Int. J. Immunopharmac., Vol. 14, No. 4, pp. 541–553, 1992.
Printed in Great Britain.

0192–0561/92 $5.00 + .00
Pergamon Press Ltd.

AROMATASE INHIBITORS REGENERATE THE THYMUS IN AGING MALE RATS

B. D. GREENSTEIN,* ELIZABETH F. DE BRIDGES and F. T. A. FITZPATRICK

Division of Pharmacological Sciences, United Medical and Dental Schools, St. Thomas's Campus, Lambeth Palace Road, London SE1 7EH, U.K.

(*Received* 14 *October* 1991 *and in final form* 22 *November* 1991)

Abstract — The thymus can be regenerated in aging rats by surgical or chemical castration and regeneration is inhibited by testosterone, which may exert this effect, at least in part, through its conversion to estradiol. An attempt has been made to regenerate the thymus in intact aging rats using inhibitors of the aromatase system, in the hope that this maneuver could lead to the use of such chemical intervention in the treatment of immunodeficiency syndromes. Young adult and aging (18-month-old) male rats were orchidectomized under ether anesthesia and 7 days later given s.c. implants of testosterone in silicone elastomer (SILASTIC) tubing. Some rats received testosterone together with a five-fold excess of the aromatase inhibitor 1,4,6-androstatriene-3,17-dione (ATD). One group of young intact rats received implants containing 25 mg ATD and a group of 18-month-old intact rats received 125 mg ATD or 25 mg of another, more powerful aromatase inhibitor 4-hydroxyandrostenedione (4-OH). On the 28th day after implanting, rats were killed and the thymus, spleen, prostate gland and seminal vesicles removed for weighing and histology. In addition, estrogen receptors were measured in the thymus. The thymus was enlarged after orchidectomy and greatly restored in aging rats. In aging rats, both aromatase inhibitors restored the thymus, which appeared normal histologically. In addition, ATD enlarged the thymus in young intact animals. Doses of testosterone which restored the accessory sex organs to weights measured in intact rats prevented the effects of orchidectomy on the thymus, and in old rats the effects of testosterone were blocked by ATD in both thymus and spleen. Available cytosolic estrogen receptors were reduced in thymus of testosterone-treated orchidectomized rats, and this effect blocked by ATD, which itself was apparently able to induce estrogen receptors. Receptors could not be detected in thymus from aging rats, but were measureable in cytosols from thymus of orchidectomized or ATD-treated old rats. It is therefore possible to restore the thymus in intact aging rats without recourse to surgical or chemical castration, and such a maneuver may possibly be of use to enhance an immune system weakened by aging or disease.

There is much evidence that the immune system is influenced by sex steroids, both physiologically and pharmacologically, in health and disease. There appears to be a sexual dimorphism in immunocompetence in that in females it is greater than in males. For example, immunoglobulin titers such as IgM are higher in women than in men, and are reduced during pregnancy (Gusdon, 1969). Certain autoimmune diseases have a strikingly higher incidence in women than in men (Ahmed, Penhale & Talal, 1985), and the hormone responsible for the enhanced immunological activity may be estradiol.

This supposition receives support from studies in animals, such as the NZB × NZW F_1 (B/W) mouse, a model of systemic lupus erythematosus; in this strain the females develop autoantibodies to nuclear components and die months before the males. They can be rescued by ovariectomy or treatment with androgens, and the males develop the syndrome if injected with estrogens (Roubinian, Talal, Greenspan, Goodman & Siiteri, 1978, 1979). Furthermore, estrogens administered in small doses enhance the immune response (Ahmed, Dauphinee & Talal, 1985), although larger doses may be immunosuppressive (Grossman, 1984).

Our attention was drawn to the effects of the sex hormones on immune tissues through the observation that the thymus of aging rats was regenerated after orchidectomy (Fitzpatrick, Kendall, Wheeler, Adcock & Greenstein, 1985). The regenerated thymus appeared normal histologically and regeneration was inhibited if orchidectomized rats

*Author to whom correspondence should be addressed. Present address: Lupus Research Unit, Rayne Institute, St Thomas' Hospital, London SE1 7EH, U.K.

were treated chronically with testosterone (Greenstein, Fitzpatrick, Adcock, Kendall & Wheeler, 1986). Estradiol was far more potent than was testosterone in inhibiting thymus regeneration, and the potent androgenic metabolite of testosterone, dihydrotestosterone (DHT), was ineffective as a regeneration inhibitor (Fitzpatrick & Greenstein, 1987). These results suggested that testosterone might have exerted its inhibitory effect on thymus regeneration partly, at least, through its aromatization to estradiol. Preliminary evidence to support this hypothesis was provided by the observation that the inhibitory effect of testosterone on thymus development in immature female rats was completely blocked by the aromatase inhibitor 1,4,6-androstatriene-3,17-dione (ATD; Greenstein, Mander & Fitzpatrick, 1988).

These results also prompted the speculation that thymus regeneration during aging might be a potentially important therapeutic maneuver, and to this end we attempted to find a drug which might regenerate this organ in aging animals which had not been orchidectomized. Initially we used an analog of the neuropeptide luteinizing hormone-releasing hormone (LHRH), and this was effective, although the animals were functionally orchidectomized during the course of the treatment (Greenstein, Fitzpatrick, Kendall & Wheeler, 1987).

Based on the knowledge of the effects of ATD on those of testosterone, and on those of the LHRH analog, we attempted to find a drug which would regenerate the thymus in intact aging rats, without producing functional castration. An attempt was also made to relate the steroid effects to estrogen receptor activity. The interaction of steroids with receptors is a primary cellular event in the action of the steroid and estrogen receptors have been described in the thymus (Grossman, 1985). In the present experiments, it was reasoned that if testosterone exerts an atrophic effect on the thymus through conversion to estradiol, this might be reflected by altered estrogen receptor activity. In this paper evidence is presented that the thymus can be regenerated using inhibitors of the aromatase system without surgical or chemical castration, and that the atrophic effect of testosterone may be effected, at least in part through estrogen receptors, which are apparently expressed in the old regenerated thymus.

