined by the decrease in serum LH and T levels. Moreover, during DHT administration (125 mg/day, percutaneously, for 10 days), the LH response to LH-releasing hormone (LHRH) is significantly higher in elderly men compared to the pretreatment response. During estradiol administration (1.5 mg/day, percutaneously for 10 days), the LH response to LHRH is decreased in elderly men but unchanged in young men, suggesting greater responsiveness to estradiol in elderly men (DESLYPÈRE et al. 1987).

According to UMBREIT (1996), there is an estrogen deficit if the level is below 20 pg/ml, and estrogen deficit is said to be frequently accompanied by high androstenedione concentrations (>3.0 ng/ml). However, exact data in a low serum-concentration range should be interpreted with some caution, since most commercially available radio- or enzyme immunoassays do not work precisely in this concentration range. Recently, however, ultrasensitive

bioassays for estradiol having sufficient sensitivity for monitoring estrogen-deficient men have been developed (KLEIN et al. 1998).

In contrast to testosterone, no diurnal rhythm was found for estradiol secretion in aged men (MURONO et al. 1982). According to JANSSEN et al. (1998), free, non-SHBG-bound estradiol is positively correlated with serum insulin-like growth factor-1 (IGF-1) levels in men. O'CONNOR et al. (1998) found that serum IGF-I levels declined with age in both men and women. In men, the decline was linear, whereas IGF-1 levels decreased faster in women less than 45 years of age than in older women or in men.

In males suffering from systemic lupus erythematosus, the estradiol levels were significantly lower than in controls (VILARINHO and COSTALLAT 1998). Chronic exercise training – in contrast to testosterone – does not cause a decline in E2 in men (HACKNEY et al. 1997).

C. Metabolism and Pharmacokinetics of the Estrogens in the Male

The main source of estrogens is the adipose tissue. In addition, however, there is a variety of other sources, such as the adrenal gland, brain, breast, hair, liver, and testis. The daily production rate of estrogens in men is comparable to that in postmenopausal women. Of the total estrogen production, the testes contribute 5% in the form of 17β-estradiol and 25% in the form of estrone production (HABENICHT 1998).

According to ZUMOFF et al. (1968), the urinary elimination of estrogen is faster in young women than in elderly women and men. However, the biliary excretion is larger in men than in women. Moreover, the conjugation of the metabolites differs between men and women.

The urinary concentrations of estrone, estradiol, and estriol in men range between 20% and 30% of those in cyclic women. However, the 2-hydroxy estrone or the 2-methoxy estrone metabolites in male urine amount to 40–90% of the quantities measured in women. These observations show that, in males, the metabolism of estrone on the ring A (in contrast to ring C) is preferred (FISHMAN 1980). Also, in blood plasma the percentage of the 2-hydroxy estrone fraction is clearly higher than that of the estrone, estradiol or estriol fractions. This is important, inasmuch as the 2-hydroxy or 2-methoxy metabolites are counted among the non-dangerous estrogens because these compounds fail to show up as mutagens in cell-culture studies or as carcinogens in animals (SERVICE 1998). So, in men, the "good" metabolic pathway of the estrogens is preferred. Hydroxyestrone shows antiproliferative activities in vitro as well as in vivo (BRADLOW et al. 1996). In every experimental model in which 2-hydroxylation was increased, protection against tumors was achieved. Correspondingly, when 2-hydroxylation was decreased, an increase in cancer risk was observed. 2-Hydroxyestradiol in men is preferably metabolized to 2-methoxyestradiol (BANGER et al. 1996). 2-Methoxyestradiol is the only

metabolite of estrogen devoid of uterotropic, vaginotropic, or tumorigenic activity in vivo and is now emerging as a potential therapeutic agent for the treatment of angiogenically based diseases. Recent studies have shown 2-methoxyestradiol to be a potent nonspecific antimitotic agent in vitro and an effective oral anti-angiogenic and antitumor agent in vivo. It may be the first endogenous chemotherapeutic compound that is a physiological metabolite in humans (Pribluda and Green 1998). In addition, it is a particularly interesting fact that the phytochemical indole-3-carbinol, a constituent of cabbage-family vegetables, strongly increases the "good" estrogen 2-hydroxylation (Telang et al. 1997; Michnovicz 1998). Wong et al. (1997), in a dose-ranging study of 60 women at increased risk of breast cancer, demonstrated a significantly increased urinary 2-hydroxyestrone-to-16α-hydroxyestrone ratio after giving subjects 300 mg indole-3-carbinol per day.

In men, 2-hydroxy estrone suppresses gonadotropins when given in doses high enough to compensate for their rapid clearance and degradation (Merriam et al. 1983). The metabolism of 2-hydroxyestradiol in men is dominated by methyl ether formation, that of 4-hydroxy estrone by direct conjugation (Emons et al. 1987). However, it should be taken into account that under physiological conditions the plasma value of 2-hydroxy estrone (approximately 15 pg/ml) is below the detection limit for most of the assays (Kono et al. 1980, 1983).

Zimmermann et al. (1994) reported on gender-specific differences in the pharmacokinetics of ethinyl estradiol (EE). In a study using an oral combination of EE (0.06 mg/day) and levonorgestrel (0.250 mg/day) in male and female volunteers, the area-under-the-curve values for EE were 2.850 pg/ml/h in males and 6.216 pg/ml/h in females. The C_{max} values were 251 pg/ml in males and 495 pg/ml in females. The elimination half-lives were 10.2 h in the males volunteers and 16.5 h in the women. These results show, obviously, that the EE absorption in males is lower or the first-pass effect greater in males than in females. Additionally, the excretion of the estrogen is faster in males. Is this phenomenon part of a "protection mechanism" of the male organism e.g. against environmental estrogens?

D. Genitourinary System

I. What Can We Learn from the ERα Knock-Out (ERKO) Mice?

Both sexes produce estrogens, but at present relatively little is known about the physiological role of the "female" hormone in males. Hess et al. (1997) showed that estrogen-receptor activation is necessary for normal male fertility, and indicated that the effects of estradiol are important for reproduction in males (Berg 1998). In a case report of a man with dysfunctional ERs, the sperm count was normal but the viability of the sperm was low (Smith et al. 1994). A low level of estrogen caused by aromatase deficiency was seen in a

24-year-old man with increased testicular volume (MORISHIMA et al. 1995), whereas a 38-year-old man with the same deficiency had small testicles and severe oligozoospermia (CARANI et al. 1997).

The first ER was cloned and sequenced in 1986. It was denoted ERα (GREEN et al. 1986) after the identification of an additional receptor (ERβ) ten years later (KUIPER et al. 1996). In 1993, a knock-out mouse model lacking a functional ERα was created (ERKO mice; LUBAHN et al. 1993). The phenotype of male ERKO mice initially appeared normal and excluded a role for ERα in the fetal development of genitalia. Adult male mice became infertile, with atrophy of the testes and dysmorphic seminiferous tubules. Sperm from the mice were abnormal, and the sperm concentration in the epididymis was low (EDDY et al. 1996). Testicles of male ERKO mice developed normally until puberty, after which they started to degenerate. A transient increase in weight occurred between 32 and 81 days of age, when the rete testis became dilated and the efferent ductules were swollen, with increased luminal area. This was caused by an increased secretion of fluid by the testis or a defective removal of the fluid secreted. After 185 days of age, the observed atrophy and decreased weight of the testes appeared to result from long-standing increased luminal-fluid pressure (BERG 1998).

The efferent ductules conduct sperm from the testis to the epididymis (for review see ILIO and HESS 1994). They are the first site of epithelial ER expression in the developing male reproductive organ in mice, and both the male rat and marmoset monkey show a pronounced immunoexpression of ERα in the efferent ductules (COOKE et al. 1991; FISHER et al. 1997). The concentration of estrogens in fluid from the testes is high and comparable to the E2 level in females of reproductive age. Interestingly, spermatids express aromatase and may convert androgens to estrogens. The number of spermatozoa in transit to the epididymis may determine the estrogenic stimulation of the cells lining the efferent ductules. These cells have a well-developed apparatus for the reabsorption of fluid, and more than 90% of the fluid continuously secreted by the seminiferous epithelium of the testes is reabsorbed in the efferent ductules. By occluding the ductal system at different levels, HESS et al. (1997) demonstrated that the apparent increase in luminal pressure in mice lacking ERα was caused by a defect in the reabsorption of testicular fluid secretion. Experiments with cultured pieces of epididymis corroborated these findings. Thus, ERα seems to be important for fluid reabsorption and normal adult function of the efferent ductules. However, wild-type efferent ductules treated with the "pure" antiestrogen ICI 182.780 in vitro did not swell like the ductules from the ERKO mice. The presence and effects of ERβ in efferent ductules and the epididymis may explain this discrepancy (HESS et al. 1997).

II. Gonads

It is well known that androgens as well as estrogens are secreted from the testis, and the Sertoli cells contain ERs (PANNO et al. 1996). Estrogens play a

pivotal role in regulating spermatogenesis, e.g. by potentiating the stimulatory effect of FSH (MacCalman et al. 1997).

As shown above, in the estrogen receptor α gene-knock out (ERKαO) mice, estrogens are necessary for mating frequency, sperm number, and several sperm functions (Eddy et al. 1996). While the necessity for ERα-mediated function in male fertility has meanwhile been well supported by the ERαKO experiments, there are still some open questions about the physiological role of ERβ. This ER subtype is expressed in the Leydig cells, elongated spermatids, efferent ductules, and the initial segment of the epididymides of ERαKO mice, but the presence of ERβ is not able to compensate for the absence of ERα in male reproductive function (Rosenfeld et al. 1998).

For a number of years, estrogens have been discussed as one of the factors regulating testicular function locally. As in the 1970s, Dorrington and Armstrong (1975), Payne et al. (1976, 1987) and Rommerts et al. (1982) demonstrated aromatase activity to be present in the testis. In the context of this investigation, an age-dependent shift in the localization of this enzyme system was shown to exist. Originally, aromatase activity was demonstrated in the Sertoli cells of immature animals. Later on, it became evident that estrogens are produced in the Sertoli cells of immature animals only whereas, in the adult ones, Leydig cells are the major source (Kmicikiewicz et al. 1997). Most of the studies were performed in rats, but there is now good evidence that the same is true for human beings (Rommerts et al. 1982; Insker et al. 1995). This has not been fully clarified yet as far as the exact function of estrogens in the male gonads is concerned, but there are a number of suggestions that estrogens has a mitogenic action on Sertoli cells and Leydig cells (Payne et al. 1987). Furthermore, an inhibitory effect on 17α-hydroxylase/c17–20-lyase has been demonstrated, indicating a local paracrine/autocrine regulatory effect on testosterone production. Recently, Lio et al. (1997) reported on a direct stimulatory effect of estrogens on rat gonocyte proliferation mediated via the plateled-derived growth factor (PDGF) pathway, indicating an essential role of estrogens in supplying the adult testis with a sufficient number of germ cells. Furthermore, data are available indicating a direct inhibitory effect of pachytene spermatocytes and early spermatides on Sertoli cell estradiol production (Dupaix et al. 1996), a finding speaking clearly in favor of a possibly important role of estrogens in the context of a cross-talk between germ cells and Sertoli cells.

However, estradiol treatment can increase germ cell apoptosis mainly at stages IV-X of the spermatogenic cycle, rather than at stage VII when apoptotic germ cell death is mainly triggered by gonadotropin withdrawal e.g. caused by hypophysectomy. Therefore, estradiol plays a specific local role in the modulation of germ cell death in the adult testis (Blanco-Rodriguez and Martinez-Garcia 1997). Sah (1998) treated 14 men with oligospermia with daily 0.044 mg EE plus 3.6 mg methyl testosterone orally over 4 months. In 9 of the 14 men (64.2%), treatment was effective; there was some improvement in the remaining 5 men. The wives of three patients became pregnant within

6 months of therapy initiation. None of the patients had any side effects. The mean post-treatment semen index was 18.6–8.4× larger than the pre-treatment level.

Spermatozoa also express ERs (Durkee et al. 1998). Interestingly, sperm themselves are sources of estrogen. Hess et al. (1995) and Janulis et al. (1996) demonstrated that developing spermatids in several species contain aromatase. This observation is the basis for the hypothesis that estrogen, synthesized by sperm, plays a role in the regulation of epididymal function proportional to the number of sperm being transported. Human epididymides of fertile men aged 14 years to 64 years express ERs whereas, in the epididymis in boys aged from neonatal to 12 years and in men over 65 years, ER was not detected (Misao et al. 1997). Furthermore, the sperm penetration into oocytes is enhanced by estradiol (Chian et al. 1996).

