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Spontaneous Pathology of the Thymus in Aging Wistar (Cpb:WU) Rats

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Abstract. Spontaneous thymic lesions were investigated in Wistar (Cpb:WU) rats. Thymic tumors were not uncommon and most showed medullary differentiation. Thymic involution was investigated in a limited group of animals in which the survival rate for males and females was similar. The histological pattern of thymic involution differed between sexes. Severe thymic involution occurred more frequently in males than in females and at an earlier age.

Understanding patterns of disease in aging rats is essential when using this species in research areas such as carcinogenesis, toxicology, and gerontology. Thymic involution and cellular immune dysfunction may influence pathogenesis of many types of diseases. Moreover, there may be a relationship between aging and the rate of decline of immune competence as indicated by increasing thymic pathology.[13,14,17] The thymus may have a non-immunological relation with aging: the organ is probably involved in regulating the development of sexual maturation[3,15] and neuroendocrine functions.[18] Extensive studies on spontaneous (background) pathology in aged rats of various strains have recently been reported.[5,8,12,24,31,33] The thymus, which is an important organ with regard to aging and a target organ of many immunotoxicants,[4,22,32] received relatively little attention in these studies. More detailed studies of the histopathological profile of the thymus in aging rats are therefore warranted. This study presents histopathological findings in the thymus of Wistar (Cpb:WU) rats.

Materials and Methods

Weanling Cpb:WU (Wistar Random) virgin rats, 400 males and 400 females, were obtained from the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, The Netherlands. These animals were used in a life-span study with interim kills in which the background pathology of this strain of rats was investigated. They were kept five to a cage in stainless steel wire mesh cages (44 × 32 × 17 cm) at 23 ± 1 C, relative humidity 40–70%, and with lighting for 12 hours a day. The rats were fed a powdered stock diet of the following percentage composition: yellow maize 29.7, whole wheat 36, soybean oil meal 11, meat scraps 4, fish meal 7, dried whey 2, brewer's yeast meal 3, grass meal 3, soybean oil 3, vitamin preparations 0.4, trace mineralized salt 0.5, and steamed bone meal 0.4. Animals had free access to food and tap water.

Food and water were regularly examined for contaminants which were always below acceptable limits.[2] Two rat groups, initially consisting of 50 males and 50 females each, were kept for 3 or 12 months, one group of 100 males and 100 females was kept for 24 months, and the remaining 200 males and 200 females were kept until 80% had died (32 months for both males and females). Animals were checked twice a day. Moribund animals were killed to prevent autolysis. At necropsy a large variety of organs and tissues were dissected and fixed in a 4% neutral phosphate-buffered formaldehyde solution. Body weights and a variety of organ weights were recorded. The thymus was weighed at 3 and at 12 months. Organs to be examined microscopically were embedded in paraffin wax, sectioned at 5 μm and stained with hematoxylin and eosin.

Correlations between thymic involution and other age-associated lesions were evaluated using Spearman's two-sided rank correlation method.[25] Age-associated lesions included testicular and ovarian atrophy, mammary gland proliferative lesions, tubular nephrosis of the kidneys, generalized periarteritis, and the presence of benign and/or malignant tumors.

Results

Survival of males did not differ significantly from that of females (Fig. 1). Frequent causes of death were nephrosis, generalized periarteritis, and tumors of the mammary gland and pituitary. Both absolute and relative thymic weights were distinctly lower at 12 than at 3 months (Table 1). Thymuses of animals killed at 24 months and at 32 months were not weighed because severe involution precluded identification of the organ. The incidence and type of microscopic lesions in the thymus in the interim-kill and life-span groups varied. (Table 2).

Involution was characterized by loss of lymphocytes and proliferation of epithelial structures (Fig. 2). The degree of involution was based on the size of the thymus and the amount of lymphocytes still present in the organ as judged from the cortex-medulla ratio which decreased when the involution became more advanced. The thymus could not always be identified at necropsy in older animals due to advanced involution.