EXPERIMENTAL PROCEDURES

Animals and treatments. Male Wistar CSE rats were bred and maintained in the Biological Services Division of the United Medical and Dental Schools of Guys and St Thomas's Hospitals (St Thomas's Campus). The rats were kept three to a cage under controlled conditions of lighting and heating (lights on from 0800–2200; 21–23°C). Young adult (10-week-old) rats were allowed unlimited access to water and Spratt's Laboratory Diet No. 1, but 18-month-old rats were given 100 g daily per cage, since it had been found that access to unlimited quantities of food resulted in a high mortality rate in these animals (Fitzpatrick & Greenstein, 1987).

Rats were orchidectomized through the scrotal route under pentobarbitone anesthesia (SAGITTAL; young: 40 mg/kg; aging: 28 mg/kg; May & Baker, Dagenham, Essex, U.K.) and caged in weight-matched groups. Some animals were sham-orchidectomized, in that the testes were exposed but not removed. On the 7th day after the operation, rats were anesthetized with ether and given s.c. implants containing the appropriate dose of testosterone (Sigma, London, U.K.) in elastomer medical grade tubing (SILASTIC; 601–335; 0.45 cm (internal diameter) × 1.5 cm; Dow Corning Corporation Medical Products, Midland, MI, U.S.A.). Young animals were given 5 mg, and aging animals 25 mg of testosterone. From previous work, it was known that these doses produced plasma levels of testosterone in the physiological range (Greenstein *et al.*, 1986). Some animals were orchidectomized and given implants containing the appropriate dose of cholesterol. Two groups of young orchidectomized rats received 25 mg of the aromatase inhibitor 1,4,6-androstatriene-3,17-dione (ATD) alone or together with 5 mg of testosterone in the same implant. Aging rats received either 125 mg of ATD alone, or a combination of 125 mg of ATD and 25 mg testosterone.

Tissues. On the 28th day after implanting the animals, they were killed by cervical dislocation and the ventral prostate gland, seminal vesicles, thymus and spleen removed, trimmed of surrounding tissues, weighed and half the thymus and the remainder of the tissues fixed in formol acetic alcohol to be processed later for histological examination. After fixation, tissues were embedded in paraffin wax and 4 μm sections cut and stained using hematoxylin–eosin. One half of the thymus tissue was retained for measurement of cytosolic estrogen receptors. The other half was used for histology.

In a further experiment, groups of intact young adult (10-week-old) and aging (18-month-old) male rats were given s.c. implants of ATD. Young rats received 25 mg and older rats 125 mg for 28 days prior to processing of the tissues. This experiment

was designed in order to address the question as to whether the aromatase inhibitor was able to induce thymus growth and regeneration in the presence of the testes.

Estrogen receptor assay. Cytosolic estrogen receptors were measured using minicolumns of Sephadex LH-20 (Pharmacia UK Ltd; Ginsburg, Greenstein, MacLusky, Morris & Thomas, 1974). Briefly, the thymus was homogenized at 0°C in a buffer containing 0.01 M phosphate – 0.25 M sucrose, pH 7.4, containing 0.1 M 2-mercaptoethanol, and centrifuged at 100,000 *g* to prepare the cytosols. Aliquots of cytosol were incubated with a range of concentrations of [^{3}H]estradiol (0.03 – 1.0 nM; Amersham International, Bucks, U.K.; 135 Ci/mmol) at 25°C for 1 h either alone or in the presence of 100-fold excess of unlabelled estradiol (Sigma) to determine non-specific binding. Bound and free hormone were separated on small columns of Sephadex LH-20 at 1°C and the bound radioactivity measured by liquid scintillation spectrometry. The binding capacity and molar dissociation constant (K_d) were estimated using the Scatchard plot (Scatchard, 1949). The binding capacity was expressed per mg cytosol protein which was determined using the Folin – phenol method (Lowry, Rosebrough, Farr & Randall, 1951).

In order to establish that any effect of an aromatase inhibitor was not in the idiosyncratic effect of a single substance, we also used the compound 4-hydroxyandrostenedione (4-OH), a potent aromatase inhibitor (Henderson, 1987), currently used to treat prostate and breast carcinoma and kindly supplied by Ciba-Geigy Pharmaceuticals, West Sussex, U.K. In a separate experiment, 12-month-old male rats were divided into two groups of five animals. In both groups, animal weight ranged from 490 to 550 g at the beginning of the experiment. The rats were not orchidectomized. Rats in one group were implanted with a SILASTIC implant containing 25 mg of cholesterol and the other with an implant containing 25 mg of 4-OH. After 28 days, the rats were killed and the thymus, spleen, ventral prostate, testes and adrenal glands removed and weighed.

Statistical analysis. Results were analyzed using the unpaired Student's *t*-test. This test was performed with the variance obtained by pooling data from all groups. The analyses were carried out using the statistical package GLIM (The Glim System Release 3.77 Manual 1985, the Numerical Algorithm Group, Oxford, U.K.; Greenstein *et al.*, 1986). Results were also analyzed using the Mann – Whitney *U*-test in order to gain a non-parametric statistical result.

RESULTS

Use of ATD

Tissue weights.

Accessory sex organs. The overall pattern of results was compared in young and aging animals. Implants of testosterone which produced plasma concentrations in the physiological range were able to restore accessory organs to weights measured in intact animals (Figs 1 and 2). Histologically, the ventral prostate and seminal vesicles from orchidectomized, testosterone-treated rats were indistinguishable from those of intact animals (not shown). Tissue weights were expressed per g of body weight. Had they been expressed as organ weights, accessory sex organ weights in intact aging rats would have been about twice those in younger animals. Young rats weighed from 290 to 320 g and 18-month-old rats weighed from 600 to 700 g.

ATD had no effect on weights of prostate or seminal vesicles in any group of animals (Figs 1 and 2). Histologically, both prostate and seminal vesicles appeared normal (not shown).