III. Epididymis

Another target where estrogens might play a role is the epididymis, as this tissue is particularly rich in ERs. Furthermore, there is some evidence of a relationship between estrogens and sperm function (see above). There are new signs indicating that estrogens have a function in the reproductive system as well as in non-reproductive organs. As shown above, these new signs originate mainly from results obtained with the ERαKO mice (Lubahn et al. 1993; Eddy et al. 1996) and first descriptions of a naturally appearing defect either in the aromatase gene and/or the ER in men (Morishima et al. 1995; Smith et al. 1994). Unexpectedly, male ERKO mice were infertile (Eddy et al. 1996). Altough spermatogenesis was mainly normal, epididymal sperm were not able to fertilize an egg. These findings further support the data of Habenicht (1998) on the role of estrogens for male physiology. Treatment of rats with antiestrogens resulted in an inhibition of motility as well as alterations of acrosomal status. Smith et al. (1994) described a man with mutation on the aromatase gene and another man with mutation on the ER gene. In these cases, a disturbance of sperm motility was evident.

IV. Prostate

ERα is found almost exclusively in stromal components, with isolated reports indicating basal epithelial cell localization as well (Ehara et al. 1995; Prins and Birch 1997). However, it is widely believed that estrogen exerts its influence on the prostate mainly via the epithelium (Farnsworth 1996). From this point of view, it is remarkable that ERα mRNA was not detected in any epithelial samples in dysplastic epithelial tissues of Noble rats. In contrast to this, both testosterone and estradiol (or both combined) can induce large amounts of ERβ in the epithelium (Kuiper et al. 1997; Lau et al. 1998). On the basis of this finding one might speculate that estrogens or selective estrogen modulators (SERMs) with preferred binding to ERβ have stronger dysplastic

effects on the prostate than ligands showing stronger binding to ERα. Also, Prins et al. (1998) found that ERβ-mRNA was localized to rat prostatic epithelial cells, which contrasts with the normal stromal localization of ERα in the rat prostate.

Estrogens are known to be involved in the etiology of benign prostatic hyperplasia (BPH), probably because of their ability to increase androgen receptors (ARs; Moore et al. 1979). We know that estrogens can be produced in the prostate by aromatization of testosterone. Aromatase is present in the prostatic stroma, and aromatase activity has been reported to increase with age (Hemsell 1974).

What is new is that estradiol (but not diethylstilbestrol) can act as a natural ligand for the AR in DU145 human prostate cancer cells. While all three known AR coactivators – ARA_{70}, steroid receptor coactivator 1, and RAC3/ACTR – can enhance AR transcriptional activity at 1 nM dihydrotestosterone, Yeh et al. (1998) demonstrated that ARA_{70} can induce AR transcriptional activity less than 30-fold only in the presence of 17β-estradiol.

It is known that unliganded SHBG binds to a receptor (R_{SHBG}) on prostate membranes. The R_{SHBG}–SHBG complex is rapidly activated by estradiol to stimulate adenylate cyclase, with a resultant increase in intracellular cyclic adenosine monophosphate (cAMP). Nakhla et al. (1997) found that dihydrotestosterone (DHT) caused an increase in prostate-specific-antigen (PSA) secretion in serum-free organ cultures of human prostates. This event was blocked by the anti-androgens cyproterone acetate and hydroxyflutamide. In the absence of androgens, estradiol added to prostate tissue whose R_{SHBG} was occupied by SHBG reproduced the results seen with DHT. The estradiol-SHBG-induced increase in PSA was not blocked by anti-estrogens, but was blocked both by anti-androgens and 2-methoxyestradiol, which prevents the binding of estradiol to SHBG. Furthermore, an inhibitor of protein kinase A (PKCA) prevented the estradiol-SHBG-induced increase in PSA, but not that which followed DHT. The conclusion of the authors is that there is a signaling system that amalgamates steroid-initiated intracellular events with steroid-dependent occurrences generated at the prostate cell membrane and that the latter signaling system proceeds by a pathway that involves PKCA.

Concerning the metabolism of 17β-estradiol, Lane et al. (1997) found that the formation of catechol estrogens is much lower in the rat prostate than in the liver. Thus, these estrogen metabolites are unlikely to be involved in the hormonally induced prostatic dysplasia.

E. Mammary Gland

The mammogenic actions of estrogen consist of indirect actions via pituitary intermediaries such as prolactin (PRL) and growth hormone (GH) and direct effects on the mammary gland. The two direct estrogenic, mitogenic pathways

concern ductal elongation (ductal growth) and ductal maintenance (ductal branching) (SILBERSTEIN et al. 1994).

The connection between estrogen replacement and the development of breast cancer is the subject of controversial discussions. Existing evidence supports a causal relationship between use of estrogens and progestins, levels of endogenous estrogens, and breast cancer incidence in postmenopausal women. Hormones may act to promote the late stages of carcinogenesis among postmenopausal women and to facilitate the proliferation of malignant cells (COLDITZ 1998), but what about the situation in men?

In the male ERαKO mouse the mammary glands were undeveloped, with only vestigial ducts present at the nipples, indicating a causal role of estrogens for the development of the male breast (KORACH 1994). Gynecomastia is common in adolescent and adult men, and reflects in some – but not in all cases – an underlying imbalance in hormonal physiology in which there is an increase in estrogen action relative to androgen action at the breast-tissue level (WILSON et al. 1980; KORENMAN 1985; MATHUR and BRAUNSTEIN 1997). According to BAUDUCEAU et al. (1993), gynecomastia is present in almost 40% of young men and is linked with reduced T and hypogonadism. This suggests an increased aromatase activity. However, SASANO et al. (1996) found relatively strong aromatase immunoreactivity in only 11 of 30 cases (37%) of gynecomastia; in contrast to this, they found such immunoreactivity in all 15 cases (100%) of mammary carcinoma. ER and progesterone-receptor (PR) expression was observed in the nuclei of ductal cells in all cases of gynecomastia.

Carcinoma of the male breast is rare and represents only 1% of the female mammary cancer rate (RIBEIRO 1985). There is strong circumstantial evidence to implicate hormonal factors in the development of male breast carcinoma (ROGERS et al.1993). However, some investigators assume that there has been no consistent evidence of increased serum estrogen or progesterone concentrations in men with breast malignancy. However, ERs and PRs are found even more commonly in breast carcinomas of men than in breast carcinomas of women (ROGERS et al. 1993; PICH et al. 1994). Altered androgen metabolism has also been proposed to be involved in the development of male breast carcinoma (LABACCARO et al. 1993). According to THOMAS (1993), it is now suggested that men with breast carcinoma may have endocrine profiles different from those of normal male subjects. Increased aromatase expression in the malignant stromal cells is considered to contribute to the increment in the in situ estrogen concentration and the development of male breast carcinoma (SASANO et al. 1996). Male breast carcinoma patients showed a positive correlation between ERs and PRs; however, there was a lack of correlation between heat-shock protein 27 and ERs or PRs. This lack of correlation differs from the results known for female breast carcinoma, suggesting that male breast carcinoma and female breast carcinoma are biologically different tumors (MUNOZ DE TORO and LUQUE 1997).

There is some evidence that the cardiac glycoside digoxin may share intriguing similarities to SERMs. This digitalis glycoside shares a structural homology with steroid hormones, and digoxin-induced gynecomastia in men is well known (SCHUSSHEIM and SCHUSSHEIM 1998).

F. Liver

I. Estrogens

Relatively little is known about gender-specific estrogen effects on the liver. In male rats, cholestasis can be induced by EE or estradiol-17β-glucuronide (ALVARO et al. 1997;TAKIKAWA 1997). Gallstone disease is known in men during estrogen treatment of prostatic carcinoma (ANGELIN et al. 1992). Hepatic calcium-binding protein regucalcin mRNA was expressed in male rats more than in females (UEOKA and YAMAGUCHI 1998), and there are profound gender-dependent differences in the regulation of sulfotransferase mRNA in female and male rats (KLAASSEN et al. 1998).

In men, LDL-receptor expression under estrogen treatment is 3× higher than in untreated controls, and the 3-hydroxy-3-methylglutaryl-coenzyme A reductase was increased two-fold (ANGELIN et al. 1992). Oral estrogen treatment induces a decrease in expression of sialyl Lewis X and α_1-acid glycoprotein in females and male-to-female transsexuals (BRINKMAN-VAN DER LINDEN et al. 1996).

There is a great deal of controversy regarding hormonal changes in hepatic cirrhosis patients. GLUUD et al. (1983, 1987), on behalf of the Copenhagen Study Group for Liver Diseases, claim that T levels in cirrhotic patients could be low, normal, or subnormal, and that estrone and E2 levels should be high. However, in most of the reports in the literature it is maintained that T levels are lower in cirrhotic patients, while estrogens are normal or a little higher in severe cases (VAN THIEL et al. 1981; WANG et al. 1991). Still other reports declare that estrogen levels are found to be lower in cirrhotic patients (WANG et al. 1993). It has been reported by GORDON et al. (1975) that hepatic extraction of testosterone decreases in cirrhosis, because testosterone circulates towards extrasplenic tissues (mainly in fatty tissues and muscles), peripheral aromatization increases, and finally the level of estrogen increases. VAN THIEL et al. (1980, 1983) have shown in rats that after diverting portal blood flow, T decreases, testicular atrophy develops, estradiol formation increases, and the hypothalamo-pituitary-gonadal axis is disrupted. It is generally accepted that the primary mechanism causing hormonal changes in cirrhosis is blocked entero-hepatic circulation and portal hypertension, disrupting the hypothalamic-pituitary-gonadal axis (VAN THIEL et al. 1974; GAVALER and VAN THIEL 1988). Nevertheless, a significant increase in the level of SHBG and a decrease in the level of free T because of the high affinity of SHBG have been reported in cirrhotic patients. CETINKAYA et al. (1998) investigated 60 patients with postnecrotic cirrhosis and alcoholic cirrhosis at age 40 and over, and 20

The male risk of osteoporosis is often underestimated. However, men will be 5 years older on average at the time of reaching the critical bone density. Therefore, men and women of the same age and the same bone density are at the same fracture risk (DELAET et al. 1997). Bone loss accelerates with age, as seen more clearly in men than in women (BURGER et al. 1998). Compared with women, elderly men presenting with hip fracture have a higher mortality and have more risk factors for osteoporosis (DIAMOND et al. 1997). Adjusting for age, body-mass index (BMI), and BMD at the trochanter in grams per square centimeter, men had a two-fold higher risk of deformity than women (LUNT et al. 1997). The bone density of the ERαKO male mice was 20 to 25% lower than in wild-type mice, suggesting a direct role for estrogen in male bone physiology (KORACH 1994).

Concerning the race- and gender-specific incidence rate of hip fractures in the United States, JACOBSEN et al. (1990) found a higher risk for white men than for black women. Since black women have a high aromatase activity, this study provides the first epidemiological indication of the importance of estrogens for the etiopathogenesis of male osteoporosis.

Reports of severe osteopenia in several men with estrogen deficieny (ER abnormality or absent aromatase activity) have raised questions concerning the role of estrogens in maintaining bone mass (SMITH et al. 1994; MORISHIMA et al. 1995). Aromatase activity is present in bone (SASANO et al. 1997), and probably the local production of estrogen may not be mirrored by serum estrogen concentrations (ORWOLL 1998). In transsexuals, estrogens can maintain bone mass in the presence of low concentrations of androgens after orchiectomy (VAN KESTEREN et al. 1996), and some reports suggest that estrogen levels are closely associated with bone mass in older men (SLEMENDA et al. 1987; GREENDALE et al. 1997). Although studies in experimental animals and in osteoblastic cells in vitro indicate that nonaromatizable androgens are potent modulators of skeletal homeostasis (WAKLEY et al. 1991; GOULDING and GOLD 1993; MASON and MORRIS 1997; WIREN et al. 1997), the important roles of estrogens – including aromatization of androgens to estrogens – is beyond question. For example, 17β-estradiol reduces IL-1β-induced IL-6 mRNA production and IL-6 secretion by human osteoblast-like cells (KOKA et al. 1998).

