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Environmental Influences on Mass Dynamics of the Cotton Rat (*Sigmodon hispidus*) Thymus Gland

Robert L. Lochmiller and Stephen S. Ditchkoff

ABSTRACT

The influence of season on thymus gland mass was examined relative to captivity, gender, and age in 921 cotton rats (*Sigmodon hispidus*) from free-ranging and laboratory populations. Age-related involution of the thymus gland was evident in free-ranging males and females and captive females. A distinct seasonal cycle in thymus mass dynamics was apparent among adult cotton rats. Mass of the thymus gland was greatest from late fall to early winter before declining 2–4 fold during spring. Thymus gland mass remained low through spring and summer in adult cotton rats when reproductive activity was maximum. No seasonal cycle in thymus mass was apparent among juveniles. Possible involvement of sex hormones in regulating thymus size is discussed.

Abstracting keywords: Cotton rat, immunity, seasonal cycle, *Sigmodon hispidus*, thymus gland.

INTRODUCTION

The thymus gland is an indispensable primary immune organ of mammals where precursor T-lymphocytes differentiate into mature immunocompetent cells (Miller, 1990). The thymus is a dynamic organ which develops to its maximum size just prior to puberty in mammals, followed by a gradual regression in size with advancing age, but remaining functional throughout life (Kendall, 1981). Thymus development has also been shown to be highly sensitive to adrenocortical and reproductive activity (Andersen, 1932; Dougherty, 1952; Kendall and Twigg, 1981; Cockburn, 1992). Regression of the thymus gland has been demonstrated in small mammals exposed to social aggression, malnutrition, infection, and population density stressors (Clarke, 1953; Christian, 1956; Myers et al., 1971; Chandra and Newberne, 1977; Twigg and Harris, 1982). Stress-induced thymic regression is frequently correlated with increased incidence of disease and mortality (Lee and McDonald, 1985). Ozoga and Verme (1978) have proposed that the thymus gland could be a useful index of nutritional condition because diet mediates the normal pattern of thymic development in some species.

Address for correspondence: S.S. Ditchkoff, Department of Zoology, Oklahoma State University Stillwater, OK 74078, USA. E-mail: steved@okstate.edu

Our laboratory has been exploring the use of thymus gland size in cotton rats (*Sigmodon hispidus*) as an index to nutritional condition and stress-induced immunosuppression in wild populations. However, with the exception of nutrition (Lochmiller et al., 1993), little is known of how other environmental factors influence thymic development in this wild rodent species. We investigated environmental-induced dynamics of thymus development in free-ranging populations of cotton rats from central Oklahoma, with particular emphasis on documenting the seasonal rhythm in thymus size as influenced by age, gender, and captivity.

MATERIALS AND METHODS

Cotton rats ($n = 621$) were captured from a wild population inhabiting a tallgrass prairie ecosystem located 8 km west of Stillwater in central Oklahoma (36°6′ N, 97°12′ W). Cotton rats (referred to as free-ranging animals) were collected every 6–10 weeks from August 1989 to May 1992 using Sherman live-traps baited with rolled oats. Collected animals were returned to the laboratory for further analysis. Thymus development in free-ranging animals was compared to known-age cotton rats from our outbred laboratory colony ($n = 300$) which consisted of free-ranging adults that were subsequently housed for >6 weeks in the laboratory or first and second generation of laboratory-born offspring. Captive animals were maintained on a 14L:10D photoperiod at room temperature and fed a standard, 23% protein, commercial, rodent chow *ad libitum*. Animals were anesthetized with ketamine hydrochloride before measuring body mass to the nearest 0.1 g; mass of the thymus gland was determined to the nearest 0.1 mg after removing adhering fat tissue and blotting the tissue dry. Age-class designations for free-ranging animals were determined on the basis of body mass (Odum, 1955): adults (≥100 g), subadults (60–99 g), and juveniles (<60 g).

Differences in thymus gland mass due to captivity (captive versus free-ranging animals), age, and gender were tested using a 3-way analysis of variance (PROC GLM, SAS Institute, Inc., 1988). Seasonal changes in thymus gland mass of free-ranging animals were examined using a 3-way analysis of variance with gender, age class, and season as main effects. In the presence of significant ($P \leq 0.05$) interaction, we analyzed simple effects using contrasts and when appropriate, means were separated using Fisher's Least Squares Differences (LSD) procedure (Hicks, 1993). There was no significant difference in thymus gland mass ($P = 0.11$) among years so we pooled data across years prior to these analyses.

RESULTS AND DISCUSSION

Age class had a substantial influence ($P < 0.001$) on mass of the thymus gland, however, there was a significant ($P = 0.019$) treatment-gender-age interaction. Free-ranging cotton rats showed the typical age-related decline in mass of the thymus gland, but captive individuals did not show a clear decline in thymus mass with advancing age (Table 1). Captive juvenile males had thymus glands of lower mass ($P = 0.016$) than their free-ranging counterparts while captive adult males had greater thymus gland mass ($P < 0.001$) than free-ranging adult males. These results could be attributed to differences in reproductive status or different nutritional histories between captive and free-ranging animals. Development of this immune organ is thought to be largely independent of exposure to antigens (Morrison et al., 1986), so we do not believe the pathogen-rich environment of free-ranging animals contributed to the observed differences.

