Proceedings of the Iowa Academy of Science

Volume 72 | Annual Issue Article 64

1965

A Pilot Study of the Thymus of Propylthiouracil-treated Rats

Maybelle G. Conklin
Coe College

also of Coe who greatly assisted in the histological interpretations. The author is also gratful to Dr. Cook and Mr. Atkins for their constructive criticism in reading this manuscript.

Literature Cited

Fregly, M. J., Galindo, O., and K. M. Cook. 1961. Spontaneous sodium chloride appetite of goitrogen-treated rats: effect of hypophysectomy and adrenalectomy. Endocrinology 69: 1060-1067.

Stone, C. T., Childers, J. H., Johnson, J. E., and I. V. Epstein. 1951. Preoperative use of, and histological changes produced by, Tapazole. Texas Reports on Biol. and Med. 9: 815-833.

Lilly Research Laboratories. 1963. Tapazole, Methimazole, U.S.P.: For the treatment of hyperthyroidism. Eli Lilly & Company. Indianapolis.

A Pilot Study of the Thymus of Propylthiouracil-treated Rats[1]

MAYBELLE G. CONKLIN[2]

Abstract. A study of the effects of propylthiouracil[3] on the rat thymus showed histological changes in that tissue. The overall weight of the thymus tissue was reduced in comparison to the thymus weight of the control group. Histologically the thymus of the PTU group showed decreased width in the cortex with fewer lymphocytes and thymic corpuscles in the medullary portion. There appeared to be an intermediate area between the cortex and medulla rather than the distinct demarcation seen between the cortex and medulla of the normal rat thymus.

The results observed in these tissues may indicate a retardation in the maturation of the thymus rather than thymic involution. Further histological studies must be made on the changes of the normal rat thymus from birth to adulthood in order to determine the extent of deviation from normal thymic growth as was observed in this experiment.

BACKGROUND

The theories on function of the thymus and relation to other organs range widely. The major controversy prior to the most recent findings on its autoimmune activity, focused on whether the thymus was a lymphoid structure or an endocrine organ. Recent studies on the thymus have linked it to the autoimmune response in the body and to antibody production. It may also

[1] This research was conducted under the auspices of the Biology Department of Coe College, Cedar Rapids, Iowa.

[2] School of Medical Technology, Presbyterian-St. Luke's Hospital, Chicago, Illinois.

[3] 6n propyl-2thiouracil, Nutritional Biochemical Co., Cleveland, Ohio.

be a precursor of the circulating lymphocytes. (Miller, 1964; Good, 1963; Burnett, 1962) 1964:

A characteristic of the human thymus and some other vertebrate thymuses is the process of involution normally occurring at puberty. This process begins with a decrease in the size of the cortex. Adipose tissue gradually replaces most of the thymus tissue, but the original shape of the organ is retained. The number of thymic corpuscles (Hassal's corpusles) increases as does the interconnective tissue of the lobules. Fat is often present in large amounts in the septa. The blood supply is decreased, especially in the medulla.

Involution occurs rapidly and prematurely during some chronic diseases, under starvation conditions, extreme or prolonged tension, alcoholic intoxication, and drug addiction. This premature involution is known to occur in the human, and similar results have been produced in laboratory animals.

Several experiments involving the response of various organs to stress conditions showed thymic involvement. During conditions of stress induced by starvation, confinement within a small area (Marsh and Rasmussen, 1960), administration of ACTH (Lee, 1964), and in other conditions where continuous demand is made upon the adrenals, the thymic weight is decreased and histological changes have been reported. Miller (1964) reported a decrease in lymphocytes during stress, and Kohnen and Weiss (1964) reported lymphocyte depletion and increase in thymic cysts from cortisone administration. Why this occurs is not understood.

Although the thymus is considered a lymphoid organ, it is under the influence of the endocrine organs. Of special concern in this paper is the effect of the thyroid and the adrenals. Thyrotropic hormone from the anterior pituitary stimulates the thyroid which produces a positive response in the thymus, increasing lymphopoetic activity. Stimulation of the adrenal cortex by adrenocorticotropin, however, has an inhibitory effect upon the thymus according to Houssay (Gajewski, 1964).

