CONTRACEPTION

THE EFFECT OF SEX STEROIDS AND HORMONAL CONTRACEPTIVES
UPON THYMUS AND SPLEEN OF INTACT FEMALE RATS

H. Kuhl, M. Gross, M. Schneider*, W. Weber, W. Mehlis,
M. Stegmüller, and H.-D. Taubert

Abteilung für gynäkologische Endokrinologie, Zentrum der
Frauenheilkunde und Geburtshilfe, and Senckenbergisches
Zentrum der Pathologie*, J. W. Goethe-Universität,
D-6000 Frankfurt am Main, F. R. Germany

ABSTRACT

In view of a possible influence of oral contraceptives
upon the immune system, the effect of chronic treatment
of intact adult female rats with sex steroids and contra-
ceptive preparations upon the thymus and the spleen was
investigated.

Daily injections with 10 µg ethinyl estradiol, estradiol
benzoate, or diethyl stilbestrol for 2 weeks resulted in
a marked but reversible involution of the thymus, while
the spleen was not affected. Androgens exerted a signifi-
cant effect at a dose of 0.3 mg, and progestogens only
when 2 mg were given. When various contraceptive prepara-
tions were injected for 4 weeks, there was a total invo-
lution of the thymus which persisted even 2 weeks after
cessation of treatment. The effect appeared to be mainly
due to the estrogenic component. Progestogens intensified
the reduction of thymic weight only at higher doses.

Histological examinations revealed that estrogen treat-
ment alone resulted in a reduction of the cortex and a
depletion of lymphocytes. When contraceptive preparations
were administered, the medulla was also reduced, and both
cortex and medulla were replaced by reticular and adipose
tissue.

The estrogen receptors of thymus cytosol showed dissocia-
tion constants between 0.34 and 0.49 nM in diestrous rats,
progesterone-treated rats and ovariectomized rats, and
binding capacities between 6.5 and 2.6 fmoles/mg pro-
tein.

It remains, however, to be shown whether the estrogen-
induced involution of the rat thymus may lead to an im-
pairment of immune responses.

Submitted for publication December 30, 1983
Accepted for publication January 24, 1984
CONTRACEPTION

INTRODUCTION

Immunological processes seem to be subject to modulation by sex steroids. This hypothesis is based on experimental and clinical evidence such as the findings that treatment of ovariectomized rats with estrogens causes atrophy of the thymus (1), and that rheumatoid arthritis may undergo remission during pregnancy (2). The latter observation is believed to be due to the action of estrogens which increase more than 1000-fold in serum during pregnancy and appear to promote immune tolerance. Moreover, treatment with hormonal contraceptives seems to offer to some degree protection against the development of this disease (3), the pathogenesis of which is believed to entail autoimmune reactions.

As hypersensitivity reactions as well as the rejection of allografts are mediated by thymus-derived cells, the thymus seems to play a central role in the immune system (4).

In view of this, we investigated the effect of chronic treatment of intact female rats with sex steroids and contraceptive preparations upon the thymus and the spleen.

MATERIAL AND METHODS

Substances

Estradiol, estriol, estrone, diethyl stilbestrol, progesterone, chlormadinone acetate, testosterone, and cortisol were purchased from Merck (Darmstadt, F.R.Germany). Estradiol benzoate, testosterone propionate, mestasterone, levonorgestrel, norethisterone, norethisterone acetate, and cyproterone acetate were obtained from Schering (Berlin, F.R.Germany). Ethinyl estradiol, tamoxifen, and medroxyprogesterone acetate were supplied by Sigma (Munchen, F.R.Germany). Lynestrenol and desogestrel were obtained from Organon (Oberschleissheim, F.R.Germany), clomiphen from Merrell (Groß-Gerau, F.R.Germany), and cortisone acetate from Ciba-Geigy (Wehr, F.R.Germany).

Experiments

Adult intact female SIV rats (Ivanovas, Kisslegg, F.R.Germany) weighing 190 to 240 g were used. The animals were kept under standard conditions of 12 h of light alternating with 12 h of darkness. The temperature was approximately 25°C, and the humidity about 55%. Food and water were offered ad libitum.