Mass of the thymus gland differed significantly across seasons of collection ($P < 0.001$), however, a significant ($P < 0.001$) season-age interaction existed (Fig. 1). Adults achieved greatest thymus mass in November ($x = 64.2$ mg; SE = 6.0) followed by a nadir in February–August. The decrease in thymus mass of adults during February-August was nearly 5-fold in males and 2-fold in females. We suggest that this spring decline in thymus mass is likely due to reproductive activity, where the breeding season of cotton rats extends from April to September in Oklahoma (Schetter, 1996), and increases in steroid hormone levels because the thymus possesses a high density of steroid receptors (Grossi and Lydyard, 1992). These negative steroidal effects on thymus mass likely negate any positive effects that nutritional intake may have on thymus mass as was noted

Table 1. Thymus mass (mg) of captive and free-ranging cotton rats (*Sigmodon hispidus*) from a population in central Oklahoma during August 1989 to May 1992.

		Age class								
		Juvenile			Subadult			Adult		
Source	Sex	*n*	$\overline{x}$	SE	*n*	$\overline{x}$	SE	*n*	$\overline{x}$	SE
Free-ranging	Female	70	46.6	2.4 A[a]a[b]	104	47.5	2.0 Aa	99	39.2	2.3 Ab
	Male	69	44.6	2.6 Aa	122	46.5	2.1 Aa	157	28.7	2.1 Bb
Captive	Female	33	38.4	3.9 ABab	35	48.1	3.5 Ab	93	38.6	2.5 Aa
	Male	24	31.4	3.2 Ba	26	45.6	3.8 Ab	89	43.8	2.9 Ab

[a]Mean values in a column with different upper case letters are different ($P < 0.05$) based on Fisher's Least Squares Differences procedure.
[b]Mean values in a row with different lower case letters are different ($P < 0.05$) based on Fisher's Least Squares Differences procedure.

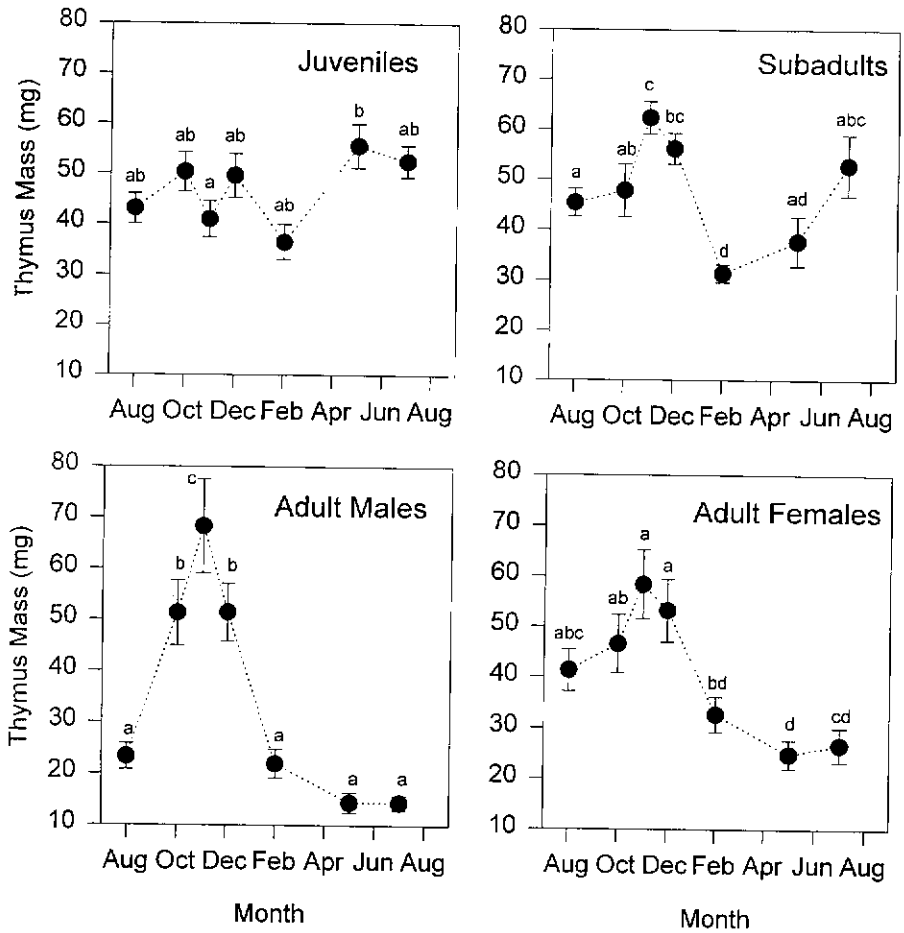

Fig. 1. Temporal changes in mass of thymus glands of free-ranging juvenile, subadult, and adult cotton rats trapped from a population in central Oklahoma from August 1989 to May 1992. Means with the same letter in the same figure are not ($P > 0.05$) different based on Fisher's Least Squares Differences procedure.