An inhibitory effect upon the thymus may also occur with the use of antithyroid drugs such as propylthiouracil. Studies by Fregley and others (1960) on propylthiouracil (PTU) have shown that it affects organs other than the thyroid. In normal rats, administration of PTU produces hypothyroidism with a compensatory hypertrophy of the thyroid (Cook, 1960).

In experiments on renal hypertension, PTU inhibited a rise in blood pressure of hypertensive rats and produced other adverse side effects, namely, reduced growth rate, anemia, and increased testicular size (Fregley and Cook, 1960).

The above experiments included observations on the thymus under these various conditions following PTU administration. Normal rats after receiving PTU showed a weight decrease in the thymus. Fregley and Cook administered thyroid hormone with PTU to rats with induced hypertension and found no significant difference between the thymus weight of the test rats and that of the control rats. Without the thyroid hormone the PTU hypertensive rats had a decrease in thymus weight of almost half the thymus weight of the controls.

In adrenalectomized rats; the weight of the thymus was increased; if PTU was given following adrenalectomy, the rats showed a decreased thymus weight (Madden, 1962).

In light of the foregoing experiments, it seemed of interest to examine histologically the thymus of normal rats which had been given propylthiouracil.

Procedure

Seven white male rats of Holtzman strain, 61 days old, were divided into two groups. Three rats were placed on a regular diet of ground Purina laboratory chow and tap water. The remaining four were placed on a diet of the same laboratory chow but which had 0.1% propythiouracil added to it, and were also given tap water. The animals were kept in separate, round, metabolic cages in a temperature-controlled room at 25°C ($\pm$ 2°). Animals, food and water were weighed daily.

At the end of the fifth week the animals were sacrificed with ether. The thymus and thyroid glands were removed and weighed intact. The thymus was cut into several pieces to be fixed in different solutions—Zenker's acetic alcohol and Bouin's. The tissues were stained with Harris, hematoxylin and eosin after the method described by Humason.

Results

As was expected the rats treated with propylthiouracil lost weight during the first week, and thereafter gained weight very slowly as compared with the control group. Their general condition was good but their appetites were less than that of the control. Also, they drank considerably more water than the controls. During the fourth week of the experiment the control group shed its winter hair. No shedding was noticed in the PTU group.

The histological examination of all the tissues was not possible due to mechanical difficulties and a time element. However, at least two slides were completley prepared of thymus tissue from each rat in both groups. The slides of the control group, stained

Table 1. Body Weights – Weekly Average.

	Initial Wgt.	Wk. 1	Wk. 2	Wk. 3	Wk. 4	Wk. 5
#1	210.5	259.7	301.7	327.3	343.7	361.1
#2	230.0	271.1	306.2	331.6	354.6	367.1
#3	218.5	263.6	287.2	306.3	319.6	328.5
#4	235.5	260.8	283.9	290.3	292.9	297.8
#5	223.0	246.6	268.4	275.4	278.6	285.7
#6	223.5	249.4	273.4	288.7	294.6	304.9
#7	213.5	250.6	270.8	287.2	291.7	298.7

Body weights ± 0.5 gms.
Rats # 1-3 are control group.
Rats # 4-7 are PTU-treated group.

Table 2. Thymus and Thyroid Weights.

	Body Wgt. at Death	Wgt. of Thymus	Thymus- mg/100 g Body Wgt.	Wgt. of Thyroid	Thyroid- mg/100 g Body Wgt.
#1	356.9	0.5525	154.5	0.0145	4.06
#2	367.8	0.5200	141.5	0.0175	4.77
#3	343.2	0.6151	179.3	0.0176	4.80
#4	303.5	0.2410	79.5	0.0910	9.10
#5	294.0	0.2325	79.1	0.1285	12.85
#6	314.0	0.3149	99.7	0.1270	12.70
#7	307.7	0.2626	86.0	0.0895	8.95

Rats # 1-3 are control rats.
Rats # 4-7 are PTU-treated rats.

with hematoxylin and eosin, showed a definite demarcation between the cortex which stained dark purple, and the medulla which was a much lighter bluish purple. In the medulla eosinophilic circular masses with concentric rings formed the thymic corpuscles. The connective tissue of the septa and interlobular areas contained occasional regions of adipose tissue.