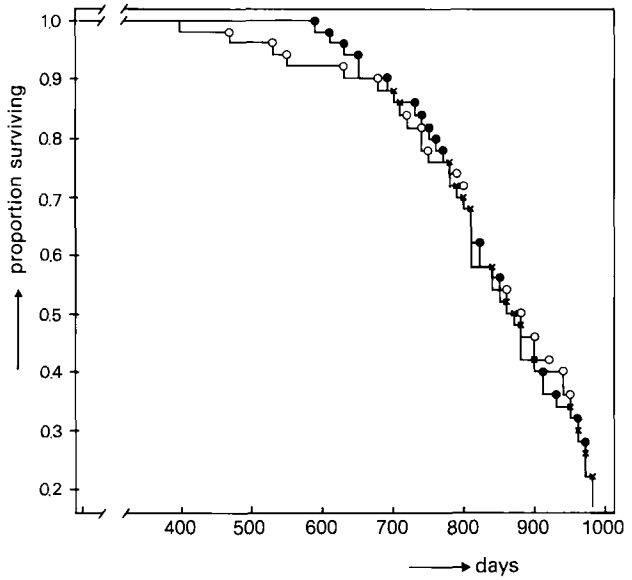

Fig. 1. Proportion of animals surviving at times during the life-span study (black dots = males, open dots = females, cross = coincidental group scores).

The presence of epithelial cells and of cysts, although thought to be part of involution, were scored separately (Table 2).

The onset of slight degrees of thymic involution occurred earlier in females than in males. In male rats that died during the study, the degree of involution was distinctly more severe than in survivors killed in week 104 or terminally. In females such a trend was not present, and both survivors and decedents showed about the same degree of involution (Table 3). Thymic involution did not correlate with histopathological changes in other organs (Table 4). Only ovarian atrophy was positively correlated with thymic involution.

Epithelial cells increased in incidence and severity with age, forming cords and tubules and only occasionally nests (Fig. 3). Small foci of epithelial cells were located in or near the interlobular septa. In more advanced stages epithelial structures were also found in the cortical and medullary region of the lymphoid tissue. The cytology of the proliferating epithelial cells was characterized by a round to irregularly shaped nucleus without prominent nucleoli and lightly stained eosinophilic cytoplasm. Cystic structures lined by epithelial cells and often containing acidophilic material were also seen in close association with epithelial cell proliferation.

Proliferation of epithelial structures and cyst formation were distinctly more pronounced in females than in males. In old males the thymus was generally composed of a few nests of lymphocytes accompanied by some clearly identifiable epithelial cords, but tubules and cysts were seldom seen.

Table 1. Mean absolute and relative weights of the thymus.

	Mean Body Weight (g)	Thymus Weights*		
		n	Absolute (g)	Relative g/100 g Body Weight
Males				
3 months	273	50	0.466 ± 0.014	0.126 ± 0.003
12 months	487	50	0.161 ± 0.010	0.034 ± 0.002
Females				
3 months	209	50	0.322 ± 0.007	0.116 ± 0.005
12 months	278	50	0.142 ± 0.006	0.051 ± 0.002

* Mean ± standard error of the mean (SEM).

Some old rats showed a persisting thymus seen as a gland with a relatively normal architecture but considerably larger than would be expected considering the animal's age. Interestingly, this phenomenon was found only in decedents.

Well-developed lymph follicles are not normally seen in the thymus. The presence of lymph follicles in the cortex was considered a pathologic condition and was called follicular hyperplasia. In most cases only a single lymph follicle was present. Follicular hyperplasia occurred only in 3-month-old animals.

Tumors in the thymic region were divided into three classes according to their histologic pattern: thymoma with medullary differentiation, thymoma without differentiation, and lymphoma. Thymoma was defined as a tumor with involvement of thymic epithelial cells in the neoplastic process. The histomorphological spectrum of the thymic tumors and tumor-like conditions ranged from lesions with only minimal epithelial involvement up to mainly epithelial lesions with pronounced neoplastic features suggesting a transition from slight to more severe degrees of epithelial involvement. Therefore, the borderline between hyperplasia and neoplasia was arbitrary. Thus, lesions showing one or more typical features of thymoma were all considered to be neoplastic.

Thymomas with medullary differentiation were generally large (up to 3 cm diameter) lesions showing extensive lymphoid hyperplasia, seen as large amounts of small uniform lymphocytes (Fig. 4). There were small areas showing medullary differentiation as evidenced by the presence of Hassal bodies. Lymphocytes were much less abundant in these areas than in the surrounding areas. The lesions were further characterized by subdivision into lobules which were partially enclosed by fibrous trabeculae. Occasionally there were proliferating epithelial cells within the trabeculae that frequently formed cords or tubules and occasionally cysts and were similar to the epithelial structures seen in normal age-associated involution. In addition there

Table 2. Spontaneous lesions of the thymus and mediastinum in the random-bred Cpb-WU Wistar rat at different ages.

