J. Sci. Soc. Thailand, **1** (1975), 49–56

QUANTITATIVE HISTOLOGICAL CHANGES IN THE THYMUS OF NEWBORN RATS TREATED WITH SEX STEROIDS

P. SOBHON, A. ANGSUSINGHA and C. CHIRASATTHUMB

Anatomy Department, Faculty of Science, Mahidol University, Bangkok

(*Received 22 January 1975*)

Summary

Exogeneously administered testosterone or estrogen resulted in a decrease of the thymic weight, a reduction of thymic incorporation of ^{3}H-thymidine and a drop in number of circulating lymphocytes in newborn rats. On the other hand, the administration of progesterone caused increases of thymic weight and ^{3}H-thymidine incorporation but a decrease in number of circulating lymphocytes. Histologically, the concentration of lymphocytes was lower than normal in the cortex but higher than normal in the medulla of the thymuses of testosterone, extrogen, and progesterone treated rats.

It was suggested that testosterone and estrogen inhibited mitotic activities and induced the death of thymic lymphocytes whereas progesterone stimulated their mitotic activities and their proliferation. Since in all cases the increased concentration of lymphocytes in the medulla occurred concurrently with the drop in number of circulating lymphocytes, it was suggested that sex steroids might reduce the release of lymphocytes from the thymus by interfering with the process of lymphocyte transport through the walls of post-capillary venules in thymic medulla.

Introduction

Thymic involution can be elicited by the administration of several steroid hormones; for example, corticosteroids[1] testosterone[2,3] and estrogen[3]. These steroids cause thymic involution by killing thymic lymphocytes, particularly the short-lived lymphocytes in the thymic cortex. In case of corsticosteroids, there has been an elaborate study showing that these hormones kill lymphocytes by blocking the action of DNA-dependent RNA polymerase, thus resulting in the decreased synthesis of constitutive enzymes needed for normal metabolic activities of the cells[4,5]. The precise thymolytic mechanisms of sex steroids are not known though it was demonstrated that estrogen and testosterone depressed the mitotic activity and caused death of lymphocytes in the thymic cortex[3]. Sex steroids may therefore influence the normal structure and function of the thymus. In the present study we investigated (a) the changes in number and distribution of lymphocyte population in various areas of the thymus following sex steroid administration; (b) the effects of various sex steroids (testosterone, estrogen and progesterone) on the proliferation of thymic lymphocytes; and (c) the effect of sex steroids on the release of thymic lymphocytes into the circulation. Newborn rats were used because their thymuses are in the phase of rapid growth and the levels of circulating endogeneous sex steroids are low. The effect of dexamethasone, a potent corticosteroid analogue, on the thymus were also studied and compared with that of sex steroids.

Material and Methods

Newborn albino rats were divided into five groups, each with twenty five animals. Starting from the first day of post-natal life, one of the four steroid hormones, viz., testosterone propionate, estradiol benzoate, progesterone and dexamethasone was subcutaneously administered to animals in each group at dosage 1 μg/g body weight/day for twenty-one consecutive days. The remaining group was used as normal control. On the morning of day 22nd, experimental animals were injected with ^{3}H-thymidine at dosage 0.5 μCi/g body weight. Two hours later the animals were sacrificed by ether anesthesia. As much blood as possible was withdrawn from abdominal aortae and the total and differential white counts were done using hemocytometer. In each group thymuses from five animals were fixed in calcium-formalin solution and stained with hematoxylin and eosin for light microscopic examination and cell counting. Thymuses from the remaining animals were weighed and then homogenized. Nucleic acid fraction in the homogenate was isolated by the method of Shibko *et al.*[6]. Thoroughly washed precipitate was resuspended in Bray's counting medium and the radioactivity was counted using Beckman liquid scintillation counter. As a result only ^{3}H-thymidine incorporated into DNA was estimated, whereas the unbound ^{3}H-thymidine was excluded. The amount of ^{3}H-thymidine incorporation was expressed as cpm./mg of thymic wet weight. In counting the number of cells, the eye-piece lens fitted with an ocular grid was used in combination with X 40 objective. At this magnification the ocular grid represents the area of 0.058 mm^2. The concentration of lymphocytes in the thymic cortex and medulla were expressed as cells per mm^2.

Results

The thymic relative weights (mg thymic weight/g body weight) of testosterone, extrogen and dexamethasone treated animals were significantly lower, whereas those of progesterone treated animals were higher than the normal controls (Fig. 1 a).