Thymus and spleen in orchidectomized rats. The thymus from young rats was, as expected, markedly increased in size after orchidectomy (Figs 1 and 2; $P<0.001$). In young rats, the thymus from orchidectomized rats was about 62% larger than that from intact animals, based on a ratio of thymus weight/body weight (see Fig. 1); in aging rats, the thymus was about twice the weight measured in intact rats. The spleen was increased in weight after orchidectomy of young ($P<0.05$) but not aging rats. Testosterone completely blocked the regenerative effect of orchidectomy on the thymus. ATD alone did not alter the effect of orchidectomy on thymus of young rats. In spleen, ATD had no statistically significant effect in young or aging rats.

The mixture of ATD and testosterone did not produce a result significantly different from the use of testosterone or ATD alone in the thymus of young orchidectomized rats, or in the spleen from young rats, although spleen weight in older rats was significantly ($P<0.001$) greater than after ATD alone. In aging orchidectomized rats, ATD significantly blocked the atrophic action of testosterone ($P<0.05$).

Intact rats. In both young and aging intact rats, ATD alone significantly increased thymus weight (Figs 1 and 2; young rats: $P<0.001$; aging rats: $P<0.001$). In aging rats, the effect was massive, the organ being about three times the size of that measured in intact animals, and a third larger than

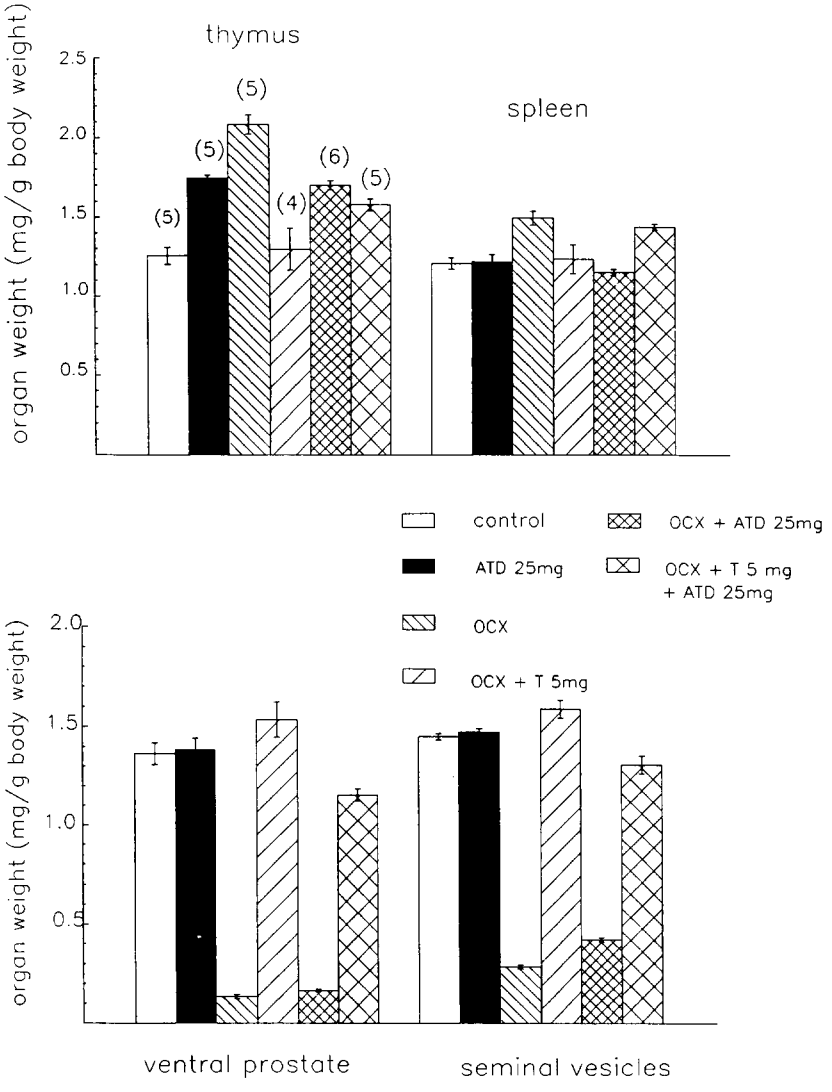

Fig. 1. Effects of orchidectomy and various steroid treatments on organ weight in 10-week-old male Wistar CSE rats. Animals were orchidectomized under Sagittal anesthesia and 7 days later under ether anesthesia given s.c. SILASTIC implants containing treatments as indicated on the figure. On day 28, the tissues were weighed. Results are shown as mean organ weight per g body weight ± S.E.M.; numbers of animals in parentheses. Key to abbreviations: OCX = orchidectomy; T = testosterone; ATD = the aromatase inhibitor 1,4,6-androstatriene-3,17-dione.

that measured in orchidectomized rats. The spleen of young intact rats was apparently unaffected by ATD, but reduced ($P<0.001$) in aging animals.

Histology of thymus.

Young adult rats. The histological appearance of the thymus in young rats is shown in Fig. 3(a). The organ was lobular, with clearly defined areas of cortex and medulla and densely packed with thymocytes. After orchidectomy, the thymus was very similar in appearance (not shown). After treatment for 28 days with testosterone, several smaller lobules appeared and the corticomedullary junction was less well defined [Fig. 3(b)]. There were signs of fat deposition and thymocyte loss. After treatment of orchidectomized rats with ATD, the medulla seemed to have shrunk considerably, and there was evidence of thymocyte loss. The organ was lobular but there appeared to be some loss of organization [Fig. 3(c)]. After treatment with testosterone and ATD together, the organ appeared better organized than with