Summarizing the preclinical and clinical arguments favoring a role of estrogens on skeletal homeostasis in the male, VANDERSCHUEREN (1996) stressed the following points:

- Both androgens (BELLIDO et al. 1995) and estrogens (GIRASOLE et al. 1992) are able to prevent osteoclastogenesis in vitro via interleukin-6 after stimulation of their specific receptors in bone marrow cells
- Male-derived osteoblasts not only possess ERs (COLVARD et al. 1989) but are also able to aromatize androgens into estrogens (BRUCH et al. 1992)
- Both androgens and estrogens prevent bone loss in the aged male rat by a similar mechanism (FRANCIS et al. 1986)

- Androgen deficiency in the aged male rat (VANDERSCHUEREN et al. 1992) has skeletal effects very similar to estrogen deficiency in the aged female rat (KALU et al. 1984).
- Both ERKO mice (LUBAHN et al. 1993) and ER deficiency in man (SMITH et al. 1994) were reported to be associated with osteopenia
- Both inhibition of aromatization in the rat (VANDERSCHUEREN et al. 1995) and aromatase deficiency in man (KENAN et al. 1995) are also associated with osteopenia

Men with a defect in the aromatase gene or in the ER gene showed incomplete epiphyseal closure, resulting in continued growth and marked osteopenia, clearly indicating an important role of estrogens for bone physiology in men (SMITH et al. 1994). Treatment of a man with aromatase deficiency with daily 0.3–0.75 mg conjugated estrogens ceased non-physiological linear growth and increased bone mass (BILEZIKIAN et al. 1998). Although androgens have direct growth-stimulating actions on the epiphysis, the final phase of skeletal maturation associated with epiphyseal closure seems to be primarily an estrogen-dependent phenomenon in men (BACHRACH and SMITH 1996). In healthy men, ERα mRNA was consistently identified in osteoblasts and chondrocytes (KUSEC et al. 1998). As early as 1994, LIESEGANG et al. succeeded in detecting two ER types in human osteoblast-like cells. Both high-affinity, low-capacity and low-affinity, high-capacity specific [^{3}H]17β-estradiol binding were demonstrable.

17β-Estradiol inhibits bone resorption by directly inducing apoptosis of the bone-resorbing osteoclasts (KAMEDA et al. 1997). In 346 men aged 23–90 years, univariate analyses showed that serum bioavailable T and E levels correlated positively with BMD at the total body, spine, proximal femur, and distal radius and negatively with urinary N-telopeptide of type-I collagen (NTx) excretion. Urinary Ntx excretion was also negatively associated with BMD. By multivariate analyses, however, the serum bioavailable E level was the consistent, independent predictor of BMD in men. These studies suggest that, in contrast to traditional belief, age-related bone loss may be the result of E deficiency not just in postmenopausal women, but also in men (KHOSLA et al. 1998).

In a case-control study, 56 men with vertebral fractures had E2 levels 30% (SD 5%, $P < 0.0005$) lower than controls (BERNECKER et al. 1995). In a cross-sectional study in 37 healthy older men with no history of bone disease, ANDERSON et al. (1996) found that BMD at the LS and hip correlated more closely with E2 ($R = 0.383$, $P < 0.03$) than with T ($R = 0.245$, $P > 0.15$). In a prospective study of 93 healthy men aged over 65 years, E2 levels were positively associated with initial BMD values at all sites (SLEMENDA et al. 1995) and were associated with significantly lower rates of bone loss at the radius and hip on twice-yearly BMD measurement over a mean of 2 years ($P < 0.05$, test for trend), whereas T levels were not predictive (SLEMENDA et al. 1996). In men with vertebral osteoporosis, E2 levels were found to be positively

correlated with BMD at the femoral neck ($R = 0.29$, $P > 0.03$; Bernecker et al. 1995) and negatively correlated with markers of bone resorption such as hydroxyproline ($R = -0.57$, $P < 0.05$). On bone morphometry, T and E2 levels were associated with differing and complementary features such as trabecular thickness and trabecular number (Selby et al. 1995). In a therapeutic trial of testosterone supplementation in men with vertebral osteoporosis (Anderson et al. 1997), pharmacological doses of testosterone were associated with proportionate rises in E2 levels. Increases in LS-BMD during testosterone therapy correlated more closely with changes in E2 than changes in T.

The essential role of estrogens for development and maintenance of the male skeleton is proved by a variety of further clinical studies. Bone density and sex steroids were measured in 93 healthy men aged over 65 years at 6-month intervals for an average of 2.1 years. Bone density was significantly positively associated with greater E2 ($R = 0.21$; $P < 0.05$) at all skeletal sites. There were weak negative correlations between T and bone density at the spine and the hip. SHBG was negatively associated only with bone density in the greater trochanter. Greater body weight was associated with lower T and SHBG and greater E2. These data indicate that, within the normal range, lower T is not associated with low bone density in men (Slemenda et al. 1997). In 534 men aged 68.6 years on average (Rancho Bernardo Study) a statistically significant positive relation was seen between bioavailable estradiol and BMD at all sites (Greendale et al. 1997). In age-related (type-II) femoral neck osteoporosis in men, the free estradiol index in 40 patients was significantly lower (1.3) than in 40 control subjects (Boonen et al. 1997).

403 healthy men living independently (aged 73–94 years) were randomly selected from a population-based sample. E2 decreased significantly with age. In contrast to this, T did not change with age. E2 was significantly related to BMD at all sites. This was independent of T (van den Beld et al. 1998; Janssen et al. 1998). This also raises the question whether or not men may have a partial aromatase deficiency. The hypothesis is supported by the study of Kaps and Girg (1998). After all, out of 302 male patients with osteoporosis, 56.7% had estrogen deficiency (E2 less than 20 pg/ml; that means practically non-detectable concentrations for conventional assays). However, only 20.5% of the patients showed testosterone deficiency (T < 2.5 ng/ml). Finally, Bernecker et al. (1995) found that mean levels of serum estradiol but not testosterone were significantly reduced in men with established idiopathic osteoporosis. Collectively, these data support the hypothesis that estrogen deficiency plays a major role in involutional bone loss in men (Riggs et al. 1998) and that the characterization of an – at least partial – aromatase defect will certainly stimulate future clinical research.

Has this knowledge already led to therapeutic consequences in the prevention and therapy of male osteoporosis? So far, unfortunately not. In view of the unknown side effects of estrogen replacement in osteoporotic men, many clinicians may prefer to use other agents, such as calcitonin, bisphosphonates, or perhaps fluorides. Nevertheless, carefully designed trials with suit-

able estrogenic drugs offer the prospect of better understanding the role of testosterone-independent estrogen deficiency in men (ANDERSON et al. 1998). Here, too, clinical studies including male-to-female transsexuals may be useful. In these patients, estrogen therapy definitely prevents bone loss after testosterone deprivation (VAN KESTEREN et al. 1998). According to SCHLATTERER et al. (1998), for male-to-female transsexual patients undergoing cross-gender hormone replacement therapy, the risk of developing osteoporosis is very low.

However, not only bone loss but also the regulation of chondrocyte metabolism is estrogen-dependent. 17β-estradiol inhibits cyclooxygenase-2 mRNA expression in chondrocytes, suggesting that estrogens could be implicated in the control of cartilage metabolism (MORISSET et al. 1998).

H. Cardiovascular System

Estrogen receptors α (ERα) were found in either cell fraction of aorta samples in men. Therefore, the male blood vessels may be considered as estrogen-sensitive (CAMPISI et al. 1993). In male Sprague-Dawley rats, ERα as well as estrogen receptor β (ERβ) were expressed in the aorta. The level of expression of ERα and ERβ-mRNA in male-rat aortas was examined before and after vascular injury using *en face* (HÄUTCHEN) preparations and in situ hybridization. Little or no change in ERα expression was observed after vascular injury in either vascular endothelial or smooth muscle cells at any time point. In contrast, ERβ-mRNA was found to be expressed markedly after balloon injury. In endothelial cells, ERβ was increased by 2 days after injury, and high levels of expression were maintained at 8 days and 14 days. Furthermore, ERβ expression was high in luminal smooth muscle cells at 8 days and 14 days after injury and had decreased to low levels by 28 days after injury. These data demonstrate the presence of ERβ in male vascular tissues and the induction of ERβ-mRNA expression after vascular injury, supporting a role for ERβ in the direct vascular effects of estrogen (LINDNER et al. 1998).

Interestingly, the story of the cardiovascular estrogen effects in men started in 1952 with the paper of BARR et al. (1952). In 16 men with advanced atherosclerosis, they found that treatment with extremely high dosages of 1 mg EE or 15 mg conjugated estrogens/day over 9 weeks decreased the plasma concentrations of total cholesterol and changed the cholesterol subfractions.

But the continuation of the estrogen story was not very promising. Estrogen supplementation in men with proven cardiovascular disease and previous myocardial infarction (MI) has been less successful (THE CORONARY DRUG PROJECT RESEARCH GROUP 1970). Unfortunately, oral estrogen dosing in this group was approximately 5–10× that used in replacement estrogen doses for postmenopausal women. At a dose of 5.0 mg of conjugated equine estrogens, a significant increase in recurrent MI, venous thrombosis, and pulmonary embolism occurred in men with established cardiovascular disease. However,

at lower doses (2.5 mg of conjugated equine estrogens) an insignificant increase in these clinical complications was noted.

Recently, there was bad news: the mean E2 level in men who had had a MI was higher ($P = 0.002$) than in men who had not had MI (Phillips et al. 1996). Estradiol supplementation augmented the flow-mediated vasodilatation and serum level of nitrite/nitrate (metabolites of NO) in women but not in men (Kawano et al. 1997).

It is well known that estrogens have also been used in the treatment of prostate cancer in men. Again, high doses of oral estrogen have been associated with an increased risk of death from cardiovascular disease. High-dose estrogens may be prothrombotic in elderly men (Byar and Corle 1988). On the other hand, this risk has not been associated with lower doses of estrogen (0.2–1.0 mg of estradiol). In addition, many of these studies in men have evaluated the cardiovascular risk in the setting of metastatic prostatic cancer. These are obviously patients with an increased risk of thromboembolic disease, as often seen in malignancies (Henriksson et al. 1990).

Fortunately, the estrogen story in men took a turn for the better. Estrogen treatment limits transplant-associated atherosclerosis in male animals by mechanisms thar may require receptor activation and transcriptional regulation of growth factors and expression of major-histocompatibility-complex class-II antigens (Cheng et al. 1991; Saito et al. 1997). Whether activation of specific ERs (α and/or β) is required for the vascular effects of estrogen to be mediated is unclear. Probably, estrogen receptor α is not required, as estrogen reduces proliferation after arterial injury and increases endothelium-mediated vasodilatation in male mice and humans deficient in this receptor (Iafrati et al. 1997; Rubanyi et al. 1997; Sudhir et al. 1997).

The cardiovascular protective effects of estrogen in men are known to be mediated by its beneficial effects on lipid metabolism as well as by its direct actions on the vessel wall (Knopp and Zhu 1997; Selzman et al. 1998). The latter can be mediated also by a specific ER present on smooth muscle cells and endothelial cells (Matsubara et al. 1997). Nevala et al. (1998) found no gender differences concerning the estrogen-induced relaxation of mesenteric artery in vitro.

Meanwhile, we know the clinical-molecular-biological basis for this phenomenon. A man with a disruptive mutation in the ER gene showed premature coronary-artery disease (Sudhir et al. 1997a). The sublingual administration of estradiol increased the brachial-artery diameter in the same manner as sublingual nitroglycerin, indicating a nongenomic mode of estrogen action (Sudhir et al. 1997b). Komesaroff et al. (1998) describe a different nongenomic action of estrogen on the cardiovascular system (CVS). To examine the time course and mechanisms of action of single doses of estrogen on skin microvasculature, two double-blind placebo-controlled cross-over studies were conducted in healthy young men using the noninvasive technique of laser Doppler velocimetry with iontophoretic application of vasodilator substances. Estradiol (2 mg sublingually) produced a significant increase in the

response to the endothelial vasodilator acetylcholine (Ach) after 15 min, but not after 20 min or 30 min. An intravenous (iv) bolus of 25 mg conjugated equine estrogens produced significant increases in the response to Ach at 15 min and 20 min but not at 30 min. There was no change in responses to the non-endothelial vasodilators sodium nitroprusside or nicotine, and administration of placebo produced no change in Ach responses at any time point. These experiments show that, at E2s within the physiological range for premenstrual women, estrogens act directly on the cutaneous microvasculature through a rapid onset – rapid offset, nongenomic mechanism that is specific to the endothelium. In addition, these experiments support the view that estrogens can act on the male CVS in a manner that is potentially clinically beneficial.