Lesions	Incidence							
	Males				Females			
	13 wk	52 wk	104 wk	139 wk	13 wk	52 wk	104 wk	139 wk
Thymus								
Number of animals	50	50	100	195	50	50	100	195
Thymuses not found	0	2	27	82	0	6	30	61
Thymuses examined microscopically	50	48	73	113	50	44	70	134
No abnormality	41	5	1	0	30	3	0	0
Involution								
Very slight	0	16	0	0	5	19	0	1
Slight	0	13	7	8	0	17	6	15
Moderate	0	10	31	33	0	5	38	44
Severe	0	0	30	32	0	0	20	34
Very severe	0	0	2	33	0	0	0	23
Epithelial proliferation								
Very slight	3	4	6	1	8	10	6	1
Slight	0	1	9	12	4	7	13	30
Moderate	0	1	4	6	0	1	5	18
Severe	0	0	0	1	0	0	1	0
Cysts	0	0	1	1	1	7	12	34
Brown pigment	0	4	6	0	0	11	5	7
Macrophages	1	0	1	0	0	0	0	0
Perivascular mast-cells	0	0	0	0	0	0	2	0
Starry sky appearance	5	3	1	0	5	1	0	1
Follicular hyperplasia	1	0	0	0	1	0	0	0
Persistent thymus	0	0	0	1	0	0	2	2
Thymoma, medullary differentiation	0	0	3	2	0	0	1	11
Thymoma	0	0	1	1	0	0	1	0
Mediastinum								
Lymphoma	0	0	0	1	0	0	1	1
Carcinoma	0	0	0	1	0	0	0	0

were varying degrees of epithelial cell proliferation occurring diffusely throughout the lesion. In some cases sheets and nodules of large, well-differentiated epithelial cells proliferated into the lymphocytic areas causing local compression. The medullary areas were closely associated with the fibrous trabeculae. A characteristic feature of this lesion was the presence of foci of large pale cells (Fig. 5). Upon electron microscopic examination, the large pale cells showed features characteristic for thymic epithelial cells: tonofilaments and large nuclei with membrane-bound chromatin. The foci were generally located in the areas with abundant numbers of small uniform lymphocytes. These tumors were well circumscribed, at least partly encapsulated, and noninvasive. Thymomas with medullary differentiation represented a local process as evidenced by occasional remnants of atrophic thymus adjacent to or in close association with the tumor.

Thymomas with medullary differentiation were present in both sexes, but especially in females, and with incidences that increased with age from week 114. They were seen in decedents as well as survivors and caused death in several cases due to compression of intrathoracic organs.

Thymomas without medullary differentiation were composed of a mixture of epithelial cells and lymphocytes (Fig. 6). The relative proportion of these two elements varied considerably from case to case and between different locations within a single tumor. The epithelial cells varied from ovoid to distinctly squamoid or spindle shapes. Most epithelial cells were large with vesicular nuclei and relatively clear cytoplasms. They occurred either isolated or in small groups or nodules causing compression of adjacent lymphocytes. The tumors were at least partly encapsulated by a thick fibrous capsule.

Two thymomas were exclusively composed of epithelial cells. In one case the epithelial cells were poorly differentiated and spindle-shaped, dispersed foci of epidermoid differentiation were present; this tumor was