The slides of the propylthiouracil group showed some apparent differences in the thymus. The cortex was thinner and there was an intermediate area between the cortex and the medulla. The medulla appeared to have fewer thymic corpuscles, and the medullary cells were more loosely packed.

Conclusion and Summary

The present experiment was conducted to study the effects of PTU on the thymus of the rat.

The material for study was obtained from seven rats of the Holtzman strain. Four animals age 61 days were used in the experimental group, while three animals of the same age were the controls. To the diet of the experimental group was added 0.1% PTU. The controls were fed the same diet without PTU. Body weights, food consumption, fluid intake of both groups were taken daily during the experimental period. At the end of the fifth week all the experimental and control animals were sacrificed with lethal doses of ether. The thymus and thyroid glands were removed immediately and weighed. Sections of

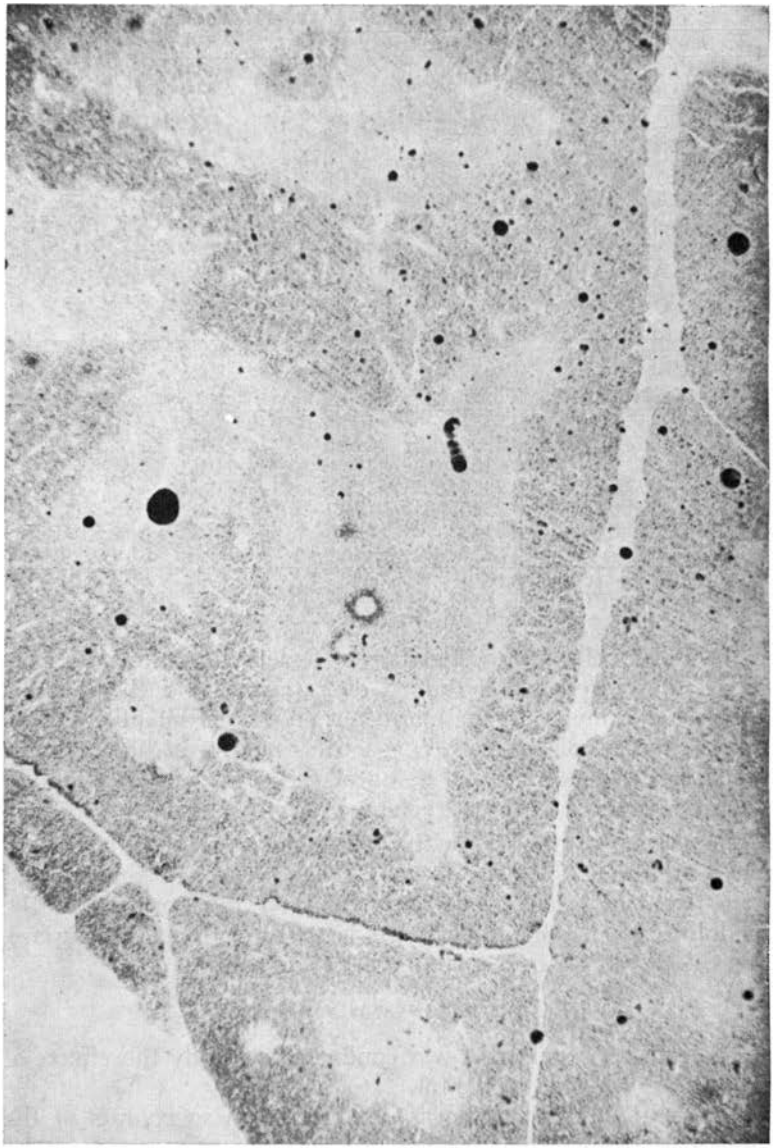

Figure 1. Thymus Control. Scanning view of normal rat thymus showing definite demarcation between cortex and medulla.

the thymus were prepared for histological study, sliced at 10-micron thickness and stained with H and E stain.