Iv conjugated estrogens (0.625 mg once) favorably modulate acetylcholine-induced changes in coronary hemodynamics in men. This suggests that estrogens may have anti-ischemic effects in men (Blumenthal et al. 1997). The results of iv conjugated estrogens (1.25 mg) in men with cardiac allografts point in the same direction. The acutely given estrogens abolished abnormal cold pressor test (CPT)-induced coronary constriction. This favorable vasomotor effect suggests that estrogen may prevent inappropriate coronary-artery constriction in men with cardiac transplants (Reis et al. 1998).

However, Collins et al. (1995) found that 20 min after intracoronary administration of 2.5 μg of 17β-estradiol into atherosclerotic, nonstenotic coronary arteries of nine postmenopausal women and seven men 52 ± 4 years old, the estrogen modulated the acetylcholine-induced coronary responses of female but not male atherosclerotic arteries in vivo.

There were significant negative associations of urinary total estradiol excretion with total cholesterol, LDL cholesterol, and apo B levels in 46 healthy Chinese men (Ooi et al. 1996). In 313 Japanese men aged 50–54 years, HDL and HDL_2 cholesterol were positively associated with E2, and E2 levels were also higher among current alcohol drinkers. Obesity, especially waist-to-hip ratio, was a strong correlate of both total and free testosterone, but not of estradiol (Handa et al. 1997). The administration of exogenous estrogen increases HDL-cholesterol levels in men (Wallentin and Varenhorst 1978).

Because the estrogen levels are rather low, they were not regarded as physiologically important until recently, when epidemiological research into heart-disease risk suggested a protective effect of endogenous estrogens in men (Khaw and Barrett-Connor 1991). This is partly explained by the fact that a lower proportion of circulating estradiol is bound to SHBG in men than in women due to competition with the higher levels of androgens (Anderson 1974). In subsequent prospective controlled trials, Bagatell et al. (1994) treated men receiving gonadotropin-releasing hormone (GnRH) agonist and testosterone replacement therapy with an aromatase inhibitor and showed that the estrogen-deficient men had a significant 8% fall in HDL cholesterol compared with the other group or normal controls, demonstrating that the

levels of estradiol normally found in men are sufficient to alter lipid profiles favorably. ZMUDA et al. (1993) showed that adding an aromatase inhibitor to supraphysiological testosterone treatment in weightlifters caused a 38% increase in lipoprotein-lipase activity, with worsening HDL levels.

It has been clearly demonstrated that the treatment of men with non-aromatizable androgens (anabolics, DHT derivatives) leads to an increase in LDL and triglyceride and a decrease in HDL, whereas treatment with aromatizable androgens such as testosterone enanthate only marginally affects the lipid system. However, when these men were treated with an aromatase inhibitor simultaneously, a decrease in HDL was observed to change the HDL/LDL ratio in favor of LDL. In summary, there were no significant effects of testosterone esters and other aromatizable androgens on HDL, but major effects on lipid levels by anabolic steroids which cannot be converted to estrogens (SUDHIR et al. 1997; FRIEDL et al. 1990; BAGATELL et al. 1994). Consequently, the administration of 17β-estradiol to a man with an ER defect shows the same benefits as estrogen replacement in postmenopausal women (SUDHIR et al. 1997). This shows that, in men, nongenomic estrogen actions are more important for the extragenital estrogen benefits. According to YEUNG (1997), the development of an estrogenic molecule that can be vascular protective yet devoid of feminizing properties could be very promising for estrogen replacement in men.

A combination of oral and parenteral estrogens administered to males with prostatic cancer profoundly lowers LDL cholesterol, apolipoprotein B (apo B), and lipoprotein (a) [Lp(a)] levels (ERIKSSON et al. 1989; HENRIKSSON et al. 1992). Surprisingly, when estrogens were given only via the parenteral route, no significant changes in serum lipid levels were observed (BERGLUND et al. 1996). This suggests that the regulatory role of estrogens on serum lipoproteins, including Lp(a) levels in men, may depend on their capability of influencing hepatic metabolic pathways.

Twenty-two healthy elderly men (age 74 ± 3 years, mean ± SD) received 0.5, 1 or 2 mg/day of oral micronized 17β-estradiol over 9 weeks. LDL-C (–6%), apo B (–9%), triglyceride (–5%) and homocysteine (–11%) concentrations decreased, whereas HDL-C (+14%) increased. Intermediate-size very-low-density lipid (VLDL) subclass concentrations were lowered, and LDL and HDL subclass levels altered, in such a way as to cause average LDL and HDL particle size to increase. Lp(a) did not change. Fibrinogen (–13%) and plasminogen activator inhibitor-1 (PAI-1) concentrations (–26%) decreased, but there were no changes in thrombotic markers including thrombin-antithrombin III complex, prothrombin fragment 1.2, D-dimer, antithrombin activity, proteins-C and S, and von Willebrand-factor antigen. Breast tenderness occurred in four men and heartburn in five, but did not require discontinuation of treatment. The conclusion of the authors is that oral estrogen in men reduces homocysteine, fibrinogen, and PAI-1 concentrations and favorably influences VLDL, LDL and HDL subclass levels without increasing markers of thrombotic risk (GIRI et al. 1998).

As in the case of osteoporosis, in the assessment of the benefits and risks of estrogen treatment on the CVS clinical experience with cross-sex hormone administration in transsexuals is very helpful. NEW et al. (1997) and MCCROHON et al. (1997) independently studied two populations of male-to-female transsexuals in Melbourne (NEW et al. 1997) and Sydney (MCCROHON et al. 1997) maintained on high-dose estrogen with and without antiandrogen therapy. The Melbourne group studied 14 male-to-female transsexuals, 14 age-matched men and 14 age-matched premenopausal women. The transsexuals had been receiving estrogen therapy for 61 ± 70 months. The daily mean oral dose of EE was 118 ± 60 μg, and the daily mean oral dose of Premarin was 1.03 ± 0.35 mg. Of the 14 transsexuals, 21% had had an orchidectomy and the rest received antiandrogen therapy. Six transsexuals had coronary risk factors (smoking, family history, diabetes, hypercholesterolemia). The flow-mediated response in the transsexuals, women, and men was 11.5 ± 1.3% vs 9.4 ± 1.1% vs 5.2 ± 1.0%, with baseline diameters of 4.1, 3.6, and 4.9 mm, respectively. The nitroglycerin response was also significantly different: 21.6 ± 1.7% vs 21.0 ± 0.9% vs 14.5 ± 1.2%. The duration of the therapy was not predictive, and only the estrogen therapy and vessel size were predictive of the brachial response. The Sydney group studied 15 male-to-female transsexuals and 15 men, well matched for age, smoking history, and rest vessel size (4.0 ± 4.2 mm). The transsexuals had been receiving estrogen for between 6 months to 21 years, with concomitant orchidectomy (50%) and antiandrogen therapy (50%). The flow-mediated response was markedly better in the transsexuals than in the control subjects (7.1 ± 3.1% vs 3.2 ± 2.8%), with an equally impressive improvement in vasodilation response to nitroglycerin (21.2 ± 6.7% vs 14.6 ± 3.3%). Both of these improvements were not related to the duration of estrogen treatment. In summary, both studies showed that male-to-female transsexuals had better arterial function (endothelial-dependent and -independent) as measured by brachial ultrasound.

Seventeen male-to-female transsexuals were treated with 0.1 mg EE and 100 mg cyproterone acetate (antiandrogen) daily. This treatment of the male subjects increased median serum leptin levels from 1.9 ng/ml before to 4.8 ng/ml after 4 months and 5.5 ng/ml after 12 months of treatment ($P < 0.0001$; ELBERS et al. 1997). Beyond this, the plasma total homocysteine level decreased from a geometric mean of 8.2 μmol/l to 5.7 μmol/l ($P < 0.001$; GILTAY et al. 1998). The same group reported that in male-to-female transsexuals mean plasma levels of tissue plasminogen activator (–4.4 ng/ml), big endothelin-1 (–0.8 pg/ml), urokinase-type plasminogen activator (–0.5 ng/ml) and PAI-1 (–26 ng/ml) decreased (all $Ps < 0.02$). The level of von Willebrand factor (vWF) decreased (+24%; $P = 0.005$), while vWF:Ag(II) did not change (VAN KESTEREN et al. 1998). A favorable endothelium-dependent dilatation in the brachial artery was seen in estrogen-treated male-to-female transsexuals (MCCROHON et al. 1997).

The mortality in male-to-female transsexuals is not increased during estrogen treatment. However, transdermal application of estradiol is

recommended, particularly in the population over 40 years, in whom a high incidence of venous thromboembolism could be expected with enhanced liver estrogenicity, which is typical of oral administration of estrogens (VAN KESTEREN et al. 1997).

These findings bring us to the following question: will only women who will benefit from the cardiovascular or gene-therapy-related benefits of estrogens? At present, we don't know the answer, since men unfortunately appear reluctant to take estrogens for prolonged periods, despite the marked salubrious cardiovascular effects of the estrogens (LUFT 1998). However, this situation can be changed in the near future.

Should we use estrogen to treat men with coronary artery disease? For now, we should say probably not. This would be too early, because the cardioprotective effects in men would be hard to demonstrate in the clinical setting without significant feminization and other side effects such as prothrombotic states (YEUNG 1997). However, our current knowledge encourages us to develop non-feminizing estrogens (see below).

I. Central Nervous System

It is sometimes believed that our fate could be influenced by our endocrine glands. Obviously, this hypothesis has never been fully tested. However, there is abundant experimental evidence suggesting that endogenous steroid hormones, mainly though not exclusively from the adrenal and gonadal glands, may exert powerful effects on the central and peripheral nervous system. These include effects on neuronal cell development and differentiation, plastic changes in the organization of synaptic connections, and modulation of the efficiency of neuronal signal-transduction events. Consequently, it is not surprising that physiological, pharmacological, or pathological changes in circulating levels of steroid hormones may induce important modifications of neuroendocrine responses related to the maintenance of general body homeostasis and appearance, behavior, mood states, and even memory (Chap. 15, 27 and 44; MIRANDA and SOHRABJI 1996; ALONSO and LÓPEZ-COVIELLA 1998).

The role of estrogens in imprinting of the brain in males is an important aspect of an involvement of estrogens in male physiology which has been known for many years. It is 17β-estradiol which mediates the male priming of the brain in terms of behavior, at least in the rat (PLAPINGER and MCEWEN 1978; MCEWEN and WOOLLEY 1994). There is no doubt that, in this species, the priming phenomenon is essentially dependent on a conversion of testosterone into estradiol in the brain itself. Interestingly, even in the adult male rat brain, aromatase activity is three times higher than in females; therefore, this aromatase activity is dependent on estrogens (ROSELLI and RESKO 1987). However, SASANO et al. (1998) found no difference between men and women in the post-mortem expression of aromatase in various regions of the brain.

17β-Estradiol, which is formed in the central nervous system (CNS) by local aromatization of testosterone, is involved in the regulation of male gonadotropin production, becoming active mainly on the hypophyseal level. Estrogens are also assumed to modulate other central nervous processes, including the psyche. The clinical proofs of the concept are the signs and symptoms of an aromatase defect. A 24-year-old man with aromatase deficiency due to mutation in exon 9 of the P450 aromatase gene (cyp19) presented normal secondary sex characteristics, macroorchidism and elevated FSH, LH, and testosterone levels associated with very low estradiol and estrone concentrations (Morishima et al. 1995). This observation gives prominence to the fact that estrogens are also important regulators of gonadotropin secretion in the male. Relatively low doses of estrogen and progestin are sufficient in men to influence gonadotropin secretion and thus to realize the concept of an orally active contraceptive (Briggs and Briggs 1974; see also section K.III).

Ogawa et al. (1997) have determined the role of ER activation by endogenous estrogen in the development of male-typic behaviors by the use of transgenic male ERKO mice. Surprisingly, in spite of the fact that they are infertile, ERKO mice showed normal motivation to mount females, but they achieved less intromissions and virtually no ejaculations. Aggressive behaviors were dramatically reduced and male-typical offensive attacks were rarely displayed by ERKO males. Moreover, ER-gene disruption demasculinized open-field behaviors. It should be noted that these changes of sexual and aggressive behaviors were not due to the reduced levels of testosterone in these mice. In the brain, despite the evident loss of functional ER protein, the androgen-dependent system appears to be normally present in ERKO mice. Together, these findings indicate thet ER-gene expression during development plays a major role in the organization of male-typical aggressive and emotional behaviors in addition to simple sexual behaviors (for review: Couse and Korach 1998).