Groups of 6 to 7 animals were injected s.c. daily with the compounds to be tested dissolved in 0.2 ml arachis oil / benzyl benzoate (6:4), or the solvent only. In some experiments rats were used which had been ovariectomized 2 weeks prior to the experiment. In most of the experiments the ani-
mals were decapitated after 2 weeks of treatment unless stated otherwise, and the thymus and spleen were removed and weighed. In the experiments upon the effect of contraceptive preparations, groups of 7 rats each were killed after 1, 2, 3 and 4 weeks of treatment, and 2 weeks after discontinuation of treatment.

Histology

For histological examination the tissues were fixed in 4% formalin/phosphate buffer (pH 7.2), and, after dehydration embedded in Paraplast. Sections of 5 μm thickness were stained with Haematoxylin-Eosine, Giemsa and PAS.

Receptor analysis

For estrogen receptor analysis, the pooled thymus glands were frozen immediately after dissection. The receptor analysis was carried out with cytosol of approximately 1 g thymic tissue. The tissue was frozen in liquid nitrogen and homogenated by means of a microdismembrator (Braun, Melsungen, F.R.Germany). The tissue powder was suspended in 4 ml phosphate buffer (0.01 M, pH 7.5; 0.005 M EDTA, 0.003 M NaN₃, 0.01 M monothioglycerol, 10% glycerol) and centrifuged at 105,000 x g for 60 min at 10°C.

The estrogen binding affinities and capacities were determined by means of a modification of the method described by Korenman et al. (5), using (6,7-3H)-estradiol (60 Ci/mMol) in 6 increasing concentrations in the range between 0.2 and 6.0 nM with or without a 500-fold excess of unlabelled diethyl stilbestrol (final volume 170 μl). After incubation for 3 h at 10°C, the bound hormone was separated from free hormone by centrifugation after the addition of 200 μl of 1% charcoal suspension (0.05% dextrane) for 15 min at 10°C.

100 μl of the supernatant was mixed with 10 ml Insta-Gel scintillant and counted in a liquid scintillation spectrometer. The binding constants were determined by means of Scatchard analysis (6).

Cytosolic protein was measured according to the method of Lowry et al. (7).

Statistical analysis

The statistical differences between means were evaluated by Student's t-test.

RESULTS

Effect of estrogens

The daily treatment of intact adult female rats with various estrogens resulted in a significant reduction of the thymus gland to less than one half of its initial weight.
Fig. 1 Effect of daily s.c. injections for 2 weeks with increasing doses of ethinyl estradiol upon thymus weight in intact female rats (mean ± S.D.).

Table I Effect of daily s.c. injections for 2 weeks with various estrogens and anti-estrogens upon thymus and spleen weight in intact female rats (mean ± S.D.)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Thymus weight (mg)</th>
<th>Spleen weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>373 ± 45</td>
<td>653 ± 54</td>
</tr>
<tr>
<td>10 µg Ethinyl estradiol</td>
<td>182 ± 58**</td>
<td>645 ± 116</td>
</tr>
<tr>
<td>100 µg Ethinyl estradiol</td>
<td>117 ± 25**</td>
<td>609 ± 76</td>
</tr>
<tr>
<td>10 µg Estradiol benzoate</td>
<td>178 ± 46**</td>
<td>556 ± 98</td>
</tr>
<tr>
<td>10 µg Estradiol</td>
<td>320 ± 49</td>
<td>681 ± 111</td>
</tr>
<tr>
<td>10 µg Estrone</td>
<td>314 ± 52</td>
<td>658 ± 45</td>
</tr>
<tr>
<td>10 µg Estriol</td>
<td>327 ± 30</td>
<td>595 ± 38</td>
</tr>
<tr>
<td>10 µg Diethyl stilbestrol</td>
<td>218 ± 44**</td>
<td>598 ± 46</td>
</tr>
<tr>
<td>10 µg Clomiphene</td>
<td>429 ± 36</td>
<td>685 ± 77</td>
</tr>
<tr>
<td>10 µg Tamoxifen</td>
<td>319 ± 35*</td>
<td>582 ± 53</td>
</tr>
</tbody>
</table>

* = P < 0.05          ** = P < 0.01
when ethinyl estradiol was administered at a dose of 10 μg or more (Fig. 1 and Table I). A similar effect could be observed after the injection of 10 μg estradiol benzoate and diethyl stilbestrol per day. The use of other estrogens or anti-estrogens resulted only in a weak or not significant effect upon thymus weight. There was no effect upon the weight of the spleen in any case.