The following major observations were made:

1) Daily body weights of both groups.
2) Quantity of food and fluid consumed daily by each group.
3) Thyroid weight compared to body weight at time of death

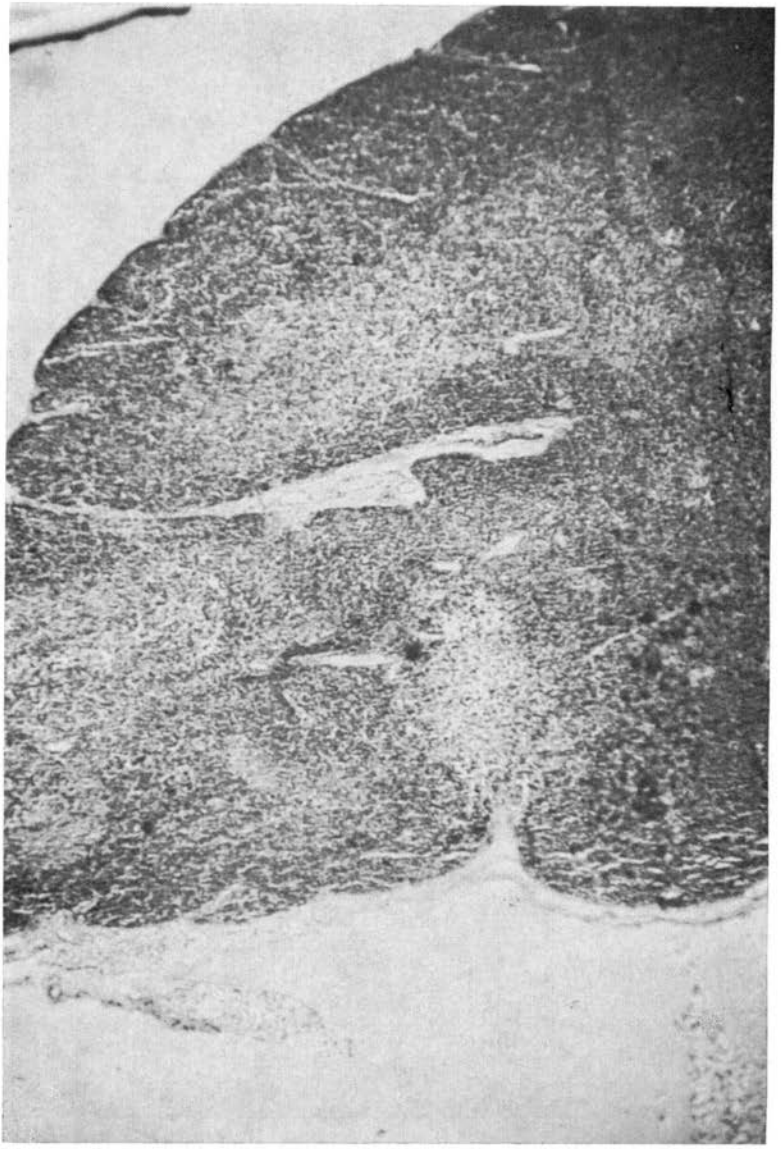

Figure 2. PTU Thymus. Scanning view of thymus from propylthiouracil treated rat showing cortex and medulla and an intermediate area.

for both group.

4) Thymus weight compared to body weight at death of the animals of both groups.
5) Histological study of the thymus of both groups.

The following are the results of these observations:

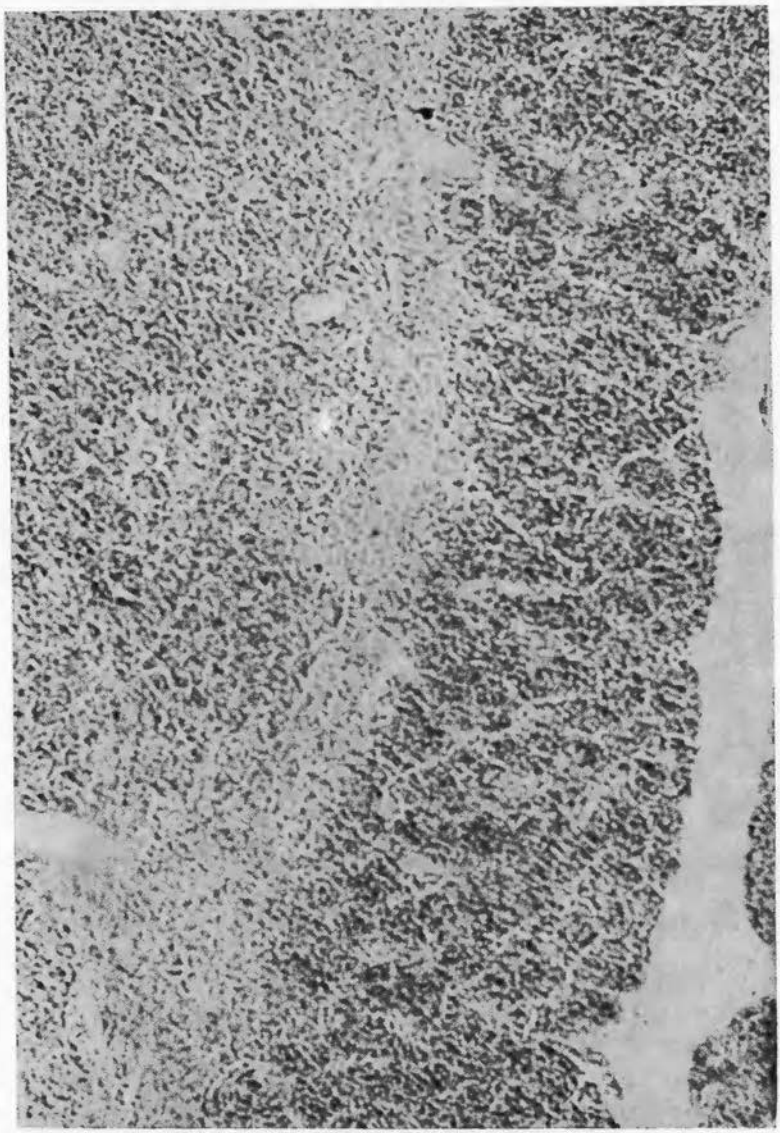

Figure 8. Thymus Control. Cortex and medulla

1) The body weights of the experimental group showed a slower weight increase and a lower body weight at the end of five weeks than the control group.
2) The average daily food consumption of the PTU group was lower than the average of the group on the normal diet. The fluid intake was increased in the rats on the PTU diet as compared with the fluid intake of the controls.