The distribution of ERα and ERβ mRNA in the CNS is asymmetrical both in female wild-type mice and in female ERα KO mice, indicating that activation of ERα as well as ERβ may have different organizational and activational effects in the CNS (Shugrue et al. 1997a, b; Laflamme et al. 1998). Using adult male ERαKO mice, Wersinger et al. (1997) showed that – in contrast to wild-type male mice – little masculine sexual behavior was displayed during testosterone replacement after gonadectomy. Therefore, it seems clear that ERα plays a key role in the expression of masculine sexual behavior. In earlier experiments using isolation-induced male agressive behavior in mice, we were able to show that pheromone-induced agressiveness was also dependent on the aromatization of testosterone to estrogens (Kurischko and Oettel 1977; Oettel and Kurischko 1978).

Can all estrogen effects in the brain be explained by transcriptional activation of either ER-α or ER-β? In this context, Shugrue et al. (1997b) conducted an interesting experiment using ERα-disrupted mice. There is only a weak affinity of ^{125}I-estradiol for ER-β. Surprisingly, the induction of PR

mRNA by 17β-estradiol in ERαKO mice was much greater than expected. Based on these observations, estrogen appears to regulate some genes in the developing and adult ERαKO brain via an unknown, non-classical ER.

Besides the PR, another estrogen-driven gene in the CNS is the oxytocin receptor (OTR). Using ERαKO mice, Young et al. (1998) demonstrated that ERα is not necessary for basal OTR synthesis, but is absolutely necessary for the induction of OTR binding in the brain by estrogen.

I. What Do We Know About Gender Differences Concerning Cerebral Blood Flow?

Mathew et al. (1986) have reported that women had higher cerebral flow than men, and the differences were most obvious in the frontal regions. This finding is confirmed by many reports of higher cerebral blood flow in females compared with males (Rodriquez et al. 1988; Daniel et al. 1988). Studies of brain metabolism have also indicated that there are sex-related CNS glucose metabolic rate differences in humans and rats. Baxter et al. (1987) have shown that women have whole-brain glucose metabolic rates that are 19% higher than those of men. The 19% difference between sexes for the brain as a whole is close to that found in the cerebral blood-flow study. Öztas (1998) hypothesize that the permeability of the blood-brain barrier differs between males and females since brain function, glucose utilization, and blood flow ehibit sex-related differences.

II. What Do We Know About the Connection Between the Serum Levels of Endogenous Estrogen and Certain CNS Functions in Men?

Regarding sexual behavior, circulating levels of estradiol – in contrast to testosterone – play only a limited role (Bagatell et al. 1994b). However, van den Beld et al. (1998) found a significant positive association between E2 and the outcome of a quality of life questionnaire in men aged 73–94 years. Low estradiol levels have been associated with decreases in verbal skills in older men (Cherrier et al. 1998), but men retain verbal skills better than women do during the initial stages of Alzheimer's disease (AD; Henderson and Buckwalter 1994).

In another study, the influence of endogenous testosterone and estrogen on memory was investigated in 33 healthy young men. Tests of visual memory, visuospatial ability, verbal memory, and attention were administered, and circulating levels of estradiol and free testosterone were measured. Participants with high levels of estradiol performed better on two measurements of visual memory than did those with normal but lower levels. There were no differences between individuals with high and low levels of testosterone on any cognitive measurement. These results support the contention that estradiol influences memory in men (Kampen and Sherwin 1996).

Finally, higher serum levels of estradiol – but not of testosterone – in women were associated with improved sequential movement as a sign of better fine-motor performance. In contrast with this, hormone levels in men's blood were unrelated to key-pressing performance (JENNINGS et al. 1998).

III. What Do We Know about the Influence of Exogenous Estrogen on CNS Functions in Men?

Treatment of four men with idiopathic hypothalamic hypogonadism with testosterone or 17β-estradiol resulted in decreased mean levels of biologically and immunologically active LH and FSH, whereas administration of DHT did not alter gonadotropin secretion. These data suggest that some of the direct effects of testosterone at the pituitary level in men are mediated by estradiol, whereas peripherally formed DHT may not play an important role in this process (BAGATELL et al. 1994c). In pubertal boys, the administration of testosterone, but not of the non-aromatizable androgen DHT, significantly influenced the secretion pattern of GH, indicating that aromatization of testosterone to estradiol in boys is the proximate sex-steroid stimulus amplifying secretory activity of the GH axis at puberty (VELDHUIS et al. 1997).

A sample of 16 young men received a patch delivering 0.1 mg of 17β-estradiol per day transdermally. They were exposed to a brief psychosocial stressor (free speech and mental arithmetic in front of an audience) 24–48 h later. The estradiol-treated subjects showed exaggerated peak adrenocorticotropic hormone ($P < 0.001$) and cortisol ($P < 0.002$) responses compared to the placebo group (KIRSCHBAUM et al. 1996). Studies by KUDIELKA et al. (1998) could support this finding. Elderly males (probably with higher estradiol levels) show larger hypothalamic-pituitary-adrenal responses to psychosocial stress than postmenopausal women (with lower estradiol levels).

In contrast to this, percutaneous admistration of estradiol (100 μg) reduced the mental arithmetic stress in 20 normal young men. The increase in epinephrine was significantly lower in the estradiol-treated groups (DEL RIO et al. 1994).

It is well known that hot flushes experienced during GnRH therapy or after orchidectomy in men e.g. with cancer of prostate can be stopped with different estrogens. Estrogens reduce adrenergic stimulation and therefore the occurrence of hot flushes. The parenteral use of estrogen preparations (e.g. patches) in men has scarcely been studied so far. However, based on the data available, it can be assumed that their use should be associated with much fewer side effects than the use of orally active estrogen drugs (KLIESCH et al. 1997).

Now, considering the fact that the involvement of exuberant oxidative mechanisms in AD is very likely (SMITH and PERRY 1995) and that meanwhile the delaying effect of 17β-estradiol replacement on the progression of senile dementia of Alzheimer's type (SDAT) can be assumed to be proven (PAGANINI-HILL and HENDERSON 1994; PAGANINI-HILL 1996), attractive

possibilities emerge for utilization of this advantage in men with the help of the so-called non-feminizing scavestrogens (Sect. M). The BEYREUTHER group in Heidelberg found that in AD, the reduction of Cu(II) to Cu(I) by Alzheimer's amyloid precursor protein (APP) involves an electron-transfer reaction and could lead to production of hydroxyl radicals. Thus, copper-mediated toxicity of APP-Cu(II)/(I) complexes may contribute to neurodegeneration in AD (MULTHAUPT et al. 1997).

Women have more strokes than men do but are more likely to recover from them. In this context, estrogen might be able to reduce brain damage from a stroke in both women and men. After ischemia, estrogen may provide protection against cellular injury related to antioxidant properties of the estrogenic molecules (HALL et al. 1991; KEANEY et al. 1994; CAULIN-GLASTER et al. 1997). TOUNG et al. (1998) gave estrogen to 36 rats and plain salt water to another 21. They then stimulated the effects of a stroke by cutting blood flow to the brain for 2 h. Female as well male rats that got the estrogen had half the brain damage. These findings demonstrate that the benefit of estrogen can be extended to the male brain, reducing tissue injury. HAWK et al. (1998) found that testosterone increases and estradiol decreases middle cerebral artery occlusion lesion size in male rats. A strong positive correlation ($R = 0.922$) between plasma testosterone concentrations and ischemic lesion size was observed. Estradiol treatment reduced the ischemic brain area of the male rats significantly.

J. The Influence of Environmental Estrogens (Xenoestrogens) on the Fertility of Men

The hormone-like activity of environmental chemicals and natural plant constituents has evoked a controversial debate in both the public and the scientific community (GREIM 1998). For the last 40 years, substantial evidence has surfaced on the hormone-like effects of environmental chemicals such as pesticides and industrial chemicals in wildlife and humans. The endocrine and reproductive effects of these chemicals are believed to be due to their ability to:

1. Mimic the effect of endogenous hormones
2. Antagonize the effect of endogenous hormones
3. Disrupt the synthesis and metabolism of endogenous hormones
4. Disrupt the synthesis and function of hormone receptors (SAFE et al. 1997; NIEMANN et al. 1998; SONNENSCHEIN and SOTO 1998)

The discovery of the hormone-like activity of these chemicals occurred long after they were released into the environment. Aviation crop dusters handling dichloro-diphenyl-trichloroethane were found to have reduced sperm counts (SINGER 1949), and workers at a plant producing the insecticide kepone were reported to have lost their libido, become impotent and have low sperm counts

(GUZELIAN 1982). Subsequently, experiments conducted in lab animals demonstrated unambiguously the estrogenic activity of these pesticides (SONNENSCHEIN and SOTO 1998). Man-made compounds used in the manufacture of plastics were accidentally found to be estrogenic because they fouled experiments conducted in laboratories studying natural estrogens (SOTO et al. 1991). For example, polystyrene tubes released nonylphenol, and polycarbonate flasks released bisphenol-A (KRISHNAN et al. 1993). Alkylphenols are used in the synthesis of detergents (alkylphenol polyethoxylates) and as antioxidants. These detergents are not estrogenic; however, upon degradation during sewage treatment they may release estrogenic alkylphenols. The surfactant nonoxynol is used as an intravaginal spermicide and condom lubricant. When administered to laboratory animals, it is metabolized to free nonylphenol. Bisphenol-A was found to contaminate the contents of canned foods; these tin cans are lined with lacquers such as polycarbonate (BROTONS et al. 1994). Bisphenol-A is also used in dental sealants and composites; up to 950 μg of this compound were retrieved from saliva collected during the first hour of polymerization. Other xenoestrogens recently identified among chemicals used in large volumes are the plastizicers benzylbutylphthalate, dibutylphthalate, the antioxidant butylhydroxyanisole, the rubber additive *p*-phenylphenol, and the disinfectant *o*-phenylphenol. Feminized male fish were found near sewage outlets in several rivers in the U.K. (for review see JOHNSON et al. 1998; SONNENSCHEIN and SOTO 1998).

Male reproductive toxicology has made some important discoveries in the past few decades. It is likely that man will increase his deliberate and incidental exposure to old and new chemicals, so it would not be surprising if, in the future, the chemicals we have discussed are replaced by others which will be a greater concern for male reproductive health (MORRIS et al. 1996).

However, it should be stated that synthetic, natural and hormonally active steroids are easily degradable in the environment by aerobic and anaerobic microorganisms, including fungi, and therefore are not an ecotoxicological risk (HÖRHOLD-SCHUBERT et al. 1995). In particular, this applies to the unjustified fear that the elimination of steroids after using oral contraceptives or hormone replacement might cause environmental pollution.

A discussion of environmental estrogens (the so-called hormonal disruptors as well as the "good" phytoestrogens) means discussing very different compounds like phytoestrogens (isoflavones, coumestane, soya), estrogenic chemicals (tetrachlorobenzodioxin, 4-octylphenol) or estrogens such as diethylstilbestrol (DES). To demonstrate a role of DES or other estrogenic chemicals, pregnant animals, including rats, were treated with these compounds and the effects on the pups investigated (MAJDIC et al. 1996). Under these circumstances, a disturbance of testicular development and an imbalance of somatic sexual organ development were demonstrated. However, these data are in very good agreement with what was shown more than 50 years ago (RAYNAUD 1940), and has since been confirmed by many investigators. Thus, Raynaud described the same effect after treatment of pregnant mice with large

doses of 17β-estradiol. However, one aspect which is of utmost importance in this context has been neglected to a great extent, and this is the relevance of the presented data in terms of fertility in context with the dose used. Negative effects on the development of the male sexual system can only be observed if extremely high doses of so-called environmental estrogens are used, and there is good evidence that the concentration necessary to induce adverse testicular effects during the time of sexual differentiation is hardly reached in men. Furthermore, it should be taken into account, and it should not be forgotten that men are used to certain amounts of estrogens during their own lives, and even more during uterine life before birth.

MÜLLER et al. (1998) estimated the estrogenic potency of 4-nonylphenol (NP) and a risk calculation for non-occupationally exposed humans was performed. The daily intake of non-occupationally exposed persons was estimated to be less than 0.16 mg/day. Risk estimates were based on this daily intake and the relative potency of NP to 17β-estradiol. Comparison of this intake with the no-observed-adverse-effect level derived from a 90-day subchronic toxicity study in animals, results in a safety factor of about 20,000. A safety margin of 3000 can be derived when comparing the resulting NP blood concentrations with 17β-estradiol levels in the blood of adult males.