Data on ^{3}H-thymidine incorporation by the thymus showed that when compared with control animals, there was a reduction in the amount of incorporation in all except progesterone-treated groups (Fig. 1b).

All steroid hormones caused a reduction of lymphocyte population per unit area in the thymic cortex (Fig. 2a); and all hormones increased the number of lymphocytes per unit area in the thymic medulla (Fig. 2b).

The result of total and differential white counts in all steoid hormone treated groups are shown in Fig. 3. From these data the absolute number of lymphocytes per unit volume of blood could be calculated for each group. It was found that all steroid hormones cause a reduction of the absolute number of circulating lymphocytes (Fig. 4).

Discussion

The thymic involution in response to the treatment with testosterone or estrogen in castrated or spayed mature rats was previously observed by many investigators[2,3,7]. In the present investigation similar result was observed when either testosterone or estrogen

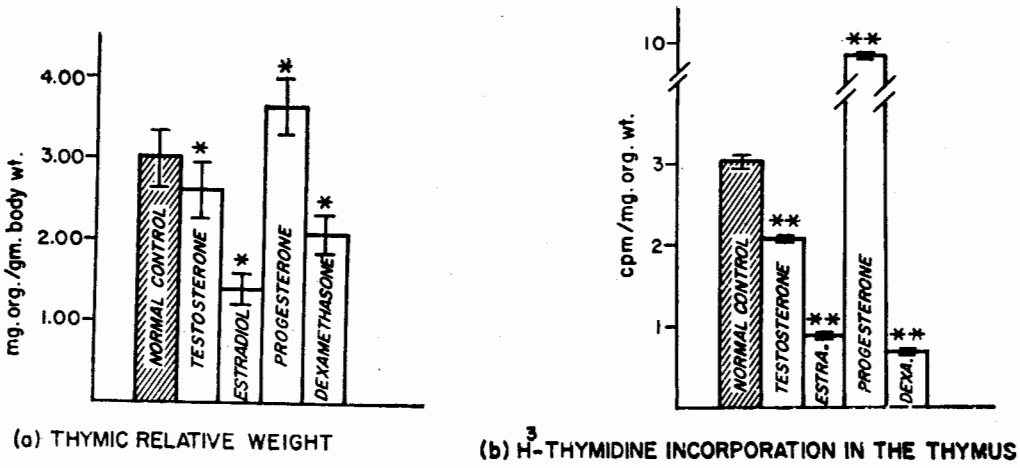

Fig. 1 Changes of (a) rat thymic relative weights and (b) thymic incorporation of ^3H thymidine (expressed as cpm./mg. organ wet weight) in response to three weeks treatment with steroid hormones. Each hormone was given daily at 1 ug/g/day.

* indicates that the difference of the results between the experimental and the control groups is significant with $P = 0.05$.

** indicates that the difference of results between the experimental and the control group is highly significant with $P = 0.001$.

I indicates standard deviation of each group with 20 rats.

was given to newborn rats. Furthermore, quantitative studies undertaken in this investigation revealed that steroid hormones affected the histology of the thymus in many respects. Following the administration of these hormones the proliferations of thymic cells were affected as indicated by ^3H-thymidine incorporation. The lower than normal incorporation of ^3H-thymidine in the thymus was observed in testosterone, estrogen and dexamethasone treated animals. This reduction corresponded with the lowering of the thymic relative weights of the groups. In contrast, progesterone enhanced the ^3H-thymidine incorporation in the thymus as well as increased the thymic relative weight (Fig. 1). The change of ^3H-thymidine incorporation (i.e. the synthesis of DNA) in the thymus could be construed as the change in mitotic activities of thymic cells. Most of dividing cells in the thymus of young rats are of lymphocytic category whereas another major type of thymic cells, the epithelial reticular cells, divide rapidly in the fetal stage and have their mitotic activities markedly curtailed by the time the animals reach weaning age. Thus it could be concluded that exogeneously administered testosterone, estrogen, and dexamethasone suppressed while progesterone enhanced the mitotic activities of thymic lymphocytes. This finding is agreement with the previous study showing that in the thymic cortex of ovariectomized rats the number of lymphocytes with mitotic figures following testosterone or estrogen administration was lower than in the control[3].

