

STEREOLOGICAL INVESTIGATIONS OF THYROID IN FEMALE
RATS TREATED NEONATALLY WITH ESTROGEN

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ABSTRACT

The height of follicular cells and the volume fractions of these cells, colloid and interstitium in the thyroid gland of female rats neonatally treated with a single or two doses of estradiol dipropionate (OeDP) were examined using the stereological method. The female rats, neonatally treated with 1 mg OeDP on the third and on the third and sixth day of life were sacrificed on days 10, 16, 38, 80, 180 and 365. The up to now obtained results have shown that the follicular cell height in the thyroid of the treated females is significantly decreased from 38 to 180 days of life. The decrease of the volume density of the follicular cells and the increase of the volume density of colloid are most clearly evident on day 80 ($P < 0,001$). The signs of increased proliferation rate of the follicular cells were evident in one year old treated animals.

INTRODUCTION

The ability of thyroid follicular cells to biosynthesise and reabsorb colloid is decreased in male rats treated with gonadal steroids (Pantić and Sekulić, 1980a) and is noticeable in the decreased quantity of polysomes, poorly developed and very often cisternoid endoplasmic reticulum and undeveloped microvilli (Pantić and Sekulić, 1980b).

The use of gonadal steroids for contraceptive and therapeutic purposes is increasing (Pantić, 1981). Having in mind data related to the role of steroids in the thyroid cell activities, the aim of this research was to investigate stereologically the influence of OeDP on the developmental and age-related changes in the thyroidal composition of follicular cells, colloid and interstitium, and to compare the data obtained with the cytological properties of thyroid tissue.

MATERIAL AND METHOD

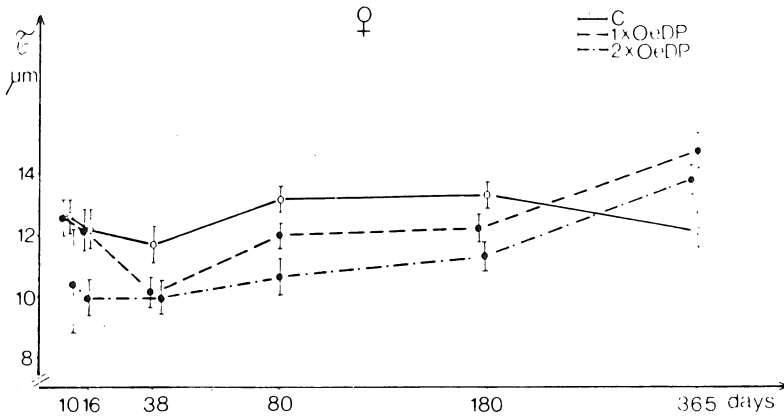
Wistar female rats treated with 1 mg OeDP on the third or on the third and sixth day of life were used for this experiment. Each age group, consisting of seven animals, had corresponding intact animals as control. The animals were kept in daylight and sacrificed on days 10, 16, 38, 80, 180 and 365.

The pieces of thyroid glands fixed in Bouin solution and stained with HE and Azan were examined using the light OPTON microscope at an objective magnification of 40X. The 5 μ m thick sections were used for determining the volume density of the follicular cells, colloid and interstitium in mm^3 . The processing of data obtained by measurement of follicular cell height was performed according to Bogataj et al., 1977 and statistically evaluated using the Student t-test.

RESULTS

a) Intact animals

The volume density of follicular cells (V_{Ve}) in the thyroid was 61% during the juvenile and 66% during the pubertal period. In six month old rats it was 56% and 49% at the age of one year. The volume density of colloid (V_{Vk}) increased as the volume density of follicular cells was decreased, and it reached its maximum (32%) in one year old rats. Comparing the volume density of interstitium (V_{Vi}) of the thyroid in female rats, an observable decrease was evident with ageing of the animals. The index of activation rate (V_{Ve}/V_{Vk}) goes from 5.19 in ten day old female rats and 1.54 in one year old rats (Graph 1.).