In this context, estrogens have been discussed in the last few years as a possible factor for declining fertility in men (SHARPE 1993; SHARPE and SKAKKEBAEK 1993). This phenomenon captured the attention of scientists, physicians, and the media. In spite of clear evidence of a major role of estrogens in the normal male physiology, estrogens have been discussed as risk factors in men, particularly in the context of male fertility. Specifically, exposure to environmental estrogens has been connected to a constant fall in sperm count over the last decades (COMHAIRE et al. 1996; MENCHINI-FABRIS et al. 1996; GIWERCMAN and SKAKKEBAEK 1992; JOFFE 1996). At present, there are the following reports concerning the decline in male reproductive health: LETO and FRENSILLI 1981; BOSTOFEE et al. 1983; BRAKE and KRAUSE 1992; CARLSEN et al. 1992, 1995; AUGER et al. 1995; IRVINE et al. 1996; VAN WAELEGHEM et al. 1996; YOUNGLAI et al. 1998.

However, it has been seriously questioned whether there is a decline in sperm count at all (NIESCHLAG and LERCHL 1996). These and other authors came to the conclusion that the re-analysis of the data, showing that male sperm counts decreased by over 40% between 1940 and 1990, indicated that inadequate statistical methods were used and that the presented data did not support a significant decline in sperm count (NIESCHLAG and LERCHL 1996; JOUANNET and AUGER 1996). All of the studies published to date were flawed in one way or another because of subject selection bias, choice of statistical model for analysis, methodological inconsistencies, inconsistencies with abstinence, quality of data (i.e., means used for skewed distributions of data not normally distributed), potential regional and ethnic variations in semen quality, or study design. These significant flaws make it imposible to conclude

anything regarding potential changes in semen quality over time (BERMAN et al. 1996; for review see LAMB 1997).

A very witty comment, of course, comes from a veterinarian: SETCHELL (1997) compared the data from 137 studies on sperm counts of bulls, boars, and rams in the literature from the early 1930s to 1995. The bull data showed no correlation of sperm count with year of publication, for the boars there was a slight but insignificant positive correlation, and for the sheep there was a slight, but significant, rise in sperm counts with time. He concluded: it would appear that, if the fall in human sperm counts is real, then it must be due to something which is not affecting farm animals.

In contrast to the controversial discussions about whether or not there has actually been a decline in sperm quality during the last five decades, there is no doubt about an increase in testicular cancer in young men (FORMAN and MOLLER 1994). The evaluation of cancer registries in several countries in north and central Europe, Australia, New Zealand, and the U.S.A. showed consistently increasing incidences, e.g. by 2–4% per year in men under the age of 50 years. Those at highest risk are men aged between 20 and 45 years. So far, it is still unclear whether the apparent increase in this type of cancer found in many countries is due to hormonally active substances (MCLACHLAN et al. 1998), changed ways of life, or other causes. For instance, the fact that there is a definite increase in testicular cancer in the U.S. white population, while in the U.S. population of Asian and African origins there is not was cited by the Danish health authority in their report of 1995 as a reason to doubt a relation between testicular cancer and the presence of environmental hormonally active substances (GREIM 1998).

We might argue further here. If environmental estrogens were to have such a negative impact on fertility and health in men, another question would have to be raised: namely, are estrogens bad for women? In terms of the reproductive system, it has also been known for decades that the treatment of pregnant animals with 17β-estradiol results in a paradoxical virilizing effect in female pups (RAYNAUD 1940; NEUMANN et al. 1974). But there are no reports about an increased virilizing phenomenon in girls or women. This is just one example of what should be expected if any relevant concentration of environmental estrogens exists (HABENICHT 1998).

K. Regimens for Estrogen and Antiestrogen Treatment in Men

I. Treatment of Prostate Cancer with Estrogens

Androgens are known to be very important for the development of the prostate gland. However, the level of testosterone decreases with age, indicating that factors other than androgens could be important for the

development of prostate hyperplasia or prostate cancer. Therefore, it is only logical to ask, what do we know about the role of estrogens in the pathophysiology of the prostate?

Long-term administration of estrogens or aromatizable androgens to either intact or castrated males of various animal species results in altered prostatic growth (SANTTI et al. 1994). The most striking morphological change caused by estrogen treatment of intact or castrated male animals is squamous epithelial metaplasia (OETTEL 1974) extending from the prostatic utricle into the epithelium covering the seminal colliculus (PYLKKÄNEN et al. 1993). Neonatal estrogenization of male mice promotes epithelial hyperplasia and dysplasia based on altered *c-fos* expression (SAITO et al. 1997). In addition, the chronic co-administration of estrogens and androgens to inbred Noble rats causes dysplastic lesions, and in some cases adenocarcinomas, in the dorsolateral lobes of the prostate and the coagulating glands (NOBLE 1977, 1980, 1982; DRAGO 1984; LEAV et al. 1988). These findings suggest that estrogens may play an important role in the control of prostatic growth and development.

Estrogens also have direct cytotoxic effects on prostatic carcinoma cells in vitro, possibly by an effect on the cell membrane, stimulating a rapid Ca^{2+} influx (WIDMARK et al. 1995; AUDY and DUFY 1996). Another interesting contribution to the nongenomic action of estrogens on the prostate comes from the Rosner group (NAKHLA and ROSNER 1996; NAKHLA et al. 1997). SHBG not only regulates the free – and therefore biologically active – concentrations of certain steroid sex hormones in plasma but is involved in a nongenomic mechanism of steroid-hormone action. It binds to a receptor on prostatic cell membranes and is activated by an appropriate steroid to initiate the generation of intracellular cAMP. In serum-free medium, both dihydrotestosterone and estradiol increase growth in the presence, but not in the absence, of SHBG.

HIRAMATSU et al. (1996) found that ER expression was only observed in the stromal cells of six out of 26 (23%) patients with prostatic carcinoma, but in none of the prostatic cells of 19 patients with benign prostatic hyperplasia (BPH). They conclude that estrogens do not have a direct genomic effect on the biological behavior of BPH or prostatic carcinoma. On the other hand, androgen deprivation leads to an upregulation of stromal ER expression in the human prostate (KRUITHOF-DEKKER et al. 1996).

In a case-controlled pair study based on 320 men who developed surgically treated BPH, GANN et al. (1995) found a positive correlation between increased E2 and risk of BPH. The excess risk associated with 17β-estradiol was confined to men with relatively low androgen levels. Contrasting with this are the findings of CETINKAYA et al. (1998). They found that, whereas E2 and the ratio of estradiol to free testosterone were increased in patients with hepatic cirrhosis, serum PSA levels and mean prostatic volume were significantly higher in the healthy control group. LAGIOU et al. (1997), too, found that E2 was not significantly related to the risk of BPH. GANN et al. (1996) also performed a case-controlled pair study based on 222 men who developed prostate cancer. High levels of circulating testosterone and low

levels of SHBG – both within normal endogenous ranges – were associated with increased risks of prostate cancer. Low levels of circulating estradiol were assumed to represent an additional risk factor. Circulating levels of DHT and 3α-androstanediol glucuronide did not appear to be strongly related to prostate-cancer risk.

What knowledge comes from the estrogen treatment of male-to-female transsexuals? In nine cases of estrogen-treated males who had undergone orchiectomy, the prostates were small only after long-term estrogens. No malignancies were found (VAN KESTEREN et al. 1996). JIN et al. (1996) measured the prostate sizes in 11 estrogen-treated male-to-female transsexuals. Compared with age-matched controls, total prostate volume, central prostate volume (CPV), ratio of CPV to peripheral prostate volume, PSA levels, and total and free testosterone were significantly reduced. There was no influence of the estrogen treatment on prostatic acid phosphatase. SHBG levels were elevated by nearly 500% ($P < 0.001$), indicating the same well-known situation of estrogen dependence of SHBG induction as in women.

The basis of palliative therapy with estrogens in advanced prostatic cancer is the systemic blockade of the androgenic stimulation of the tumor. This is achieved, among other things, by using estrogens (HUGGINS and HODGES 1941; HUGGINS et al. 1941a, b), which inhibit the pituitary secretion of LH, and therefore indirectly the testicular secretion of testosterone. Further observations include a rise in SHBG, induction of pituitary prolactin secretion, and decreased DNA synthesis in carcinoma cells of the prostate.

Unfortunately, estrogen therapy has fallen into disrepute, since numerous side effects are allegedly due to estrogens, such as cardiovascular and hepatic toxicity, salt and water retention, hyperprolactinemia, endocrine-determined psychological disturbances, immunosuppression, etc. (MELLINGER et al. 1967; BYAR 1973).

It is still the general consensus that if estrogens are to be used, contraindications, especially thrombophilia and a history of embolism, must be excluded. A general thrombosis prophylaxis with low-dose heparin is recommended, particularly in the case of iv injection. In addition, in order to prevent gynecomastia, mamillary radiation with 10–20 Gy is necessary before the beginning of therapy (ZINGG and HEINZEL 1968). On the other hand, MAUERMAYR et al. (1978) recommended pretherapeutic andromastectomy.

DES is the only estrogenic drug still used in primary therapy in the USA, with recommended doses from 0.2 mg up to 1 mg/day maximally. In previous studies, even 5 mg DES was used (THE VETERANS ADMINISTRATION COOPERATIVE UROLOGICAL RESEARCH GROUP, VACURG 1967). High-dose oral estrogens were associated with an increased risk of cardiovascular death. A dose of 1 mg DES daily is not associated with an increased risk of cardiovascular death (COX and CRAWFORD 1995). Besides the suppression of LH, a direct, local antitumor effect has also been demonstrated for DES in rats (CUI et al. 1998).

The rationale behind the use of DES diphosphate in doses of 200 mg each day, or sometimes greater, was that the free, unconjugated, presumably active

DES was released directly into the prostate by the high levels of phosphatases present in the tissue. But there is little evidence that DES diphosphate accumulates in the prostate or that DES is released in any substantial amount to elicit a cytotoxic intraprostatic effect (Griffiths et al. 1994). However, in 36 patients with advanced prostate cancer there was a stronger suppression of serum testosterone and FSH levels by DES diphosphate than by castration or a GnRH agonist (Kitahara et al. 1997). Premarin (2.5 mg t.d.s.) is also clinically effective (Boyns et al. 1974). Synthetic EE (0.15 to 1.0 mg/day), too, has had its proponents (Griffiths et al. 1994).

The long-acting, intramuscular polyestradiol phosphate (PEP) is popular in Scandinavia and does not show an increased rate of cardiovascular complications in patients suffering from cancer of the prostate (Haapiainen et al. 1990; Stege et al. 1995). Patients with prostatic carcinoma and who are on oral estrogen therapy presumably have an altered coagulation system and suffer cardiovascular side effects. The reason is that estrogens – especially oral estrogens – are potent inducers of liver-synthesized proteins, including coagulation factors. Henriksson et al. (1990) have assessed the effect of non-oral estrogen on the coagulation system in patients with prostatic carcinoma. Monthly intramuscular injections of 320 mg PEP were given to 12 patients. No change was found in any of the coagulation factors, including factor VII, with the exception of a significant decrease in antithrombin III. No patient, including another 38 patients treated with PEP, had any cardiovascular complications after an average follow-up period of 12.9 ± 0.7 months; 76% of the patients responded to treatment.

A distinct optimization of oral estrogen therapy was achieved with the development of ethinyl estradiol sulfonate (EES; Turisteron; Schwarz and Weber 1970; Schwarz et al. 1975; Chemnitius and Onken 1976; Oettel et al. 1981). EES is an orally active depot estrogen and is administered once per week in a dose of 1–2 mg (Guddat et al. 1979; Buschmann and Guddat 1997; Höfling and Heynemann 1998). The good tolerability of EES compared to the mother compound EE is remarkable. That is why a 3-sulfamate was substituted for the sulfonate in position 3 of the estrogen molecule. This resulted in the so-called estrogen sulfamates, with distinct, low hepatic estrogenicity (Elger et al. 1995, 1998). However, the excellent preclinical results need to be confirmed in patients with prostatic cancer.

Numerous studies have been performed to investigate estramustine phosphate after tumor progression, but also in primary therapy. Estramustine, a conjugate of nor-nitrogen mustard and estradiol, is an effective chemotherapeutic agent that is presently mainly used to treat metastatic, hormone-refractory prostate cancer (Hudes et al. 1992). Apart from inhibiting LH, this drug shows a direct cytotoxic activity based on its ability to attack the microtubular system and on the tau expression of the cell (Tew and Stearus 1987; Laing et al. 1998; Lidström et al. 1998; Sangrajrang et al. 1998). Its activity is dose-dependent (Benson and Gill 1986). Infusion of 300–450 mg daily over 10 days is recommended; for oral use, up to 840 mg per day is recommended.