![Graph](image)

**Fig. 2** Effect of daily s.c. injections of 10 μg ethinyl estradiol upon thymus and spleen weight (mean ± S.D.) in ovariectomized rats during a treatment period of 14 days, and for 14 days after discontinuation (open circles = spleen weight, black circles = thymus weight of estrogen-treated rats; black triangles = thymus weight of control rats).

The time-dependent effect of daily injections with 10 μg ethinyl estradiol upon thymus and spleen weight of rats ovariectomized 4 weeks ago is depicted in Fig. 2. As early as 3 days after the beginning of estrogen treatment, the thymus weight - which is elevated in castrated animals - was decreased significantly (p < 0.01) from 936 ± 124 mg to 698 ± 134 mg. The continuation of daily injections up to 14 days resulted in a further involution to 266 ± 56 mg. For three days after cessation of estrogen treatment the thymus weight remained at a nadir. Thereafter, it in-
creased slowly but remained at a significantly lower level on Day 14 after discontinuation as compared to untreated castrated rats (p < 0.01).

The histological examination revealed that the daily injection of ethinyl estradiol brought about a progressive involution of the cortex of the thymus. This was accompanied by the appearance of large, round macrophages with a starry-like pattern containing basophilic corpuscles. The concentration of lymphocytes in the cortical tissue decreased up to the 14th day of treatment. The border between cortex and medulla became poorly demarcated. The medulla began to contain large, round macrophages, too. There was no replacement by adipose tissue. The epithelial structures of the thymus were not affected by the treatment. Similarly, there was no effect upon the cortical mast-cells.

Table II Effect of daily s.c. injections for 2 weeks with various steroids upon thymus and spleen weight in intact female rats (mean ± S.D.)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Thymus weight (mg)</th>
<th>Spleen weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>461 ± 64</td>
<td>589 ± 58</td>
</tr>
<tr>
<td>0.36 mg Cortisol</td>
<td>328 ± 40**</td>
<td>568 ± 71</td>
</tr>
<tr>
<td>0.39 mg Cortisone acetate</td>
<td>412 ± 37</td>
<td>548 ± 40</td>
</tr>
<tr>
<td>0.34 mg Testosterone</td>
<td>394 ± 65</td>
<td>585 ± 75</td>
</tr>
<tr>
<td>2.00 mg Testosterone</td>
<td>216 ± 44**</td>
<td>611 ± 96</td>
</tr>
<tr>
<td>0.34 mg Testosterone propionate</td>
<td>337 ± 66**</td>
<td>587 ± 23</td>
</tr>
<tr>
<td>0.30 mg Mesterolone</td>
<td>330 ± 115*</td>
<td>626 ± 86</td>
</tr>
<tr>
<td>2.00 mg Mesterolone</td>
<td>252 ± 48**</td>
<td>665 ± 70</td>
</tr>
<tr>
<td>0.31 mg Progesterone</td>
<td>402 ± 51</td>
<td>575 ± 96</td>
</tr>
<tr>
<td>0.31 mg Levonorgestrel</td>
<td>404 ± 99</td>
<td>593 ± 106</td>
</tr>
<tr>
<td>0.28 mg Lynestrenol</td>
<td>389 ± 62</td>
<td>565 ± 39</td>
</tr>
<tr>
<td>0.30 mg Norethisterone</td>
<td>396 ± 37</td>
<td>570 ± 58</td>
</tr>
<tr>
<td>2.00 mg Norethisterone</td>
<td>239 ± 65**</td>
<td>610 ± 82</td>
</tr>
<tr>
<td>0.40 mg Norethisterone acetate</td>
<td>491 ± 56</td>
<td>567 ± 83</td>
</tr>
<tr>
<td>2.00 mg Cyproterone acetate</td>
<td>334 ± 68*</td>
<td>507 ± 75</td>
</tr>
<tr>
<td>0.40 mg Chlormadinone acetate</td>
<td>518 ± 67</td>
<td>568 ± 90</td>
</tr>
<tr>
<td>2.00 mg Chlormadinone acetate</td>
<td>364 ± 60*</td>
<td>529 ± 44</td>
</tr>
<tr>
<td>0.39 mg Medroxyprogesterone acetate</td>
<td>482 ± 118</td>
<td>594 ± 53</td>
</tr>
</tbody>
</table>