Graph 1. The height of the thyroid follicular cells (\bar{h}) in female rats from 10 to 365 days; intact rats (C); neonatally treated with a single (1xOeDP) or two doses of OeDP (2xOeDP) ($\bar{h} \pm 1SE$)

With the ageing of the intact animals the number of macrofollicles increases. The changes observed using the stereological methods were closely correlated with the follicular cell ultrastructure. Colloid density and staining affinity showed decreased thyroid activity.

b) Animals treated neonatally with a single dose of OeDP

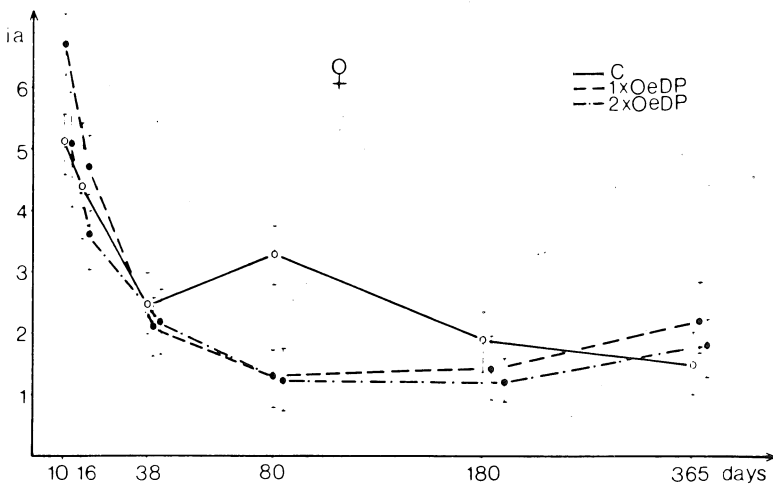
Examining the chosen parameters of follicular cells, colloid and interstitium in the thyroid of the female rats neonatally treated with OeDP, the following results were obtained:

- The follicular cell height was decreased in the thyroid of the animals from 38 days to 180 days.

When compared with the corresponding controls the

decrease of follicular cell height was highly significant in 80 day old rats ($P < 0,001$).

- The follicular cell volume density (V_{Ve}) was significantly decreased while the colloid volume density (V_{Vk}) was significantly increased in the thyroid of the treated animals. However, the value obtained was highly significant in 80 day old rats. Volume density of the interstitium was highest in the females at the ages of 10 and 16 days, amounting to 27% and 21% respectively. The smallest percentage of V_{Vi} (12%) was in the thyroid glands of 80 day old rats. The index of activation value of all the examined rats was found and its smallest rate (1.35) was observed on day 80 (Graph 2).



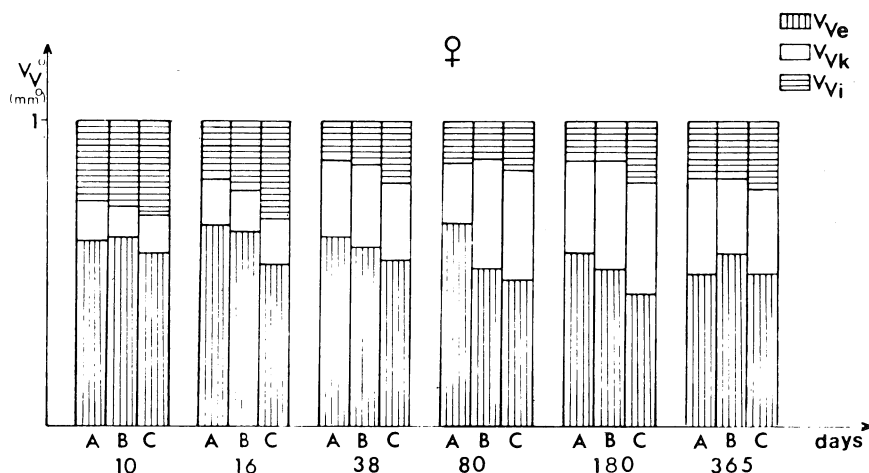
Graph 2. Index of activation (ia) in the thyroid of female rats from 10 to 365 day; intact (C); neonatally treated with a single (1xOeDP) or two doses of OeDP (2xOeDP) ($ia \pm 1SE$)

c) Animals treated neonatally with two doses of OeDP

- The follicular cell height was significantly decreased in the thyroid of all the animals examined and varied from 10,5 μ m on day 10 to 11,3 μ m on

day 180.