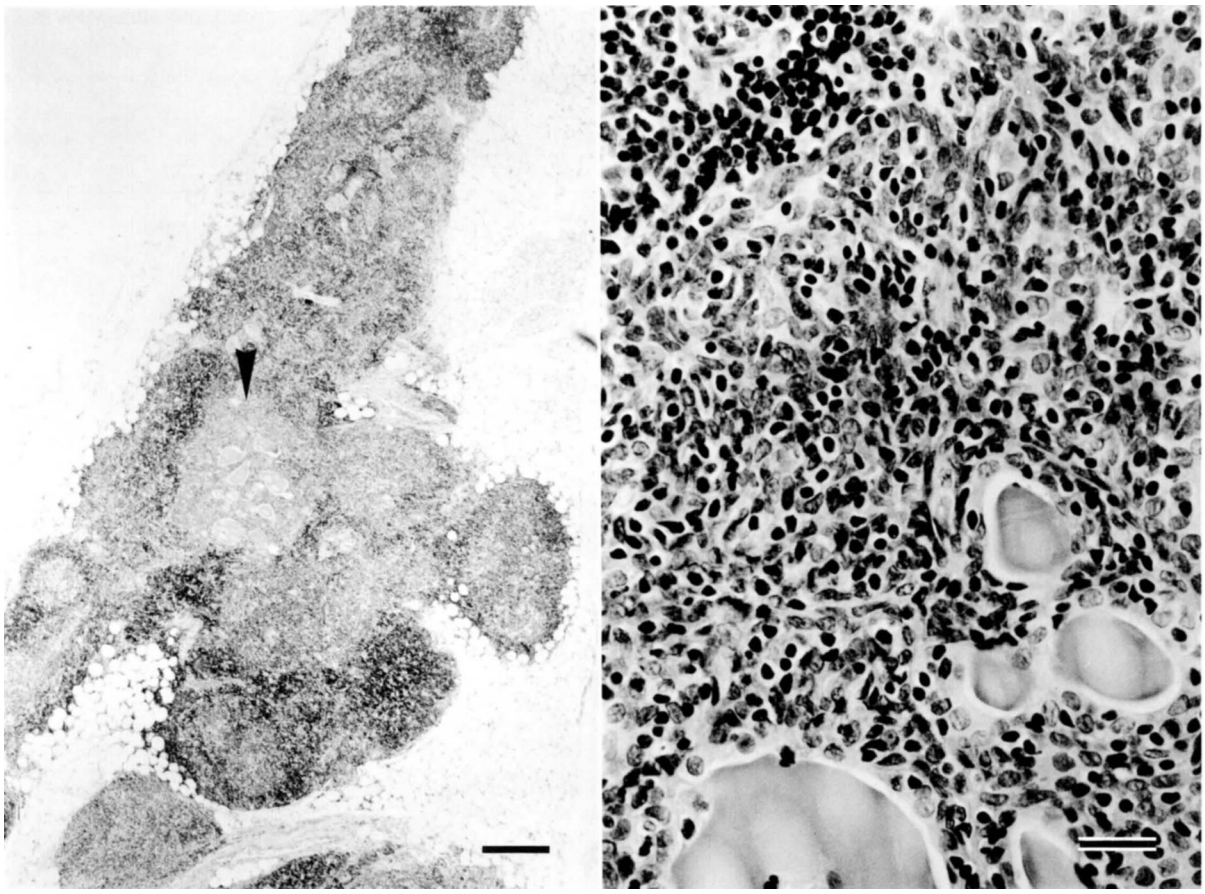

Fig. 2. Moderate thymic involution, female rat. Arrowhead indicates area given in detail in Fig. 3. HE. Bar = 250 μm.
Fig. 3. Epithelial cell proliferation. Detail of Fig. 2. HE. Bar = 25 μm.

locally invasive. The other tumor consisted of well-differentiated epithelial cells and was well circumscribed, growing in sheets and cords (Fig. 7).

None of the thymomas showed metastases. Thymomas without medullary differentiation were seen only in decedents from week 84 and on and were all likely to have caused the animals' death. A few lymphomas were seen in the mediastinum. Due to their large size, the exact site of origin, i.e., thymus or mediastinal lymph node, could not be ascertained. Lymphomas, all of which occurred in decedents, consisted of large areas of lymphocytes and/or lymphoblast-like cells intermingled with macrophages which contained cellular debris. There was no indication of medullary differentiation or epithelial structures. The tumors frequently showed a multinodular growth pattern. Sheets of tumor cells invaded the surrounding adipose tissue, but the tumor mass was restricted to the mediastinum. In one case extra-mediastinal lymphoid tissues were involved. The first lymphoma was found at an age of 37 weeks.

Discussion

Sex differences are reported in the rate and histological pattern of thymic involution in rats.[5,6] The present findings are in agreement with those of Kruisbeek et al.[13] in WAG/Rij rats, indicating that severe thymic involution occurs earlier in life in males than in females. In their study, male WAG/Rij rats showed earlier mortality and an earlier onset of many age-associated lesions than did females. The authors speculated that thymic involution might be causally related to early appearance and rapid development of age-associated alterations, thus increasing mortality. We confirmed the sex difference in age-specific appearance of thymic involution but did not find differences in mortality between males and females. On the other hand, thymic involution in males was generally more severe in decedents than in survivors (Table 4). Hence, there could be an association between early death and early onset, and more severe progression of thymic involution. However, this phenomenon may also be related

Table 3. Incidence and degree of involution of the thymus in decedents in the life-span group and survivors in 2-year and life-span groups.