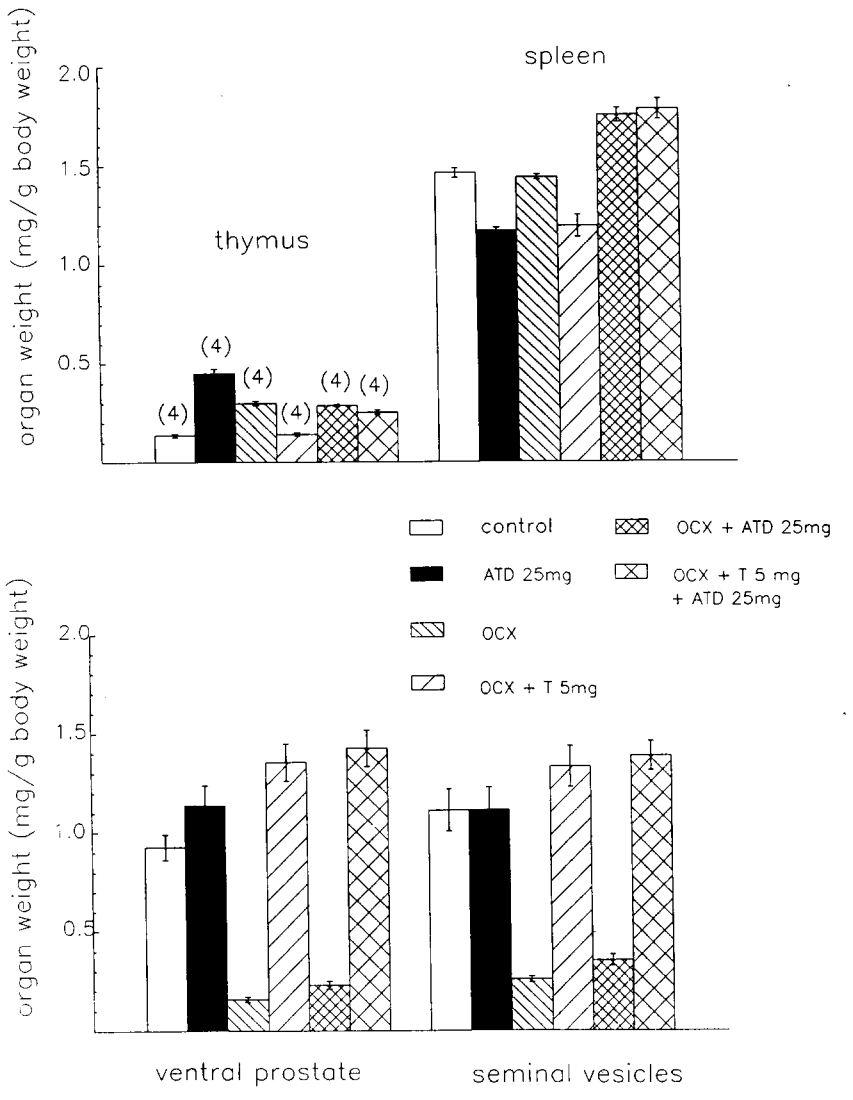

Fig. 2. Effects of orchidectomy and various steroid treatments on organ weight in 18-month-old male Wistar CSE rats. See legend to Fig. 1 for explanation of treatments and abbreviations.

testosterone alone, although the medullary areas were considerably shrunken [Fig. 3(d)].

Aging rats. The histological appearance of the thymus in an 18-month-old male rat is shown in Fig. 4(a). The organ was lobular, but organization was minimal, and there was much fat deposition. In general, sections from these thymuses showed poor organization and very little vascularity. By 28 days after orchidectomy, there was evidence of organization into cortex and medulla, although there was still fatty tissue present, and the organ clearly was accumulating lymphocytes once more [Fig. 4(b)]. Testosterone treatment of orchidectomized rats produced a thymus histologically identical to that from an intact animal (not shown).

The thymus from an old intact rat treated for 28 days with ATD is shown in Fig. 4(c). The organ was discernibly organized into cortex and medulla, although the corticomedullary junction was not well defined and there were areas of fatty tissue. The thymus appeared well vascularized and filled with thymocytes. A similar picture was obtained after

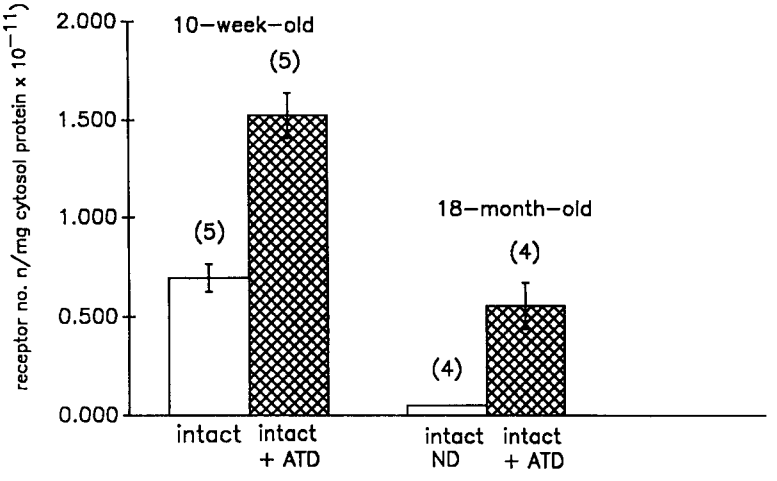

Fig. 6. Effects of the aromatase inhibitor ATD on cytosolic estrogen receptors in thymus of 10-week and 18-month-old intact male rats. Results are shown as the numbers of specific estradiol-binding sites per mg cytosol protein (mean ± S.E.M.; numbers of animals in parentheses). Abbreviations: ATD = 1,4,6-androstatriene-3,17-dione; ND = not detectable.

treating orchidectomized rats with ATD [Fig. 4(d)]. The gross appearance of the exposed thorax in 18-month-old rats treated either with cholesterol or ATD is shown in Fig. 5.

Use of 4-hydroxyandrostenedione (4-OH)

After treatment with 4-OH, the thymus was greatly increased in weight and in histological appearance resembled the thymus from an orchidectomized rat (not shown). Thymus weights were: control: 76 ± 6 mg (n = 4); 4-OH: 231 ± 42 mg (n = 5; mean ± S.E.M.). These results were significantly different from each other (P<0.001). Weights of accessory sex organs and adrenals were unchanged by the treatment (not shown).

Estrogen receptors

Intact rats. Numbers of receptors in thymus are shown in Fig. 6. Estrogen receptors were measurable in cytosols from 10-week-old but not 18-month-old thymus. After treatment with ATD, however, estrogen receptors were greatly (P<0.001) increased in cytosols from both age groups.