In addition to a decreased ^3H-thymidine incorporation it was also observed that there was an increase of dead lymphocytes in the cortex after testosterone, estrogen

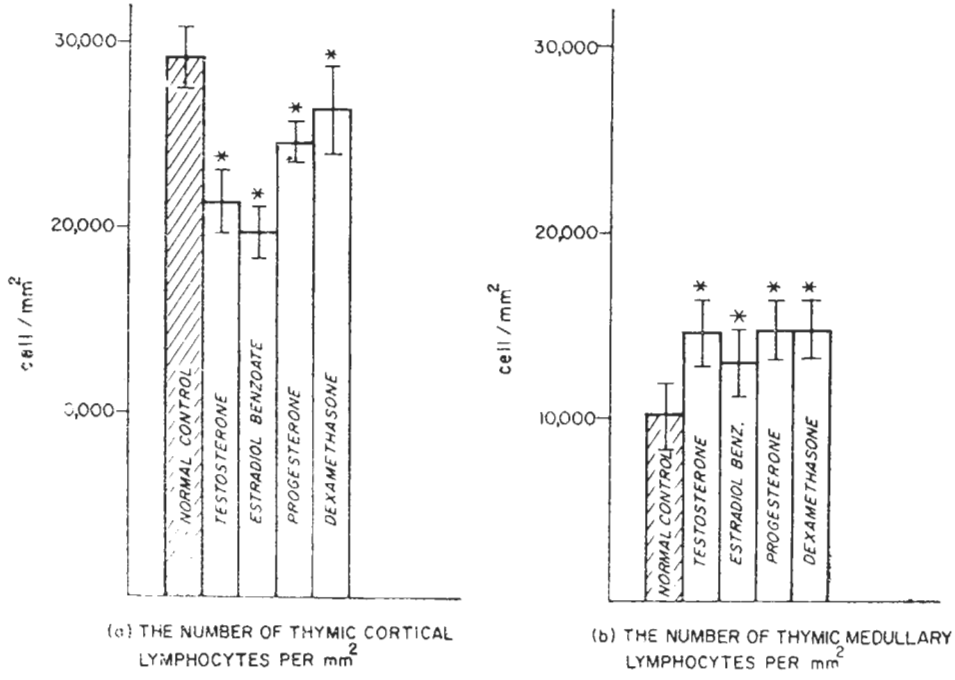

Fig. 2 Changes in the number of lymphocytes in (a) the cortical and (b) the medullary areas of the thymus induced by 3 weeks treatment with different steroid hormones. Each hormone was given daily at 1 ug/g body weight/day. The number of cells is expressed per sq. mm.

* indicates that the difference of results between the experimental and control groups is significant with $P = 0.05$.

I indicates standard deviation of each group with 5 rats.

and dexamethasone treatments. The mechanism by which testosterone and estrogen inhibit mitosis and cause the death of lymphocytes has not yet been known. This mechanism may be similar to that of corticosteroids which has already been studied in detail. Corticosteroids inhibit the mitosis and bring about the death of lymphocytes by causing a repression of active genomes and a decrease of RNA polymerase activities.[4,5]. Presumably, these effects in turn reduce or inhibit the synthesis of many proteins. Suppression of the synthesis of the enzyme DNA polymerase and the proteins constituting mitotic spindles could lead to the inhibition of mitosis. Suppression of the synthesis of other proteins, particularly the constitutive enzymes essential for the metabolic processes, could lead to the death of lymphocytes.

Results obtained in the present study revealed that after testosterone, estrogen or dexamethasone administration, the number of lymphocytes per unit area increased in the medulla and decreased in the cortex. The decreased concentration of lymphocytes in

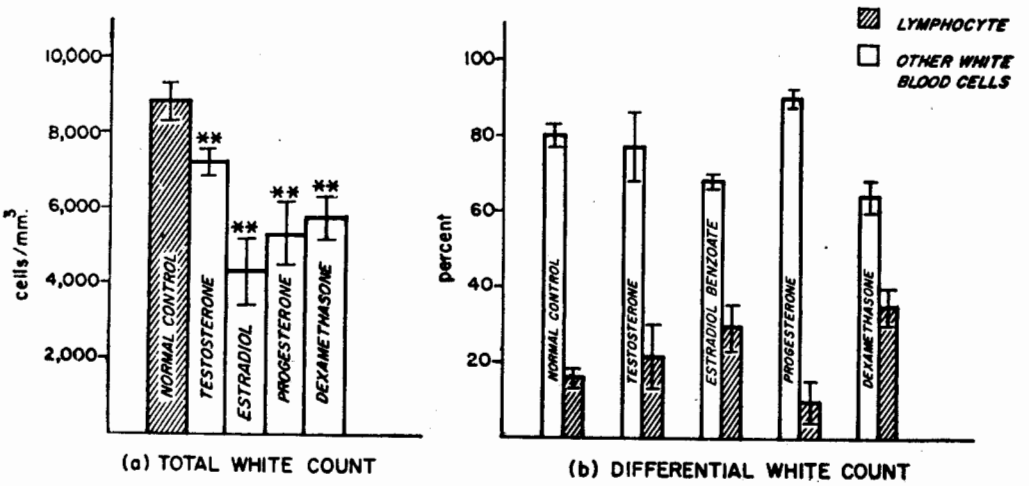

Fig. 3 Changes of the rat total (a) and differential white blood cell count (b) in response to there weeks treatment with different steroid hormones. Each hormone was given daily at 1 ug/g body weight/day.