Lesions	Incidence of Lesions					
	Decedents	Survivors*	Decedents			Survivors†
Week:	1–104	104	104–122	122–139	104–139	139
Males						
Number of animals examined	16	53	61	63	124	45
Involution						
Slight	1‡	6	1	2	3	4
Moderate	5	27	10	6	16	13
Severe	2	18	11	13	24	8
Very severe	7§	1	39	42‡	81‡§	20
Females						
Number of animals examined	20	54	56	60	116	43
Involution						
Slight	6	4	3	2	5	6
Moderate	8	34	16	13	29	8
Severe	1	14	14	12	26	7
Very severe	2	0	22	33	55	18

* Killed intercurrently (24-month period).
† Killed terminally (32-month period).
‡ $P < 0.03$ (Fisher exact test). Incidence of very severe involution of survivors compared with decedents in previous periods.
§ $P < 0.02$ (Maxwell contingency test). Trend test for degree of involution of survivors of wk 139 compared with decedents of wk 104–139. Other groups could not be analyzed because the analysis was disabled by the too low number of animals in most of the categories.

to the preterminal conditional decline of the decedents. Thymus involution has been associated with tumor formation in mice,[10,26,29] diabetes in man,[7] and sex hormone activity.[21,28]

Thymic hyperplasia has been associated with hypertensive vascular disease[30] and nephrosis.[11,23] We investigated correlations between the degree of thymic involution and the presence of tumors, nephrosis, gonadal atrophy, and generalized periarteritis but could only find a slight positive correlation between thymic involution and ovarian atrophy. This finding warrants the investigation of correlations between the degree of thymic involution and the onset of ovarian atrophy.

Table 4. Correlations between thymic involution and other parameters measured in 54 male and 38 female Wistar rats of the life-span group (Spearman Rank Correlation Test).

	Variable	Males	Females
		Correlation Coefficient	Correlation Coefficient
Thymic involution	Testicular atrophy	0.1624	—
	Nephrosis	0.1701	0.2436
	Generalized periarteritis	0.0234	−0.0753
	Malignant tumors	0.0000	−0.1076
	Benign tumors	−0.1315	−0.2238
	Thymic epithelial proliferation	—	0.2003
	Ovarian activity	—	0.3203*
	Mammary gland activity	—	0.1935

* $P = 0.05$.

Proliferation of epithelial elements with advancing age while the lymphoid tissue gradually declines is an interesting phenomenon of thymic involution, especially in females. The relationship between the epithelial proliferations and the thymic lymphoid cells is investigated by Cherry et al.[6] especially with respect to hormone activity. In reaction to certain noxious treatments and hormone applications, epithelial and lymphoid elements behaved similarly, indicating that the epithelial thymus is involved with lymphoid cell function. This congruency in reaction, however, was modified by sex, age, and hormonal activity of the animals. Until now, the functions of epithelial structures were not completely understood.

Epithelial thymic tumors were designated thymomas. A few lymphomas were found in the mediastinum, but the place of origin could not be ascertained. Thymomas were divided into two categories, dependent on the presence of medullary differentiation. Thymoma without medullary differentiation was extremely rare (less