Orchidectomized rats. Estrogen receptors were not increased in number after orchidectomy of young rats, and were measurable in cytosols from thymus of 18-month-old rats (Fig. 7). Testosterone apparently reduced receptor number in cytosols from both age groups, and they were not detectable in aging rat thymus. Surprisingly, both ATD alone and ATD in combination with testosterone produced large increases in receptor number, particularly in thymus from aging rats.

The affinity of the binding reaction was unaffected by any of the treatments, the molar dissociation constant (K_d) varying from 1.5 to 4.5 × 10^{-10} M.

DISCUSSION

The regeneration of the thymus observed in aging rats after orchidectomy, and the inhibition of this phenomenon by testosterone confirms results reported previously (Greenstein *et al.*, 1986; Fitzpatrick & Greenstein, 1987). It should be pointed out, however, that testosterone alone is unlikely to account for the age-related atrophy of the thymus. It has been shown elsewhere, using mice (Bellamy, 1967) and rats (Chioidi, 1940), that the thymus continues to atrophy after removal of the gonads. Therefore, some other as yet unknown factor(s) dictates thymus atrophy with aging, although testosterone presumably exerts a regulatory effect on thymus size, structural organization and perhaps function.

Rats were considered to be "aging" at 18 months. In this colony rats were dying of apparently natural causes, at this age, although a restricted diet reduced

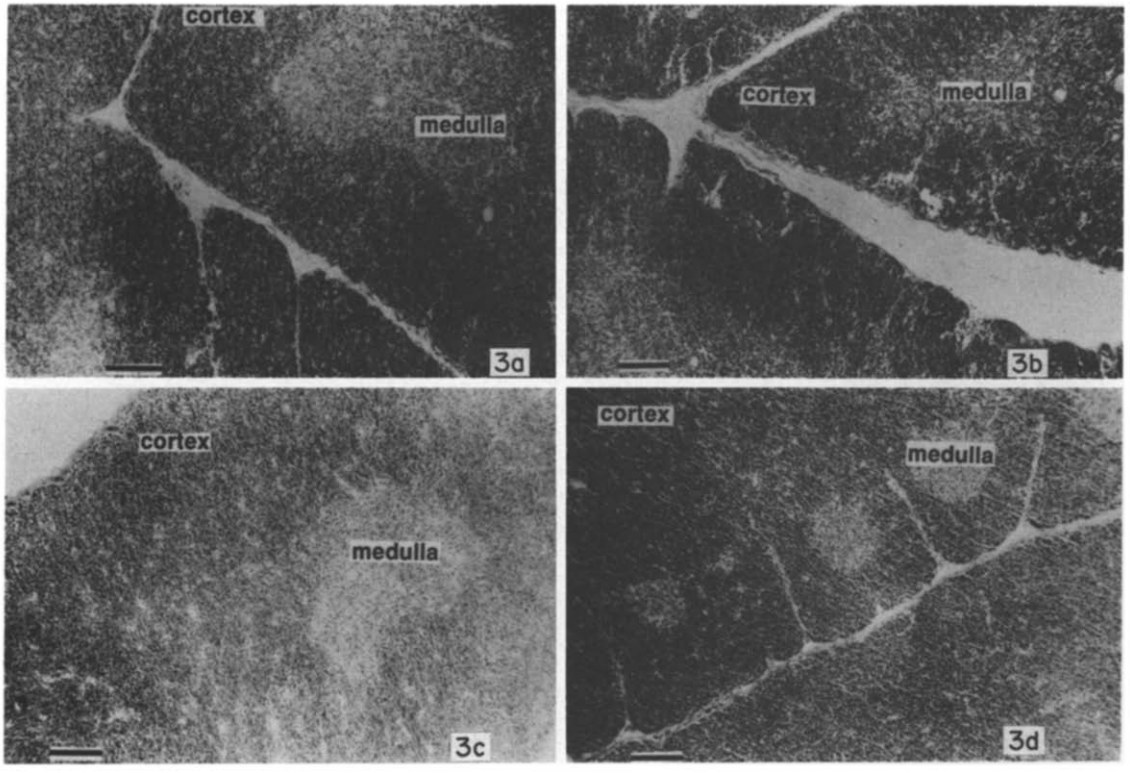

Fig. 3. (a) Photomicrograph of a section through the thymus of a 10-week-old intact male rat. The outer cortex and inner medullary areas are indicated, and lobes are clearly visible. The sections have been magnified 375 × and the horizontal bar indicates 40 μ; (b) after orchidectomy (OCX) and implant of 5 mg testosterone (T) for 28 days. Gaps in cortex and medulla indicate cell loss. Note deposition of adipose tissue; (c) after OCX and implant of 25 mg aromatase inhibitor 1,4,6-androstatriene-3,17-dione (ATD) for 28 days. Note blurring of corticomedullary junction and cell loss; (d) after OCX and 5 mg T/25 mg ATD for 28 days. Note presence of small lobes with shrunken medullary areas and cell loss. Contrast with (b).