* = p < 0.05  ** = p < 0.01
The spleenic weight was also significantly reduced in the ovariectomized rats during estrogen treatment with a minimum being reached after 14 days (p < 0.01). One week after the discontinuation of treatment, the suppressive effect was still noticeable, even though it was abolished after one more week (Fig. 2).

**Effect of progestogens and androgens**

When various progestogens were injected daily for 2 weeks into intact female rats at doses between 0.28 and 0.4 mg (= 1 μMol), neither the thymus nor the spleen were significantly affected (Table II). At a higher dosage (2 mg), however, both the progesterone derivatives chlormadinone acetate and cyproterone acetate as well as the nortestosterone derivative norethisterone caused a significant involution of the thymus while the spleen remained unaffected.

Contrary to this, the application of androgens resulted in a reduction of thymus weight even at a dose as low as 0.3 mg when mesterolone or testosterone propionate were used; testosterone was effective only at a higher dosage (Table II).

The weight of the spleen of intact rats was not altered during treatment with androgens.

The daily injections with cortisol brought about only a moderate involution of the thymus.

**Effect of contraceptive preparations**

The treatment with various contraceptive steroid preparations for 4 weeks revealed that the estrogenic component is mainly responsible for the reduction of the thymus and that the maximal effect is attained after 2 weeks of daily injections (Table III).

The progestogenic component intensified the suppressive action of the estrogen only at higher doses. This is exemplified by the effect of a combination of 50 μg ethinyl estradiol with either 0.5 mg levonorgestrel, 2 mg chlormadinone acetate, 2 mg cyproterone acetate, or 2 mg norethisterone which caused a drastic involution of the thymus (Table III). The suppressive effect persisted even 2 weeks after cessation of the treatment.

Histologically, changes identical to those observed during daily treatment with 10 μg ethinyl estradiol were demonstrable in the 2 groups of animals treated with 50 μg ethinyl estradiol and 2 mg norethisterone (Fig. 4), or 2 mg cyproterone acetate, respectively. Only remnants of the parenchymacould be identified. Cortex and medulla could not be distinguished any more by morphologic means in that large portions of either component have been replaced by reticular and adipose tissue. Within these structures, typical mast-cells abounded, which were also distributed throughout the remaining lymphatic, thymic
Table III  Effect of daily s.c. injections of various contraceptive steroid preparations upon female rat thymus weight (mean ± S.D.) during a treatment period of 4 weeks, and for 2 weeks after discontinuation (EE = Ethinyl estradiol, NG = Levonorgestrel, DG = Desogestrel, NET = Norethisterone, CMA = Chlormadinone acetate, CPA = Cyproterone acetate)