** indicates that the difference of results between the experimental and the control groups is significant with P. = 0.001.

I indicates standard deviation of each group with 20 rats.

the cortex was the result of the mitotic inhibition and increased cell death induced by the above steroids. The concentration of lymphocytes in the medulla is regulated by three major factors. First, lymphocytes in the medulla can divide and multiply to certain extent though with much lower rate than lymphocytes in the cortex[3]. The mitotic division of medullary lymphocytes was probably not a significant factor in causing the increased concentration of lymphocyte in the medulla since the administration of the above steroids tended to suppress such activity. The other two factors which govern the concentration of lymphocytes in the medulla are the migration of lymphocytes from the cortex to the medulla and the release of lymphocytes into the circulation. In a normal situation, the thymic cortex is the site where lymphocytes divide rapidly. Most of the daughter lymphocytes are short-lived and die within the cortex. The remaining lymphocytes migrate to the medulla where they enter the circulation[8]. Lymphocytes leave the thymus to enter the circulation by passing through special blood vessels of the medulla which are known as post-capillary venules. Lymphocytes pass through the wall of these venules by pushing their way through the cytoplasm of endothelial cells into the lumen of bood vessels. This process is generally termed diapedesis. In contrast, capillaries and venules of the thymic cortex do not allow cells or even macromolecules to pass through their walls. Such property of cortical vessels constitutes what is termed "blood-thymus barrier" as proposed by Raviola and Karnovsky[9].

Following the treatment with testosterone, estrogen or dexamethasone, there should be fewer lymphocytes migrating from the cortex to the medulla because these hor-

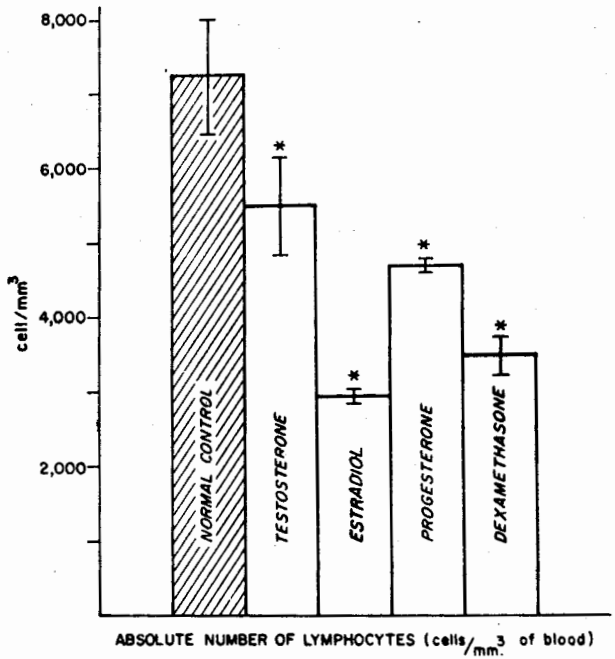

Fig. 4 Changes in absolute number of circulating lymphocytes/mm3. of blood after 3 weeks treatment with different steroid hormones. Each hormone was given daily at 1 ug/g body weight/day.
* indicates that the difference of results between the experimental and the control group is significant with P= 0.05.
I indicates standard deviation of each group with 20 rats.

mones reduced the number of cortical lymphocytes by inhibiting their mitosis as well as increasing their death. Thus the concentration of lymphocytes in the medulla was expected to decrease. However, the opposite result was observed. The increased concentration of lymphocytes in the medulla could occur only if there were fewer lymphocytes leaving the thymus. One possibility was that steroid hormones might interfere with the process of diapedesis and reducing the number of lymphocytes released into the circulation through the wall of post-capillary venules. If this occurred fewer lymphocytes would be allowed to leave the thymus. Cells which normally would be released into the circulation were instead accumulated in the thymic medulla. Thus the lymphocytes per unit area in the thymic medulla was observed to increase despite the lower rate of lymphocyte proliferation.