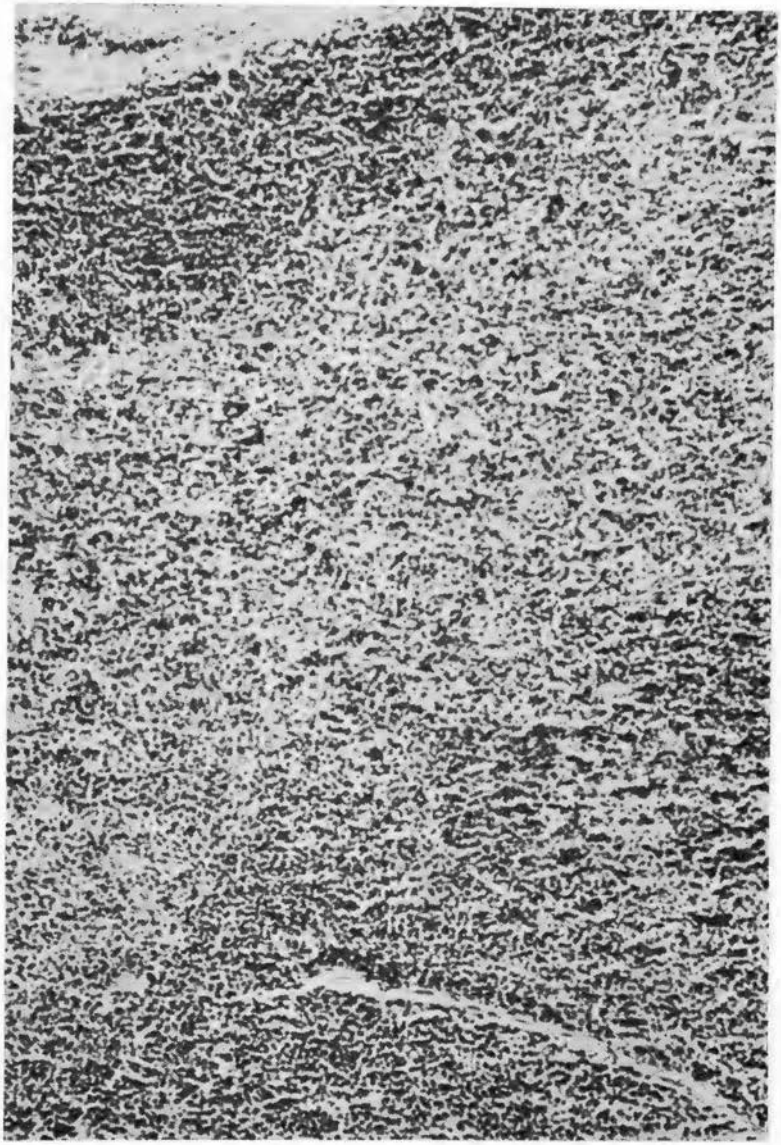

Figure 4. PTU Thymus. Cortex, medulla and intermediate area.

3) Thyroid-weight to body-weight ratio was markedly increased in the rats on the PTU diet.
4) In the rats of the experimental group the thymus-weight to body-weight ratio was decreased as compared with the ratio of the control group.
5) Histological examination of the thymus of the PTU group