<table>
<thead>
<tr>
<th>Preparation</th>
<th>1 week</th>
<th>2 weeks</th>
<th>3 weeks</th>
<th>4 weeks</th>
<th>2 weeks after discontinuation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>335 ± 88</td>
<td>302 ± 38</td>
<td>291 ± 27</td>
<td>273 ± 74</td>
<td>262 ± 60</td>
</tr>
<tr>
<td>50 μg EE</td>
<td>134 ± 26**</td>
<td>71 ± 13**</td>
<td>75 ± 42**</td>
<td>67 ± 20**</td>
<td>158 ± 40**</td>
</tr>
<tr>
<td>125 μg NG</td>
<td>330 ± 58</td>
<td>317 ± 58</td>
<td>270 ± 39</td>
<td>303 ± 66</td>
<td>320 ± 48</td>
</tr>
<tr>
<td>125 μg DG</td>
<td>368 ± 90</td>
<td>357 ± 59</td>
<td>315 ± 46</td>
<td>285 ± 52</td>
<td>272 ± 27</td>
</tr>
<tr>
<td>30 μg EE + 125 μg NG</td>
<td>122 ± 32**</td>
<td>83 ± 22**</td>
<td>60 ± 6**</td>
<td>85 ± 12**</td>
<td>95 ± 15**</td>
</tr>
<tr>
<td>30 μg EE + 250 μg NG</td>
<td>129 ± 16**</td>
<td>88 ± 11**</td>
<td>56 ± 8**</td>
<td>87 ± 9**</td>
<td>102 ± 15**</td>
</tr>
<tr>
<td>50 μg EE + 125 μg NG</td>
<td>108 ± 46**</td>
<td>106 ± 23**</td>
<td>64 ± 29**</td>
<td>64 ± 28**</td>
<td>117 ± 18**</td>
</tr>
<tr>
<td>50 μg EE + 500 μg NG</td>
<td>90 ± 12**</td>
<td>35 ± 10**</td>
<td>17 ± 8**</td>
<td>39 ± 18**</td>
<td>41 ± 20**</td>
</tr>
<tr>
<td>30 μg EE + 125 μg DG</td>
<td>156 ± 32**</td>
<td>55 ± 13**</td>
<td>52 ± 7**</td>
<td>70 ± 13**</td>
<td>111 ± 21**</td>
</tr>
<tr>
<td>50 μg EE + 125 μg DG</td>
<td>97 ± 19**</td>
<td>78 ± 14**</td>
<td>53 ± 8**</td>
<td>128 ± 38**</td>
<td>118 ± 34**</td>
</tr>
<tr>
<td>50 μg EE + 2 mg CMA</td>
<td>90 ± 29**</td>
<td>33 ± 7**</td>
<td>22 ± 9**</td>
<td>17 ± 3**</td>
<td>95 ± 35**</td>
</tr>
<tr>
<td>50 μg EE + 2 mg CPA</td>
<td>81 ± 18**</td>
<td>47 ± 17**</td>
<td>29 ± 8**</td>
<td>31 ± 14**</td>
<td>90 ± 40**</td>
</tr>
<tr>
<td>50 μg EE + 2 mg NET</td>
<td>78 ± 21**</td>
<td>23 ± 14**</td>
<td>41 ± 18**</td>
<td>22 ± 6**</td>
<td>42 ± 39**</td>
</tr>
</tbody>
</table>

** = P < 0.01
Fig. 3  Section of thymus from intact female control rat. Cortex (dark) and medulla (bright) are sharply differentiated. The cortex contains numerous small lymphocytes. Near to the center, an epithelial island is cut (Haematoxylin, x 200).

Fig. 4  Effect of daily injections of 50 μg ethinyl estradiol + 2 mg norethisterone for 2 weeks upon thymus of intact female rat. Cortex and medulla cannot be distinguished. In the cortex and also the medulla there are large, round macrophages (lightly stained). The capsular fibrous tissue is thickened (Haematoxylin-Eosine, x 200).
CONTRACEPTION

Fig. 5 Effect of daily injections of 50 μg ethinyl estradiol + 2 mg chlormadinone acetate for 2 weeks upon thymus of intact female rat. There are only small cystic epithelial structures with some interspersed lymphocytes. In the central area, there are macrophages with light-grey cytoplasm (Haematoxylin-Eosine, x 200).