The combination of the vinca alkaloids vinblastine or vinorelbine with estramustine phosphate is active and well tolerated in hormone-resistant prostate cancer and supports the therapeutic strategy of combining agents that impair microtubule function (Batra et al. 1996; Carles et al. 1998). The metabolites of estramustine phosphate perform as androgen antagonists of ARs, an additional mechanism involved in the therapeutic effect of the parent compound in patients with prostate cancer (Wang et al. 1998). Fosfestrol 1000–1200 mg daily is now only used for alleviation of pain in tumor progression, mostly as 10-day infusion therapy.

Disillusioned about the marginal therapeutic progress achieved with the expensive complete androgen blockage (GnRH analogs), we turn to Scandinavia and the UK again, where estrogen therapy is still among the standard treatments of metastatic prostate cancer. So far, the side effects which have become known since the publication of the VACURG study in 1970 have deterred us from estrogen therapy. These side effects mainly include edema, cardiovascular toxicity, thrombosis, and the development of male breast (Presti 1996). They can be controlled symptomatically, the cardiovascular effects may possibly be reduced or prevented by ASS dosage or the systemic use of the estrogens with circumventing of the enterohepatic circulation, or the modern development of estrogen sulfamates. If we succeed in freeing a modern estrogen therapy of prostate cancer from the stigma of threatening cardiovascular death, it would be easy to predict a revival of the use of estrogens, since their influence on prostate cancer is at least equal to that of all other therapeutic methods. The distinctly lower economic load this therapeutic alternative involves should not be underestimated (Mohren 1998).

Prostate-cancer patients who underwent orchiectomy or GnRH treatment experience hot flushes. Estrogens, owing to their binding to ERs while simultaneously showing antitumor activity, seem to reduce adrenergic stimulation and therefore the occurrence of hot flushes. However, unlike oral administration, the parenteral use of estrogen preparations in men has altogether been scarcely investigated so far. The data available suggests a definitely lower rate of side effects for the parenteral than for the conventional, orally active estrogen drugs (Kliesch et al. 1997).

II. Estrogen Replacement in Men

No area of hormone replacement in elderly males is so speculated on and so little understood as the sense and usefulness of estrogen therapy in males. As mentioned above, previous negative experiences in terms of adverse effects of estrogen therapy of prostate cancer are totally useless. Estrogen is used here without preceding measurement of endogenous estrogens. What is more, synthetic products, such as DES or EE are used in unphysiologically high doses. These, of course, must have adverse effects on breast tissue, the clotting system, and the vascular system. A good example is the comparison of the rate of side effects of the synthetic estrogen EE in oral contraceptives with the natural

estrogens in the different hormone-replacement regimens used in postmenopausal women. The risk of deep venous thromboembolism in postmenopausal estrogen replacement using natural estrogens appears to be smaller compared with the risk from contraception using EE. Mimicking natural estrogen sulfation by sulfonation of EE in position 3 (Turisteron) considerably reduces the cardiovascular risk of large-dose estrogen treatment in men suffering from cancer of the prostate (Buschmann and Guddat 1997). There is an exogenous supply of aromatizable androgens, e.g. testosterone esters; at the same time, aromatization will always lead to increased endogenous serum concentrations of 17β-estradiol and estrone (Sih et al. 1997).

It is crucial to know whether there is an estrogen deficit in men (section B). Men with a complete aromatase defect are devoid of any estrone and 17β-estradiol in serum (absolute estrogen deficiency), although FSH and LH are increased. The skeletal age seems retarded, with incomplete epiphyseal closure. The clinical picture corresponds to osteoporosis. Moreover, increased basal insulin levels and a decreased HDL/LDL ratio are detected (Bulun 1996). At present, only speculations about the frequency of complete or partial aromatase defect in men (normal testosterone concentrations and lacking or very low estrogen concentrations) are possible.

Remarkably, there are regional differences in fatty-tissue ER concentrations of men and women. While the numbers of estrogen receptors in visceral fat are the same, the number of ERs in the abdominal subcutaneous fatty tissue in men is double that of women (Pedersen et al. 1996).

An interesting proposal is made by Kuhl et al. (1994). The daily oral intake of 2–10 mg estriol (t.i.d.) is said to be appropriate for the prevention and treatment of arterial diseases in men. It being well known that, due to its short half-life, estriol has little or no "classical", i.e. genomic (nucleus-mediated) estrogenic actions; the estriol effect must be based on other mechanisms of action. As we have found out, estriol has distinct antioxidative (i.e. nongenomic) effects which, with regard to the inhibition of lipid peroxidation in synaptosomal membranes, are about as strong as vitamin E (Römer et al. 1993). Generally, nongenomic actions of estrogens on the vascular system (Farhat et al. 1996) or in the CNS (Ramirez et al. 1996) are a very interesting starting point for the development of novel drugs (Sect. M).

III. Male Contraception with Estrogens

The disruption of spermatogenesis by the estrogen treatment of experimental animals has been widely used as a useful approach in the study of spermatogenesis regulation (Blanco-Rodríguez and Martínez-García 1996). Steroidal estrogens and nonsteroidal estrogenic compounds such as DES can suppress spermatogenesis in man so that, in the end, only Sertoli cells and spermatogonia remain in the thickened seminiferous tubules (Jackson and Jones 1972). Nevertheless, the small number of publications so far which have dealt with the use of estrogens for male contraception is surprising. Too little is

known to definitely either recommend or reject the use of a low-dose estrogen component (besides androgens or progestins). The cautiousness in using or recommending estrogens certainly results from the negative experience gained with previous estrogen therapy of prostate cancer, where it should be considered that the estrogen doses chosen were much too high (Sect. D.IV.). While it is possible that the diminished libido and sexual potency could be improved by concomitant androgen replacement, the occurrence of symptomatic gynecomastia and other metabolic side effects, such as those demonstrated in women, make it unlikely that high-dose estrogens will be accepted in formulations designed to interrupt male fertility (DE KRETSER 1974). An example of previous, extremely high estrogen dosage is reported by HELLER et al. (cited by DE KRETSER 1974). Mestranol (0.45 mg/day) led to azoospermia, but this was accompanied by decreased libido and potency together with the universal occurrence of painful gynecomastia.

In men, the role of testosterone and estradiol in a feedback regulation has been established. The very low daily dose of 15 μg EE obviously is the borderline amount for decreasing plasma LH and testosterone levels in normal males. LH and testosterone are reliably reduced by 30 μg EE whereas, in patients suffering from Klinefelter's syndrome, the EE dose must be approximately five times higher (SMALS et al. 1974). On the other hand, KULIN and REITER (1997) found that daily 32 μg and 42 μg suppressed FSH in urine and blood, respectively. A significant suppression of LH was not obtained. A more powerful decline in T was seen with daily 50 μg EE plus 0.5 mg norgestrel over 9 days (KJELD et al. 1977). However, after checking the individual components, it became evident that the stronger effect regarding the decrease in testosterone emanated from norgestrel/levonorgstrel (KJELD et al. 1979). Using a combination of 0.02 mg EE and 10 mg methyltestosterone per day, KULIN and REITER (1972) and BRIGGS and BRIGGS (1974) found a suppression of pituitary secretion of FSH sufficient to stop the sperm production in normal adult men. Curiously, these experiments have not been carried on.

High-dose sex-steroid administration had marked effects on adrenal androgen levels, which decreased by 27–48% in males treated daily with 0.1 mg EE orally and increased by 23–70% in females treated with 250 mg testosterone enanthate intramuscularly every 2 weeks (POLDERMAN et al. 1995).

IV. Treatment of Male-to-Female Transsexuals with Estrogens

Transsexualism belongs to the group of gender dysphoric disorders. These have been known from antiquity onward across national and cultural boundaries. Formally, the prevalence of transsexualism has increased over the past years. This – in our current opinion – is due to the improvement of therapies and to an increased public acceptance of this phenomenon (SCHLATTERER et al. 1996). Cross-gender hormone treatment of transsexual patients is carried out to suppress secondary sex characteristics of the original sex and to induce those of the opposite sex. This kind of therapy is life-long. Prescription of

mediated by androgens and/or ERs (HABENICHT and EL ETREBY 1992). As a consequence, the treatment with either an ER and/or AR antagonist does not only inhibit the effect of androgens or estrogens at the corresponding peripheral target organ but also at the brain, inducing a counter-regulatory increase to compensate for a supposedly deficient hormone synthesis. The same happens under treatment with inhibitors of androgens or estrogens, such as, for instance, aromatase inhibitors.

In the rat, feedback regulation is dependent almost exclusively on androgens. Thus, treatment with an AR antagonist such as flutamide results in an immediate and permanent increase in LH and testosterone levels. Conversely, treatment with an aromatase inhibitor leads only to a transient increase in serum testosterone and LH concentration. Anordrin is an antiestrogen with agonist activity and is used as a postcoital contraceptive in China. Anordrin and its metabolite anordriol act in rats like fully active estrogen agonists. A significant decrease in serum LH, FSH, and testosterone levels occurred in treated animals as compared to controls. The compound adversely affected spermatogenesis and induced moderate to severe degenerative changes in the epididymis and prostate, and atrophy of the glands of the seminal vesicles (VANAGE et al. 1997).

The SERM raloxifene did not compete for binding of the androgen [^{3}H]-R1881 (methyltrienolone) in cytosolic extracts of the ventral prostate in rats. It produced a significant dose-dependent regression of the ventral prostate and seminal vesicles. This was associated with a decline in T. Raloxifene antagonizes testosterone-induced increases in the prostate weight of castrated rats, but does not bind to AR or affect prostatic 5α-reductase or testicular steroid 17α-hydroxylase activity (BUELKE-SAM et al. 1998). Administration of estradiol to castrated male rats stimulated four-fold increases in in vitro ventral prostatic binding of [^{3}H]-R1881. When raloxifene was co-administered with estradiol, the compound markedly antagonized the estrogen-induced increase in prostatic binding of [^{3}H]-R1881, confirming its antiestrogenic properties in male rats. Raloxifene increased serum prolactin and decreased serum FSH, while leaving LH unaffected (NEUBAUER et al. 1993). There was no indication in either male rat study that raloxifene caused important changes in sperm production, sperm quality, or male reproductive performance at doses as high as 100 mg/kg/day orally (HOYT et al. 1998).

In dogs, feedback regulation is also exclusively dependent on estrogens (WINTER et al. 1983). Therefore, in this species, treatment with an aromatase inhibitor results in immediate and permanent increases in LH and testosterone, whereas treatment with an antiandrogen such as flutamide does not induce counter-regulatory increases in LH and testosterone secretion. Subhuman primates, as well as men, have a position somewhere in between these two extremes. Thus, in the monkey, treatment with the antiandrogen flutamide induced a slight increase in T, while combined treatment with an aromatase inhibitor plus an antiandrogen resulted in complete opening of the feedback loop (HABENICHT and EL ETREBY 1992; HABENICHT et al. 1992).

For the assessment of antiestrogenic activities in the brain, it is important that while certain antiestrogens do overcome the blood-brain barrier, others do not. For example, tamoxifen, clomiphene, RU 58668, and UC 23469 act on the brain after systemic use (Vagell and McGinnis 1997; Ortega et al. 1993), while the "pure" antagonists, e.g. ZM 182780 (formerly named ICI 182780), ICI 182780, and ZK 164015, do not (Wade et al. 1993; Fuhrmann et al. 1998).

How about the therapeutic value of antiestrogens in men? Certain forms of male gynecomastia seem to respond to antiestrogens (Eversmann et al. 1984; Glass 1993; Vizner et al. 1994). The use of a tamoxifen therapy in oligospermia is being discussed controversially. The mechanism of action in male infertility treatment is presumably based on a competitive displacement of estrogens from the ERs in the hypothalamus and pituitary. Since estrogens inhibit pituitary gonadotropin secretion via a negative feedback mechanism, blockage of the estrogen effect may cause an increase in gonadotropins. Increase in LH and FSH is assumed to improve spermatogenesis (Nieschlag et al. 1993).

Rolf et al. (1996) analyzed clinical studies including a total of 1586 infertile men treated with tamoxifen. Six studies were randomized and placebo-controlled. The randomized study with the largest sample size provided no data about the frequency of pregnancies but showed a significant increase in sperm concentration and the number of viable sperm in ejaculate (Kotulas et al. 1994). No significant changes in the sperm parameters examined were stated in the remaining randomized studies. Rolf et al. (1996) conclude that there is no proof of any improvement in fertility chances by tamoxifen. According to Sterzik et al. (1993), there is a lack of correlation between a rise in hormone levels and improvement of sperm quality, which suggests that tamoxifen is of questionable value in men with idiopathic oligozoospermia (Breznik and Borko 1993).