A significant reduction in the number of circulating lymphocytes (Fig. 3 b and 4) was actually observed following sex steroids and dexamethasone administration. This result is in agreement with the observation of Eurnstrom and Larsson[10] who found that the release of lymphocytes from the thymus into the circulation decreased after the administration of steroid hormones. In post natal period lymphocytes released from the thymus

contribute up to 10–15% of the circulating population[11]. Hence it is expected that any stimuli which reduce the proliferation of lymphocytes in the thymus will result in the reduction of circulating lymphocytes. This happended as predicted in the cases of treatments with testosterone, estrogen or dexamethasone. However, it is surprising to observe a decrease in number of circulating lymphocytes in progesterone treated animals, even though progesterone was shown to increase the thymic relative weight and the thymic incorporation of thymidine. The observed effect could be explained if it is assumed that progesterone also reduced the release of lymphocyte from the thymus by acting on the post-capillary venules of the medulla to suppress the process of transport of lymphocytes through their walls. The influence of steroid hormones on the process of diapedesis, which directly controls the release of lymphocytes from the thymus into the circulation, remains to be further investigated.

References

1. Santisteban, G.A. and Dougherty, T.F. (1954) *Endocrinology* **54,** 130–146.
2. Wettstein, M.F. and Craddock, C.G. (1970) *Brit. J. Hacmatol.*, **35,** 257–271.
3. Sobhon, P. and Chirasatthumb, C. (1974) *Acta Anat.* **89,** 211–225.
4. Darzynkiewicz, Z. and Andersson, J. (1971) *Exp. Cell. Res.* **67,** 39–48.
5. Darzynkiewicz, A. and Andersson, J. (1971) *Exp. Cell. Res.*, **67,** 49–60.
6. Shibko, S., Koivistoinen, P. Tratnyek, C.A. Newhall, A.R. and Friendman, L. (1967) *Anal. Biochem.* **19,** 514–528.
7. Weaver, J.A. (1955) *J. Path. Bac.* **69,** 133–139.
8. Joel, D.D., M.W. Hess, and H. Cottier, (1972) *J. Exp. Med.* **135,** 907–923.
9. Raviola, E. and Karnovsky, M.J. (1972) *J. Exp. Med.* **136,** 466–498.
10. Eurnstrom, U., and Larsson, B. (1967) *Acta. Path. Microbiol. Scand.* **79,** 371–384.
11. Beauvieux, Y.J. (1963) *C.R. Acad. Sci.* (*Paris*). **256,** 2914–2916.

บทคัดย่อ

การฉีดฮอร์โมนเพศชนิด testosterone หรือ estrogen แก่หนูแรกเกิด ทำให้น้ำหนักขนาด การร่วมสาร ^{3}H–thymidine ในเซลล์ของต่อมไทมัสและจำนวน lymphocytes ในเลือดของหนูลดลง ส่วนการฉีดฮอร์โมนเพศชนิด progesterone ทำให้ขนาดของต่อมและการร่วมสาร ^{3}H–thymidine ในเซลล์ของต่อมไทมัสเพิ่มขึ้น แต่ทำให้จำนวน lymphocytes ในเลือดของหนูลดลง ในด้านการเปลี่ยนแปลงทางจุลกายวิภาคนั้น การฉีดฮอร์โมนเพศทั้ง ๓ ประเภท ทำให้จำนวน lymphocytes ในส่วนนอก (cortex) ของต่อมไทมัสลดลง แต่ขณะเดียวกันทำให้จำนวน lymphocytes ในส่วนใน (medulla) ของต่อมเพิ่มขึ้น

จากผลการทดลองนี้สรุปได้ว่า testosterone และ estrogen สามารถห้ามการแบ่งตัวและสามารถทำให้ lymphocytes ตายได้ ส่วน progesterone มีผลตรงกันข้าม เนื่องจากในทุกกรณีพบว่ามีการคั่งของ lymphocytes ในส่วน medulla ของต่อมเกิดขึ้น พร้อมกับการลดจำนวนของ lymphocytes ในเลือด จึงทำให้ตั้งข้อสันนิษฐานว่าฮอร์โมนเพศอาจจะลดจำนวน lymphocytes ที่ถูกปล่อยจากไทมัสออกสู่เลือด ด้วยการลดจำนวน lymphocytes ที่เคลื่อนเข้าสู่เส้นเลือดโดยผ่านผนังเส้นเลือดชนิดพิเศษ ที่มีชื่อว่า post-capillary venules