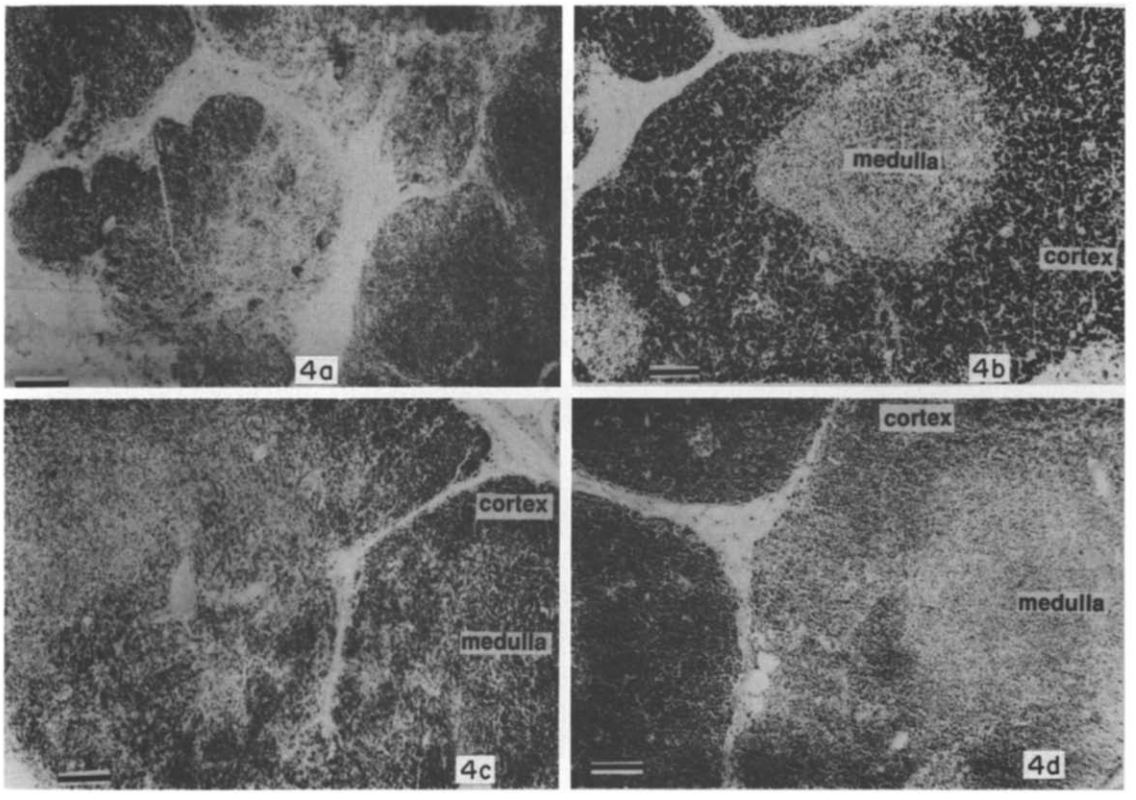

Fig. 4. (a) Photomicrograph of a section through the thymus of an 18-month-old intact male rat. Lobules are present, but there is no organization into cortex and medulla, and there is much adipose tissue (lower left corner). All sections are shown magnified 375 ×, and the horizontal bar represents 40 μ; (b) after OCX for 28 days. Cortical and medullary organization are evident, although gaps are present; (c) after treatment of intact aging rats with 125 mg ATD for 28 days. The organ is organized, and adipose tissue is much reduced. But gaps are present. Contrast with (a); (d) after OCX and 125 mg ATD for 28 days.

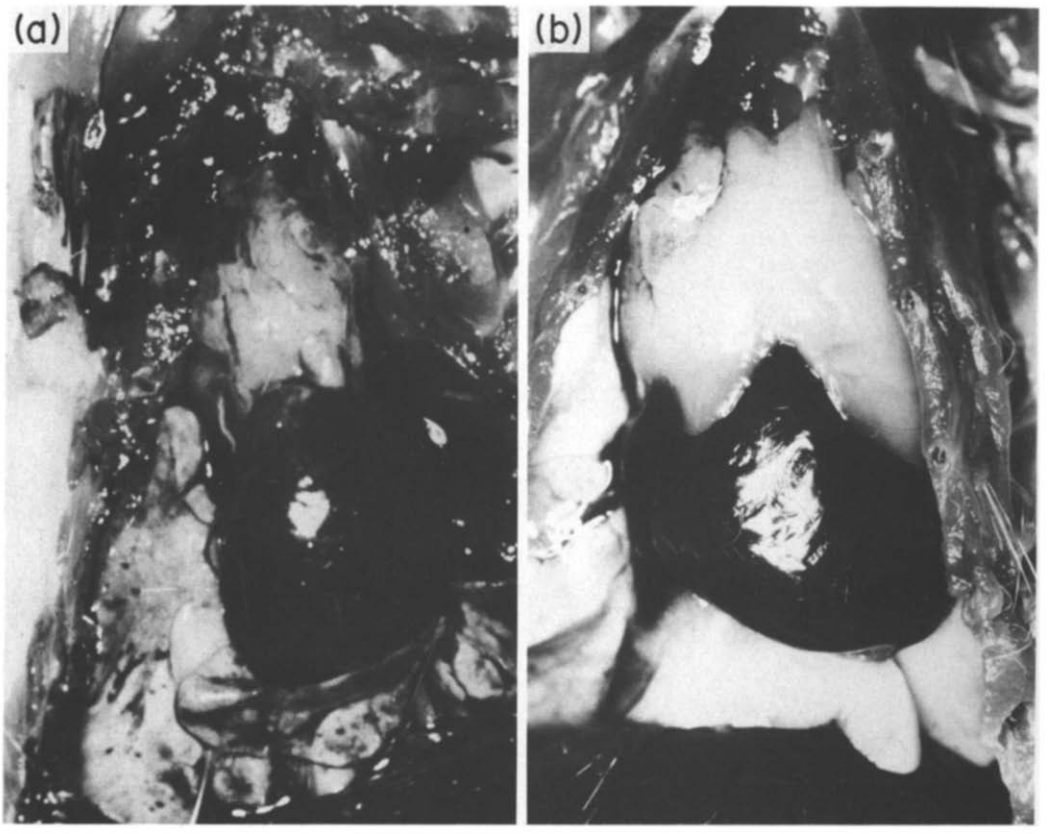

Fig. 5. Photographs of exposed thorax of 18-month-old rat treated for 28 days s.c. with (a) cholesterol, or (b) 125 mg of the aromatase inhibitor 1,4,6-androstatriene-3,17-dione (ATD). In (a) no thymus is visible, while in (b) a large lobed thymus overlies the heart.