On the other hand, more favorable results have been reported with clomiphene citrate (Hammami 1996) or with the combination of tamoxifen citrate plus clomiphene citrate (Suginami et al. 1993). The prospective randomized clinical study with 80 oligozoospermic men by Adamopoulos et al. (1997) yielded a similar result. The authors found that the combination of testosterone undecanoate with tamoxifen citrate enhanced the effects of each agent given independently on seminal parameters in men with idiopathic oligozoospermia.

The therapeutic value of aromatase inhibitors in infertile men, at present, cannot yet be assessed. The aromatase inhibitors CGS 16949A as well as CGS 20267 suppress the E2 and increase FSH, LH, and T dose-dependently in men (Bhatnagar et al. 1992; Trunet et al. 1993).

Another interesting aspect is changes in estrogen biosynthesis by concomitant administration of other drugs. Herzog et al. (1991) found total and non-SHBG-bound E2 levels to be significantly higher among phenytoin-treated men with epilepsy than among untreated epileptic men or normal controls. Consequently, treatment using a combination of testosterone and the

aromatase inhibitor testolactone may have significantly better effects on sexual function and seizure frequency than testosterone alone (HERZOG et al. 1998).

M. The Concept of Non-Feminizing Estrogens

The utilization of the benefits of estrogen on bone (Sect. G), CVS (Sect. H), and CNS (Sect. I) without feminizing effects on the genitourinary system (Sect. D) or mammary gland (Sect. E) is doubtless a big challenge and might be a great gain for hormone therapy in men. The story began with the phytoestrogens (MURKIES et al. 1998; PRZYREMBEL 1998). Phytoestrogens emerged from their esoteric role in animal husbandry following the hypothesis that the western human diet is relatively deficient in these substances compared with societies where large amounts of plant foods and legumes are eaten. Evidence is beginning to accrue that they may begin to offer protection against a wide range of human conditions, including breast, bowel, prostate and other cancers, cardiovascular disease, impaired brain function, alcohol abuse, osteoporosis, and menopausal symptoms. Of the two main classes of these weak estrogens, the isoflavones are under intensive investigation due their high levels in soyabean. Like the "antiestrogen" tamoxifen, these seem to have estrogenic effects in human subjects in the CVS and bone. Although previously only available from food, isoflavones are now being marketed in health-food supplements or drinks, and tablets may soon be available over the counter as "natural" hormone-replacement therapy. In cancer, antiestrogenic effects are thought to be important, although genistein especially has been shown to induce wide-ranging anticancer effects on cell lines independent of any hormone-related influence. There are few indications of harmful effects at present, although possible proliferative effects have been reported. In infants, the effects of high levels of isoflavones in soya-milk formulas are uncertain. The second group of phytoestrogens are the lignans. They have been less investigated despite their known antiestrogenic effects and more widespread occurrence in foods (ADLERCREUTZ and MAZUR 1997; BINGHAM et al. 1998; THAM et al. 1998).

Epidemiological studies, as well as in vitro and in vivo experiments, provide evidence suggesting that isoflavonoids and lignans are protective and lower the risk of prostate cancer. The protective effect seems to occur mainly during the promotional phase of the proliferative disease. However, as long as the role of estrogens in prostate cancer is unclear (Sect. D.IV.), the potential beneficial effects of phytoestrogens in lowering prostate-cancer risk remain hypothetical (ADLERCREUTZ and MAZUR 1997).

Investigation into the possible benefits of phytoestrogens is hampered by lack of analytical standards and, hence, inadequate methods for the measurement of low levels in most foods (BINGHAM et al. 1998). This has induced scientists to search, *inter alia*, for other non-feminizing estrogens for the treat-

ment of men. As a first example, SERMs may be cited (BRYANT and DERE 1998; GRESE and DODGE 1998; HOYT et al. 1998).

Recent advances in our understanding of the molecular mechanism of action of ER agonists and antagonists have indicated that the pharmacology of SERMs is complex, and have highlighted the fact that not all estrogens are the same. These advantages have led to the conclusion that ERs do not merely change from inactive to an active forms upon binding a ligand but are quite malleable and can exist in several different induced conformations. Importantly, the transcription apparatus within a cell is configured so as to be able to distinguish between these different complexes (MCDONELL 1998).

In cholesterol-fed male rabbits, the oral administration of estradiol or the SERM levormeloxifene reduced cholesterol accumulation in the aortic arch by approximately 50%. The other SERM, *d*-ormeloxifene, was ineffective in this respect (HOLM et al. 1997).

We found that 17α-estradiol and analogs of this epimer of the "classical" estrogen, 17β-estradiol and a derivative of 17α-estradiol – J861 –, show the same or more powerful antioxidative potential as 17β-estradiol, but possess only marginal proliferative activities on endometrium and uterus (Fig. 5). This observation correlates very well with the low binding affinities of 17α-estradiol to ER. The daily administration of 2 mg 17α-estradiol to elderly men prolonged significantly the lag time for ex vivo oxidation of LDL by copper. No signs of "classical" estrogenicity (no changes in serum levels of 17β-estradiol, estrone, SHBG, prolactin, or testosterone or subjectively recorded breast tenderness) were seen (OETTEL et al. 1995, 1996a and b). WASHBURN et al. (1996) apparently followed the same strategy. Using a different 17β-estradiol epimer, 17α-dihydroequilenin, in male rhesus monkeys, they were successful in preventing arteriosclerosis without feminizing effects. In contrast to female rhesus monkeys, the serum insulin concentrations were decreased by 17α-dihydroequilenin, and an insulin-receptor resistance was overcome.

Reactive oxygen species play an important role in both the physiology and pathology of the human spermatozoon (AITKEN 1995). Physiological action of LH in the testis causes lipid peroxidation and maintains high activities of peroxide-metabolizing enzymes in the gonadal interstitial tissue. The steroidogenic steps regulated by P450 enzymes are the most likely sites of free-radical generation (PELTOLA et al. 1996).

All estrogens have an antioxidative potential. This means that they protect, e.g., the cell membrane against the oxidative attack of free-radical oxygen species (ROS). No influence on ROS-generating enzyme systems has become known so far. The synthesis of $\Delta^{8,9}$-dehydro derivatives of 17α-estradiol has made it possible that the formation of ROS is blocked too (RÖMER et al. 1997). The so-called scavestrogens are novel derivatives of 17α-estradiol and combine nongenomic (antioxidative, radical-scavenging) effects (Fig. 5) with classical genomic (ER-mediated) activities in the brain while having a low (and clinically non-relevant) estrogenic effect outside the CNS. These scavestrogens distinguish themselves by a CNS-targeting

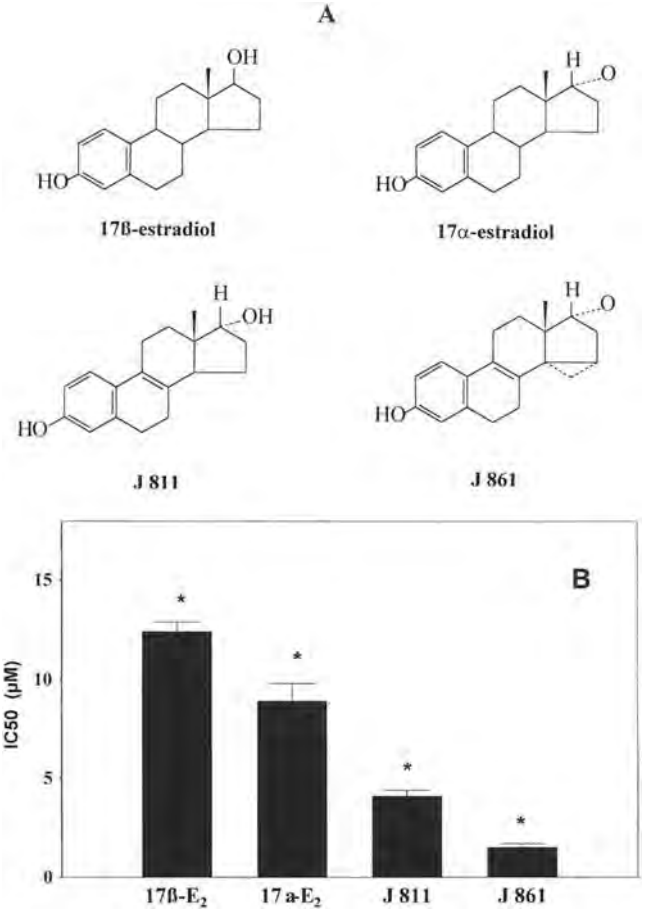

Fig. 5A,B. Chemical structure of the so-called scavestrogens J 811 and J 861 in comparison to 17β- and 17α-estradiol (**A**) and inhibition of lipid peroxidation in synaptosomal membranes from rats (**B**) (RÖMER et al. 1997)

behavior and are a completely new alternative for estrogen replacement with non-feminizing estrogens in elderly men.

In several in vitro as well as in vivo studies, we compared the estrogen-like effects of J 861 in the genital tract and the CNS with those of 17β-estradiol. The scavestrogen displayed strongly reduced affinity for, and marginal transactivational efficacy at, the "classic" ERs (ERα as well as ERβ). However, while their uterotropic efficacy in vivo was 100–1000 times lower than that of 17β-estradiol, several observations following chronic administration in ovariectomized rats are indicative of CNS-selective estrogen-like activity. Thus, J 861 regulated the secretory activity of the pituitary-adrenal axis in an estrogen-like fashion and significantly stimulated the transcription of estrogen-dependent genes in the CNS (corticotropin-releasing hormone,

oxytocin, Bcl-2; see Fig. 6) at doses which were unable to promote uterine enlargement, unlike 17β-estradiol. Chronic treatment with J861 in doses which barely affected the genital tract produced estrogen-like anxiolysis and improved the retention of conditioned avoidance behavior. Moreover, pretreatment with J861 was able to attenuate scopolamine-induced learning impairment in an estrogen-resembling manner. Finally, J861 was as potent as 17β-estradiol in protecting nerve cells from oxidative stress in vitro, and significantly decreased NO production and NOS activity in rat brain upon administration in vivo. These results indicate that non-feminizing estrogens like J861 act as selective neurotropic and, probably, neuroprotective agents, combining estrogen-like genomic effects with enhanced radical-scavenging

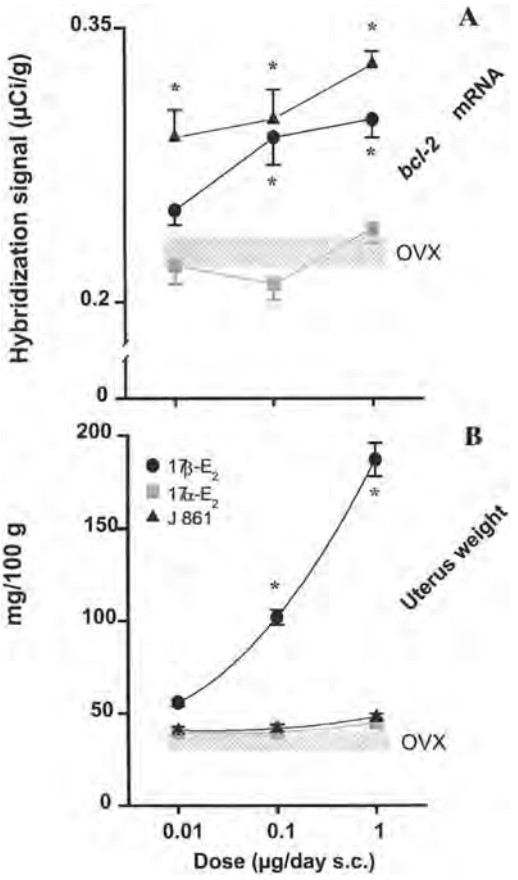

Fig. 6A,B. Non-feminizing estrogen J 861 enhances the expression of the estrogen-driven anti-apoptotic gene *Bcl-2* in the rat hippocampus (**A**), whereas J 861, like the parent compound 17α-estradiol, doesn't show any estrogenic activity on the uteri of the same animals (**B**) (Patchev et al. 1998)

capacity (PATCHEV et al. 1998). Thus, the non-feminizing estrogens emerge as prime candidates for gender-independent estrogen-replacement therapy.

Thus, all the pharmaceutical tasks of a specific brain-enhanced delivery of estradiol become more or less superfluous (SIMPKINS et al. 1998). A goal for the future is to find an answer to the question of whether scavestrogens can effectively exert their effects even in the presence of normal estradiol levels in men, and thus expand the therapeutic applications of these compounds.

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