- The follicular cell volume density (V_{Ve}) was significantly decreased in the thyroid of the animals during all stages of their development i.e. from 16th to the 180th day. The exception was observed in one year old animals in which a certain percentage of follicular cells lost polarity and did not organize a follicular structure. The colloid volume density increase (V_{Vk}) was evident in the thyroid of 80 and 180 day old animals, being significantly decreased in one year old animals. Comparing the interstitium from the thyroid of the animals treated with two doses with the interstitium of the animals treated with a single dose, a highly significant increase was observed in the rats examined between the 16th and 180th day (Graph 3).



Graph 3. The volume density of follicular cells (V_{Ve}), colloid (V_{Vk}) and interstitium (V_{Vi}) in the thyroid of the intact animals (A), neonatally treated once with a single dose of OeDP (B) and animals neonatally treated with two doses of OeDP (C) between the 10th and 365th day ($V_{Ve}, V_{Vk}, V_{Vi}, \pm SE$)

The index of activation was reduced in the animals from 16 to 180 days when it was minimal (1.23). The cytological signs of a more pronounced decrease of follicular cell activity and the increase of follicular lumen colloid density were most clearly expressed in the animals treated with two doses of OeDP.

DISCUSSION

Stereological methods are very useful for determining the volume fractions of follicular cells, colloid and interstitium in female rats during their juvenile, pubertal and adult periods and for comparing the obtained results of the intact animals with those treated neonatally with a single or two doses of OeDP.

In the analysis of our results we have taken into consideration the data reported by Bykov, 1979., Chen and Walfish 1978. and Vigoroux 1976. The increased number of macrofollicles and the lower follicular cell height in old animals were observed by Bykov, 1979. Chen and Walfish (1978) concluded that adult female rats had a lower level of total T_4 , free T_3 and T_4 and a lower TSH response to TRH. The maximal plasma concentration T_4 was found in 16 day old rats (Vigoroux, 1976). Examining the OeDP effect on TSH secretion, D'Angelo (1968), supports the opinion that the estrogen influences hypophysial hormone secretion via multiple loci and mechanisms of action.

The decreased reabsorptive ability of follicular cells is a result of an increased number of macrofollicles and follicular lumen colloid. The significant decrease of follicular cell height and volume density in the animals neonatally treated with OeDP, undoubtedly appears as a result of the long-term effect of OeDP.

Cytological analysis of the follicular cells and colloid density has pointed out that the activity of this gland is less expressed in OeDP treated female rats than in corresponding intact animals. However, in one year old animals neonatally treated with OeDP, a significantly decreased number of macrofollicles and an increased number of depolarized follicular cells which had lost the ability for follicular organization were observed. The long-term effect of OeDP in treated female

rats was more strongly expressed in the animals treated with two doses of OeDP.

REFERENCES

Bogataj M, Kališnik M, Poredoš P. A simplified indirect method of the determination of the average shell thickness of the hollow spheres from samples in sections. *Folia Anat Jugosl* 1977; 6: 45-49.

Bykov VL. Histophysiology of the thyroid gland in postnatal ontogenesis. *Arch Anat Hist Embr* 1979; 76: 80-94.

Chen HJ, Walfish PG. Effects of age and ovarian function on the pituitary-thyroid system in female rats. *J Endocrinol* 1978; 78: 225-232

D'Angelo SA. Simultaneous effects of estradiol on TSH secretion and adrenocortical function in male and female rats. *Endocrinol* 1968; 82: 1035-1041.

Pantić VR. Sensitivity of pituitary gonadotropic cells and gonads to hormones. In: *Reproductive Processes and Contraception*, Edited by McKerns KW, Plenum Publishing Corporation 1981; 47-89.

Pantić VR, Sekulić M. a) Dugotrajno delovanje estradiola na štitastu žlezdu. *Jug Simp o štitastoj žlezdi*. Zlatibor 1980; 1-4.

Pantić VR, Sekulić M. b) Reactions of thyroid follicular cells in rats neonatally treated with estrogen. *2nd Int Congr Cell Biol Berlin (West)* 1980; p.572.

Vigorous E. Dynamic study of post-natal function in the rat. *Acta Endocrinol* 1976; 83: 752-762.

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