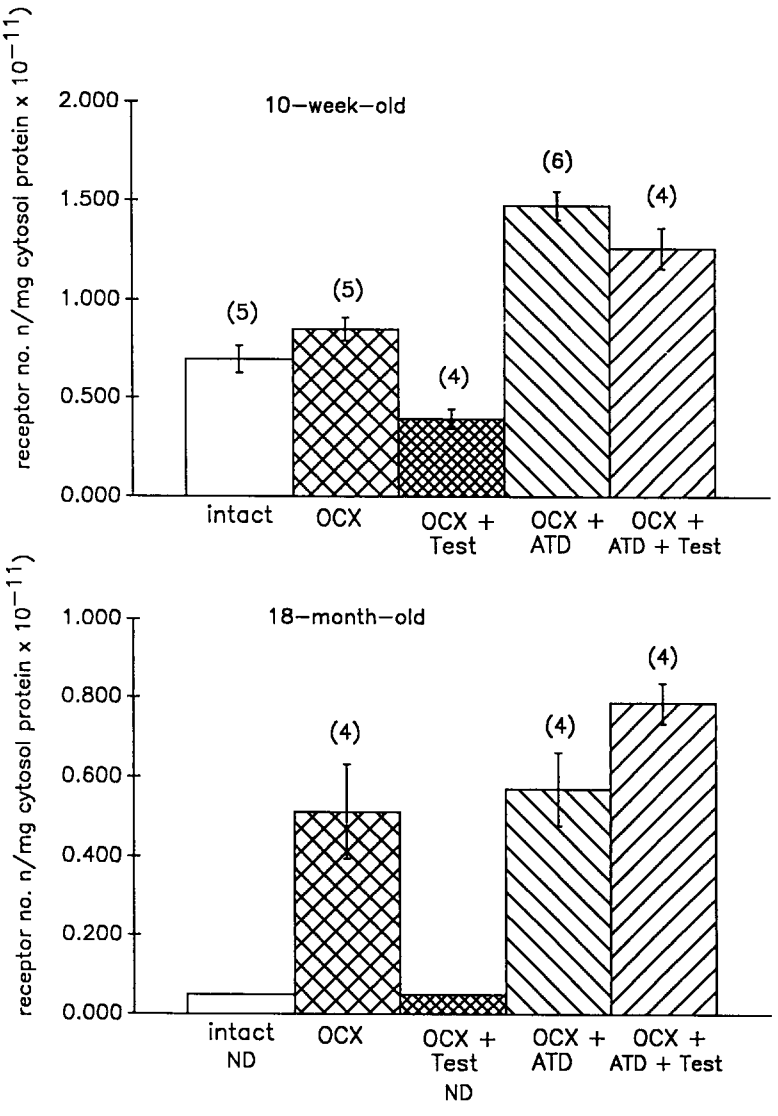

Fig. 7. Effects of orchidectomy and various steroid treatments on cytosolic estrogen receptors in thymus of 10-week and 18-month-old Wistar CSE rats. See text and legend to Fig. 1 for experimental details. Results are shown as the numbers of specific estradiol binding sites per mg cytosol protein (mean ± S.E.M.; numbers of animals in parentheses). Abbreviations: OCX = orchidectomy; TEST = testosterone; ATD = 1,4,6-androstatriene-3,17-dione.

the mortality rate. Nevertheless, we hesitate to term the rats "old" since some in the colony did survive to 2 years or more. We used 18-month-old rats since their thymuses have atrophied virtually completely and because the cost of maintaining the animals is prohibitive.

We show here for the first time that the aromatase inhibitor ATD enlarged the thymus in young rats, and caused thymus regeneration in older animals. In both age groups, ATD inhibited the atrophic action of testosterone. Therefore, the hormone might produce this effect, at least in part, through its conversion to estrogens. The compound was also active in the spleen, which was significantly decreased in weight. An explanation for this is difficult, but it may be surmised that a re-allocation of T-lymphocytes from spleen to the newly regenerated thymus might have occurred. Any

possible functional significance of this is presently unknown. The spleen has been, in this laboratory, relatively refractory to the hormonal manipulations which so profoundly affect the thymus. Clearly this organ is not discernibly affected by the aging process to the same extent as is the thymus, nor does testosterone in the doses used here produce spleen atrophy. That the effect was not peculiar to ATD was confirmed using another, more potent aromatase inhibitor, 4-OH, which is already in use in human patients (Henderson, 1987). Neither inhibitor had any apparent adverse effect on accessory sex organs, whose weights were included in the results section to confirm this. We have been able to restore the thymus in aging rats using another chemical intervention, the LHRH analog Zoladex, but this also caused chemical castration and would therefore be a less acceptable maneuver if thymic regeneration was required.

The presence of estrogen receptors in the thymus supports the hypothesis that estrogens are active in this tissue. The present results confirm the finding by Grossman (1985) that the thymus contains estrogen receptors. In addition, we present evidence that these receptors may mediate the atrophic action of testosterone on the thymus. The situation is complicated, however, by the finding that the aromatase inhibitor itself appeared to increase available receptor numbers. This could possibly be explained by the endogenous inhibition of estrogen production, which might lessen nuclear translocation of receptors, thus leaving more available for measurement. It is equally possible, however, that ATD might stimulate estrogen receptor synthesis. There is a precedent, conceptually, since it is well known that estrogens stimulate progesterone receptor synthesis (Al-Khouri & Greenstein, 1985). We are currently studying this problem by measuring the nuclear occupancy of estrogen receptors after the different treatments. The stimulant effect of ATD on estrogen receptors correlates well with its effect on thymus weight, although the possibility of a direct and general stimulant effect on thymus growth and regeneration cannot be ruled out.

The sex hormones are not alone in mediating thymus atrophy and regeneration, which has also been achieved by infusion of pituitary adenoma cells, which secrete growth hormone (Kelley, Brief, Westley, Novakofski, Bechtel & Walker, 1986). It is also conceivable that different sex and other hormones have differential effects on the thymus. Sobhon & Jirasattham (1974) found that testosterone was most potent in decreasing lobule size and cortical width, while in another study, Glucksman & Cherry (1968) reported that estradiol caused proliferation of thymis epithelial cords. The finding here that ATD restored zonal organization to the aging thymus of intact rats suggests that estradiol, possibly as a metabolite of testosterone, might be responsible for the breakdown of thymus organization, although whether this is confined to cortex or medulla is not known. In future studies, it will be necessary to vary doses of the hormones and antagonists, and to identify active metabolites of the steroids in the tissues. Even more importantly, the functional significance of the steroidal effects must be studied. In retrospect, we could have carried out further tests on the tissue, for example a simple cell count in the thymus.