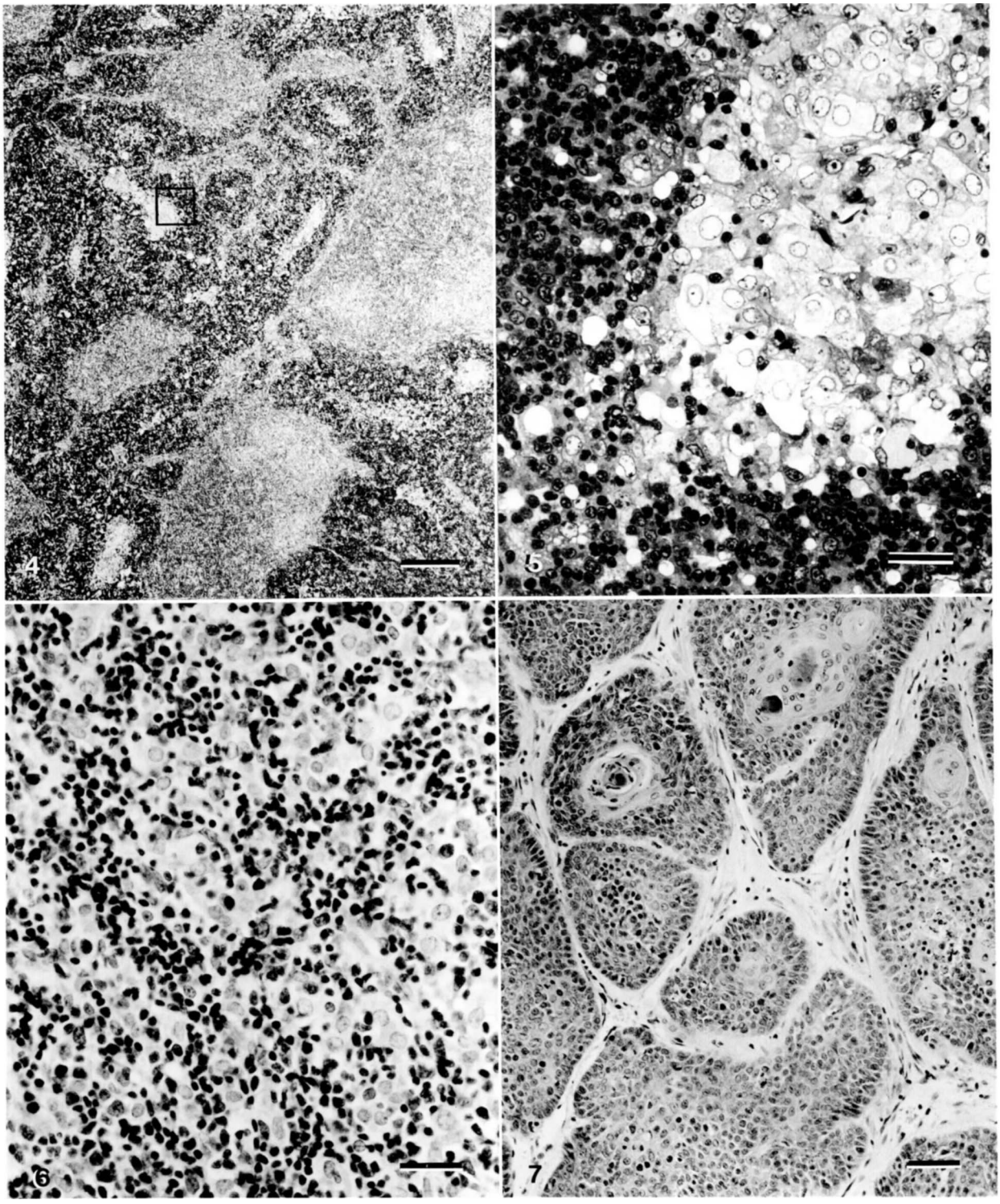

Fig. 4. Thymoma with medullary differentiation. Toluidine blue. Bar = 250 μm.

Fig. 5. Thymoma with medullary differentation, square area of Fig. 4; focus of large, pale epithelial cells. Toluidine blue. Bar = 25 μm.

Fig. 6. Thymoma; large proportion of lymphocytes. HE. Bar = 25 μm.

Fig. 7. Thymoma; epithelial cells in sheets and cords; squamous differentiation. HE. Bar = 50 μm.

than 1%). This is in agreement with incidences of thymomas reported in literature for several rat strains such as WAG/Rij,[5] ACI/N,[16] and F344.[8] A higher number (3.5%) of thymic tumors was reported for male ACI/SegHapBR rats[33] and (4.6%) for the fat sand rat (*Psammomys obesus*).[20] Most of these tumors were predominantly lymphocytic. Unfortunately, they were not described in more detail but could have been lesions similar to thymoma with medullary differentiation described in this paper. Thymoma with medullary differentiation is relatively common in our strain of rats (Table 2) and appears to be a rather uniform condition. Thymic tumors showing a comparable distribution of lymphoid cells have been described in man, however the characteristic foci of large pale cells was absent.[19] In rats these foci have been seen in thymomas without medullary differentiation.[9] Distant metastases of thymic tumors were not found in this study although they have been previously reported in rats.[1]

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