Fig. 6 Effect of daily injections of 50 μg ethinyl estradiol + 0.5 mg levonorgestrel for 2 weeks upon thymus of intact female rat. The parenchyma is completely atrophic, and contains cystic epithelial elements (Haematoxylin-Eosine, x 200).
The parenchymal rests of the thymus contained aggregations of large immunoblasts and plasma cells, i.e., cellular elements of the B immune system. There were also macrophages, some of which contained iron or lipofuscin. The general appearance of the remaining thymic parenchyma was that of lymphatic tissue found in lymph nodes. After 2 or more weeks of daily injections with 50 µg ethinyl estradiol and 0.5 mg levonorgestrel, or 2 mg chlormadinone acetate, respectively, the involution of the thymic parenchyma was even more pronounced (Fig. 5 and 6). The atrophy was nearly complete in that the thymus was practically replaced by connective and adipose tissue. The structure of the former lobuli was still vaguely outlined by aggregations of mast-cells, macrophages, histiocytes, and some lymphocytes. The epithelial components of the thymus had developed into small, cystic structures.

Contrary to the pronounced response of the thymus, no significant effect upon the spleen could be observed when intact female rats were treated for 4 weeks with estrogen/progestogen combinations (not depicted).

Table IV  Estrogen receptor concentrations and dissociation constants in cytosol prepared from thymus of diestrous rats, ovariectomized rats and intact rats treated for 2 weeks with daily s.c. injections of 0.31 mg progesterone (mean ± S.D.)

<table>
<thead>
<tr>
<th></th>
<th>Dissociation constant</th>
<th>Receptor concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K_D (nM)</td>
<td>(fmoles/mg protein)</td>
</tr>
<tr>
<td>Diestrous rats</td>
<td>0.34 ± 0.16</td>
<td>6.5 ± 3.4</td>
</tr>
<tr>
<td>Progesterone-treated rats</td>
<td>0.39 ± 0.18</td>
<td>3.6 ± 1.6*</td>
</tr>
<tr>
<td>Ovariectomized rats</td>
<td>0.49 ± 0.26</td>
<td>2.6 ± 1.3**</td>
</tr>
</tbody>
</table>

* = P<0.05  ** = P<0.01

The determination of the binding properties of the estrogen receptor in the thymus cytosol resulted in similar dissociation constants in the range between 0.34 and 0.49 nM in diestrous rats, progesterone-treated intact rats, and ovariectomized rats (Table IV). The binding capacity of the thymic receptor appeared to be relatively low in diestrous rats and was further decreased during treatment with progesterone for 2 weeks, and after ovariectomy.
The present results demonstrate that the involution of the thymus induced by sex steroids occurs both in intact and ovariectomized rats in a time- and dose-dependent manner. Even though a nearly maximal suppressive effect was already obtained after 2 weeks of treatment with estrogens alone or in combination with progestogens, the effect proved to be reversible only with considerable delay. Ethinyl estradiol and estradiol benzoate were the most effective steroids in intact as well as in ovariectomized rats, while androgens and progestogens caused a significant reduction of thymus weight of intact rats at doses which were higher by one to two orders of magnitude. Differences in the effective dose of sex steroids between intact and castrated animals had previously been reported in that castration-induced hyperplasia of the thymus was completely abolished by a dose of androgens which was totally devoid of an effect in intact animals (8, 9).

The present results demonstrate that the dose of the estrogen component is the essential factor with respect to the involution of the thymus of intact rats, as the combination with lower doses of norgestrel or desogestrel had no additional effect. A synergistic effect of synthetic progestogens became evident only at higher doses, and lasted for a longer time after cessation as compared to ethinyl estradiol alone. Contrary to this, it was shown that treatment of ovariectomized rats with 0.35 mg estradiol benzoate + 0.35 mg progesterone had a more pronounced thymus-depressive effect than 0.7 mg of the estrogen alone (10).

The histological examinations carried out in our study as well as those of previous investigators (10, 11, 12) clearly showed that particularly the cortex of the thymus is altered by chronic treatment with sex steroids. Lymphocytes in the cortex appear to be more susceptible to the destructive influence of estrogens and androgens than medullary lymphocytes, and reticular cells are not affected. It had been observed that sex steroids reduce the mitotic activity in the thymic cortex in a similar manner as corticosteroids (11), and thus suppress the proliferation of short-life lymphocytes in the cortex which are the most actively dividing lymphocytes. During treatment with high-dosed combined preparations, the medulla became nearly depleted of lymphocytes, too, resulting in a pronounced atrophy of the organ.