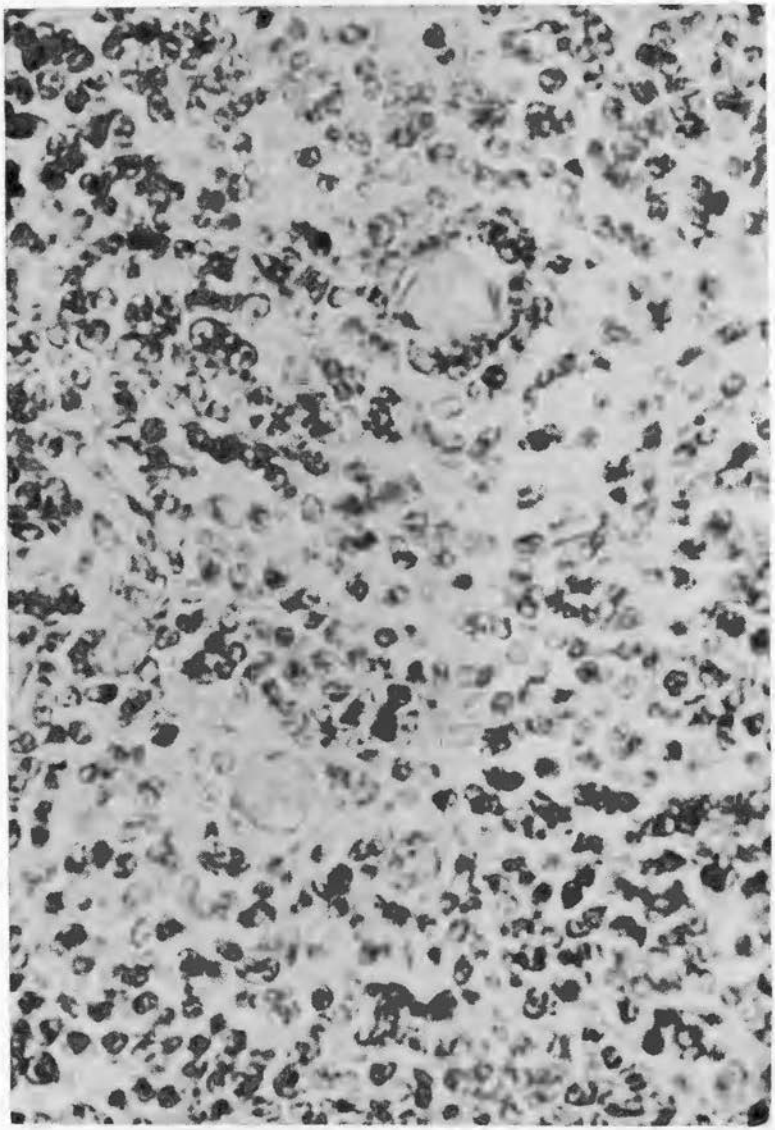

Figure 5. Thymus Control. Thymic corpuscles in medulla.

showed a thinner cortical region, a diffuse cortico-medullary boundary, fewer thymic corpuscles in the medulla, and more loosely packed lymphocytes with greater amount of intercellular spaces than the thymuses examined in the control group.

The weight loss or retarded weight gain, the loss of appetite and the increase in water consumption have been discussed in

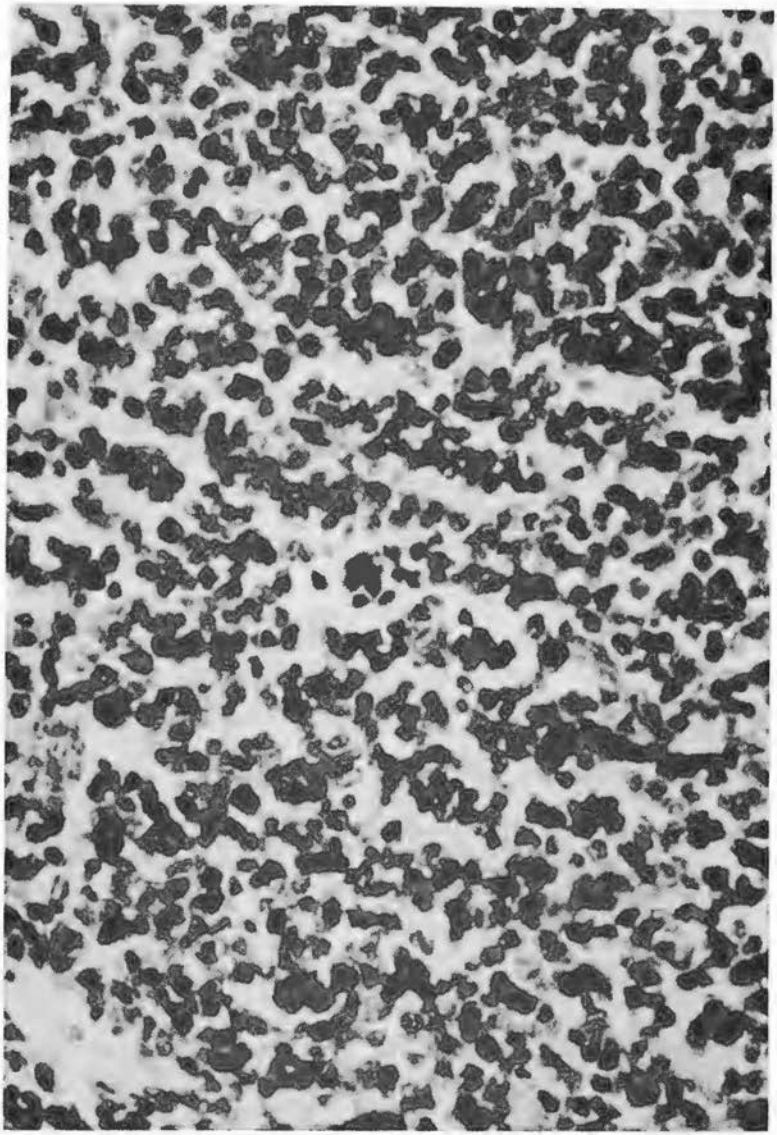

Figure 6. PTU Thymus. No large thymic corpuscles visible.

the work of Fregley and Cook (1960), and Madden (1962), who considered these to be side effects of propylthiouracil acting on the anterior pituitary and hypoactivity of the thyroid (Fregley et al. 1960).