It is felt that a contribution of this work is to draw attention to the potential use of steroids such as hormone antagonists and enzyme inhibitors in the treatment of diseases related to immune function. There is currently much interest in the relationship between sex hormones and autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus. Evidence of a role for sex hormones in the mediation of these diseases is growing (Lahita, 1990; Ahmed & Talal, 1990) and estradiol appears to be very potent in precipitating or exacerbating certain autoimmune states in humans and certain murine models. Nevertheless, it should be borne in mind that not all autoimmune diseases occur predominantly in women (Ahmed & Talal, 1990; Lahita, 1990). Sexual dimorphism of both humoral and cell-mediated immune responses is well documented (Grossman, 1989). Aromatase inhibitors are already used to treat breast and prostate carcinoma (Henderson, 1987), and their potential as therapeutic agents for diseases involving certain immune-related diseases might be worth exploring.

Acknowledgements — This work was supported by grants from the Cancer Research Campaign and the Research (Endowments) Committee of St. Thomas's Hospital (STH) to B.D.G. We thank Dr A. V. Swan of the Department of Community Medicine in the Medical School for advice on analysis of the data.

REFERENCES

AHMED, A. S., DAUPHINEE, M. J. & TALAL, N. (1985). Effects of short term administration of sex hormones on normal and autoimmune mice. *J. Immun.*, **134**, 204–210.

AHMED, A. S., PENHALE, W. J. & TALAL, N. (1985). Sex hormones, immune responses and autoimmune diseases: mechanism of sex hormone action. *Am. J. Path.*, **121**, 531–551.

AHMED, S. A. & TALAL, N. (1990). Sex hormones and the immune system — part 2. Animal data. Bailieres clin. Rheumat., **4**, 13–31.

AL-KHOURI, H. & GREENSTEIN, B. D. (1985). Progesterone receptors in rat brain and uterus: dependence on the hormonal milieu. *J. Endocr.*, **107**, 159–162.

BELLAMY, D. (1967). Hormonal effects in relation to ageing in mammals. *Symp. Soc. exp. Biol.*, **21**, 427–453.

CHIOIDI, H. (1940). The relationship between the thymus and the sexual organs. *Endocrinology*, **26**, 107–117.

FITZPATRICK, F. T. A. & GREENSTEIN, B. D. (1987). Effects of various steroids on the thymus, spleen, ventral prostate and seminal vesicles in old orchidectomized rats. *J. Endocr.*, **113**, 51–55.

FITZPATRICK, F. T. A., KENDALL, M. D., WHEELER, M. J., ADCOCK, I. M. & GREENSTEIN, B. D. (1985). Reappearance of the thymus of ageing rats after orchidectomy. *J. Endocr.*, **106**, R17–R19.

GINSBURG, M., GREENSTEIN, B. D., MACLUSKY, N. J., MORRIS, I. D. & THOMAS, P. J. (1974). An improved method for the study of high affinity steroid binding: oestradiol binding in rat brain and pituitary. *Steroids*, **23**, 773–792.

GLUCKSMAN, A. & CHERRY, C. P. (1968). The effect of castration, oestrogens, testosterone and the oestrous cycle on the epithelium of the thymus in male an female rats. *J. Anat.*, **103**, 113–133.

GREENSTEIN, B. D., FITZPATRICK, F. T. A., ADCOCK, I. M., KENDALL, M. D. & WHEELER, M. J. (1986). Reappearance of the thymus in old rats after orchidectomy: inhibition of regeneration by testosterone. *J. Endocr.*, **110**, 417–422.

GREENSTEIN, B. D., FITZPATRICK, F. T. A., KENDALL, M. D. & WHEELER, M. J. (1987). Regeneration of the thymus in old rats treated with a stable analogue of LHRH. *J. Endocr.*, **112**, 345–350.

GREENSTEIN, B. D., MANDER, B. J. & FITZPATRICK, F. T. A. (1988). Role of aromatase system in testosterone-induced atrophy of the thymus. *J. Endocr.*, **119**, 65–67.

GROSSMAN, C. J. (1984). Regulation of the immune system by sex steroids. *Endocr. Rev.*, **5**, 435–454.

GROSSMAN, C. J. (1985). Interactions between the gonadal steroids and the immune system. *Science*, **227**, 257–261.

GROSSMAN, C. J. (1989). Possible underlying mechanisms of sexual dimorphism on the immune response, fact and hypothesis. *J. Steroid Biochem.*, **34**, 241–251.

GUSDON, J. P. (1969). Fetal and maternal immunoglobulin levels during pregnancy. *Am. J. Obstet. Gynec.*, **103**, 895–900.

HENDERSON, D. J. (1987). Aromatase inhibitors: their biochemical and clinical potential. *J. Steroid Biochem.*, **27**, 905–914.

KELLEY, K. W., BRIEF, S., WESTLEY, H. J., NOVAKOFSKI, J., BECHTEL, S. J. & WALKER, E. B. (1986). GH3 pituitary adenoma cells can reverse thymic aging in rats. *Proc. natn. Acad. Sci. U.S.A.*, **83**, 5663–5667.

LAHITA, R. G. (1990). Sex hormones and the immune system — part 1. Human data. *Bailieres clin. Rheumat.*, **4**, 1–12.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin–phenol reagent. *J. biol. Chem.*, **193**, 265–275.

ROUBINIAN, J. R., TALAL, N., GREENSPAN, J. S., GOODMAN, J. R. & SIITERI, P. K. (1978). Effect of castration and sex hormone treatment on survival, anti-nucleic acid antibodies, and glomerulonephritis in NZB × NZW F1 mice. *J. exp. Med.*, **147**, 1568–1583.

ROUBINIAN, J. R., TALAL, N., GREENSPAN, J. S., GOODMAN, J. R. & SIITERI, P. K. (1979). Delayed androgen treatment prolongs survival in murine lupus. *J. clin. Invest.*, **63**, 902–911.

SCATCHARD, G. (1949). The attraction of proteins for small ions and molecules. *Ann. NY. Acad. Sci.*, **51**, 660–672.

SOBHON, P. & JIRASATTHAM, C. (1974). Effect of sex hormones on the thymus and lymphoid tissues of ovariectomized rats. *Acta Anat.*, **89**, 211–225.