It has been postulated that the two subpopulations of thymus-derived lymphocytes represent successive stages of thymocyte maturation. The more immature and steroid-sensitive lymphocytes are located in the thymic cortex. The more mature subpopulation is steroid-resistant, and is mainly found in the medulla. The transition of the imma-
ture into the more mature lymphocyte seems to be induced by a humoral factor, thymosin (13), which is thought to be secreted by the thymic epithelium. It was demonstrated that a thymosin fraction is inhibited by testosterone and estradiol, and that the stimulatory action of thymosin upon Pre-T-cells known to be the precursors of the thymus-derived lymphocytes (T-cells), is also inhibited by the sex steroids (14).

The existence of specific estrogen receptors as demonstrated previously (15, 16, 17) and by the present results indicates that there is a direct effect of estrogens upon the thymus, although an involvement of pituitary hormones in the regulation of the immune system has been suggested (19, 20). In the thymus, binding sites for estrogens were found to be located particularly in the epithelial cells (18) which, in all probability, secrete thymosin.

As a consequence, it may be assumed that the conversion of bone-marrow Pre-T-cells into the short-life T1-cells, and into immune competent lymphocytes (T2-cells) mediated by thymosin, is inhibited by estrogen-containing preparations.

Abnormalities in the function of T-cells are considered to be involved in the pathophysiology of infections, and there is increasing evidence indicating a T-cell deficiency as a major etiological factor in the development of several autoimmune conditions in rodents (4).

It remains, however, to be shown whether or not the chronic treatment of the human with estrogen-containing preparations may result in an essential alteration of the immune system. There are some data indicating that the elevation of estrogen and progesterone levels which occurs physiologically during pregnancy, and as a pharmacological phenomenon during treatment with oral contraceptives can lead to cell-mediated immuno-suppression by means of depressed lymphocyte transformation (3). The more frequent occurrence of lupus erythematosus in women as compared to men suggests endocrine factors in the pathogenesis of this disease, and there are case reports on exacerbations while taking oral contraceptives (21). On the other hand, symptoms of rheumatic disease have rarely been observed to develop in women during treatment with oral contraceptives, even in the presence of a positive L.E. cell test (22), antinuclear antibodies, rheumatoid factor, and C-reactive protein (23). The Royal College of General Practitioners' Oral Contraception Study even revealed a decrease in the incidence of rheumatoid arthritis in women taking hormonal contraceptives (3), indicating a suppression of the autoimmune response.

A statistically significant correlation between the incidence of other diseases involving immune deficiencies and the use of oral contraceptives has, however, not yet been found.
ACKNOWLEDGEMENT

We wish to thank Mrs. Carola Lins for her excellent technical assistance.

REFERENCES


sin-induced differentiation of murine thymocytes in 
14. Deschaux, P., Paucod, J.C., and Ardail, D.: Interac-
tion between thymosin, testosterone and estradiol on 
natural killer cell activity in mice. Tohoku J. exp. 
15. Gillette, S. and Gillette, R.W.: Changes in thymic es-
tragen receptor expression following orchidectomy. Cell 
16. Reichman, M.E. and Villee, C.A.: Estradiol binding by 
rat thymus cytosol. J. Steroid Biochem. 9: 637-641 
(1978).
17. Imanishi, Y., Haruki, Y., and Seiki, K.: Estrogen recep-
tor in rat thymus cytosol. Tokai J. Exp. Clin. Med. 5: 
263-267 (1980).
18. Grossman, C.J., Sholiton, L.J., Blaha, G.C., and Nathan, 
P.: Rat thymic estrogen receptor - II. Physiological 
19. Mysliwska, J.: Reactivity to estradiol of thymic cells 
from female mice before and after puberty. Endokrino-
20. Berczi, I., Nagy, E., Kovacs, K., and Horvath, E.: Regu-
lation of humoral immunity in rats of pituitary hormo-
21. Chapel, T.A. and Burns, R.E.: Oral contraceptives and 
antibodies, rheumatoid factor and C-reactive protein 
in serum of normal women using oral contraceptives. 