The cause of the histological changes is not clear. Several workers have reported similar results in studies with ACTH. When ACTH was administered to rats, a study of their thymus

showed a reduced width of the thymus cortex, the migration of lymphocytes from the medulla, and increased cell proliferation at the corticomedullary border, indicated by positive alkaline phosphatase staining of these cells (Lee, 1964). However, other effects reported in Lee's study were not observable in this study: cortical pitting, pyknotic cells, loss of cytoplasmic RNA, and phagocytosis of DNA positive nuclear debris. These effects may have been present since different staining techiques were used in the two studies.

The nature of the thymic corpuscles is not understood nor are the circumstances of their formation. In this study, apparently fewer and smaller corpuscles were in the thymuses of the PTU rats than in the controls. Harland's reports on the histogenesis of the rat thymus indicate no thymic corpuscles present at birth but they may develop later (Harland, 1940). This may indicate that the thymuses of the PTU group were less mature than those in the control group. No definite conclusions can be made from this without futher study of the maturation of the thymus after birth.

Further studies on the thymus should be conducted to determine when thymic corpuscles develop. In conjunction with this study, histochemical studies using alkaline and acid phosphatase stains would be useful to determine the sites of greatest cell proliferation.

In conclusion it may be said that histological differences were noted in the thymuses of propylthiouracil treated rats but the relationship of these changes to the known effects of the drug is obscure. Futher studies may help to distinguish whether this is a response to stress induced by PTU administration, or an inhibition of lymphopoetic activity due to reduced thyroid activity.

ACKNOWLEDGEMENTS

I would like to acknowledge the inspiration given me by Dr. Kenneth Cook, the technical assistance of Dr. Ruth Siemer, and Mr. Charles Atkins of Coe College, and the advice and aid of Dr. Ephraim Axelrod, Miss Marjorie Stumpe, Dr. Vinod Sanghani and Miss Gabriella, of Presbyterian-St. Luke's Hospital, Chicago. I am grateful to them all for their time, patience and encouragement, and the suggestions they offered.

Literature Cited

Burnett, Macfarlane. 1962. Thymus Gland. Sci. 207:50-59.

Cook, Kenneth M. 1960 The Influence of the Anti-thyroid Drug Propylthiouracil on Reproduction in Rats. Proc. Iowa Acad. Sci. 67:463-470.

———. 1962. The Influence of the anti-thyroid drug Propylthiouracil on gestation and lactation in rats. Proc. Iowa Acad. Sci. 69:629-635.

Fregley, Melvina and Cook, K. M. 1960 Role of the thyroid gland in development and maintainance of renal hypertension in rats. Acta Endocrinol. 34:411-429.

Gajewski, J. E. (ED) 1964. The thymus Therapeutic Notes 72: 6-12.

Good, Robert A. 1963. Thymus abnormalities linked to leukemia and antibody production. Sci. Newsletter 84:50.

Harland, Margaret. 1940. Early histogenesis of the thymus in the white rat. Anat. Record 77:242-271.

Humason, Gretchen 1962. *Animal Tissue Techniques.* W. H. Freeman and Co., San Francisco.

Kohnen, Paul and Weiss, Leon, 1964. An electron microscopic study of the thymic corpuscles in the guinea pig and the mouse. Anat. Record 148: 29-57.

Lee, Jr., Do. 1964. Histological and histochemical studies of the rat thymus following adrenalectomy and ACTH, corticosteroid administration. Am. J. Anat. 114:93-105.

Madden, Ross A. 1962. Effects of the antithyroid drug propylthiouracil on organ weights, NaCl intake, and blood glucose levels of adrenalectomized white rats. Proc. Acad. Sci. 69:616-624.

Marsh, James T. and Rasmussen Jr., A.F. 1960. Response of the adrenals, thymus, spleen, and leucocytes to the shuttle box and confinement stress. Soc. Exptl. Biol. Med. Proc. 184:181.

Miller, J. F.A.P. 1964. Thymus and Development of Immunological Responsiveness. Sci. 144:1544-1551.