with juveniles. Seasonal rhythms where thymus mass is generally lowest during the peak of reproductive activity have been observed in several species (Sealander and Bickerstaff, 1967; Ozoga and Verme, 1978; Kendall and Twigg, 1981), and reductions in thymus mass of laboratory rats following administration of sex steroids has been reported by several authors (Dougherty, 1952; Weaver, 1955; Reilly et al., 1967; Miholjcic et al., 1990). The dramatic recovery of thymus mass in November following the breeding season is a phenomenon previously described for other species (Bacchus and Kendall, 1975) and can occur within a few weeks (Aronson, 1989). Subadults showed similar temporal trends in thymus mass with peak mass occurring in November (x = 62.3 mg; SE = 3.2) followed by a decline February–May; mass increased slightly during summer.

In contrast, the size of the thymus gland of juveniles did not vary greatly across seasons. The only difference that we observed for juveniles was that animals collected in May (x = 55.6 mg; SE = 4.4) had larger (P = 0.027) thymus glands than those collected in November (x = 41.0 mg; SE = 3.6). This was likely due to reproductive immaturity and greater nutritional stress during November compared to May. Davis et al. (1995) demonstrated that thymus mass is positively related to nutritional intake in cotton rats. Adult thymus mass of both males (x = 68.35; SE = 9.18) and females (x = 58.35; SE = 6.84) exceeded ($P < 0.05$) that of juveniles in November. However, during February–July, juvenile thymus mass was greater ($P < 0.05$) than both adult males and females. The remarkable differences in thymus mass of adults and juveniles in early spring is likely due to the combined effects of reproductive maturity of adults and age-related atrophy of the thymus (Kendall, 1981). Differences in thymus gland mass in fall and winter indicate that factors other than reproductive steroids also influence this immune gland. One plausible explanation is that it reflects a stress-induced response mediated by glucocorticoids (Dougherty, 1952; Money et al., 1952; Weaver, 1955; Miholjcic et al., 1990). Winter stress in small mammals can result in a variety of physiological changes and coincides with high over-winter mortality of adults (Mihok and Schwartz, 1989).

Among adult free-ranging animals, thymus gland mass of males was 26% less than that of females ($P < 0.001$). Differences associated with gender among free-ranging cotton rats could be a reflection of hormonal status (Thompson, 1981). Several studies have demonstrated that androgenic steroids cause greater thymic involution than oestrogenic steroids (Money et al., 1952; Weaver, 1955). Gender differences ($P > 0.05$) were not found with captive individuals or free-ranging juveniles or subadults.

This study confirms the idea that although there is a general age-related decline in thymus mass, many other factors mediate the normal seasonal rhythm of thymus development. Controlling variables such as reproductive status and age are capable of influencing the normal seasonal rhythm of thymus gland size. Knowledge of this type will improve the sensitivity of this gland as an index of animal condition for use in the field.

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REFERENCES

ANDERSON, D.H. (1932): The relationships between the thymus and reproduction. Physiol. Rev. 12: 1–22.

ARONSON, M. (1989): Reversible thymic collapse following acute stress is beneficial: a hypothesis. Thymus 14: 61–63.

BACCHUS, S., and KENDALL, M.D. (1975): Histological changes associated with enlargement and regression of the thymus glands of the red-billed quelea, *Quelea quelea* L. (Placeidae: weaver birds), Proc. Trans. Roy. Soc. London B 273: 65–78.

CHANDRA, R.K. and NEWBERNE, P.M. (1977): Nutrition, immunity, and infection. Plenum Press, New York, 246 pp.

CHRISTIAN, J.J. (1956): Adrenal and reproductive responses to population size in mice from freely growing populations. Ecol. 37: 258–273.

CLARKE, J.R. (1953): The effect of fighting on the adrenals, thymus and spleen of the vole (*Microtus agrestis*). J. Endocrinol. 9: 114–126.

COCKBURN, A. (1992): Evolutionary ecology of the immune system: why does the thymus involute? Functional Ecol. 6: 364–370.

DAVIS, R.L., LOCHMILLER, R.L. and WARDE, W.D. (1995): Splenocyte subpopulations of weanling cotton rats (*Sigmodon hispidus*) are influenced by moderate protein intake. J. Mammal. 76: 912–924.

DOUGHERTY, T.F. (1952): Effect of hormones on lymphatic tissue. Physiol. Rev. 32: 379–401.

GROSSI, C.E. and LYDYARD, P.M. (1992): Thymus. In: Encyclopedia of immunology. I.M. Roitt and P.S. Delves (eds.). Academic Press, New York, pp. 1451–1453.

HICKS, C.R. (1993): Fundamental concepts in the design of experiments. Fourth ed. Saunders College Publishing, New York, 509 pp.

KENDALL, M.D. (1981): Age and seasonal changes in the thymus. In: The thymus gland: symposium 1 of the Anatomical Society of Great Britain and Ireland. M.D. Kendall (ed.). Academic Press, London, pp. 21–25.

KENDALL, M.D. and TWIGG, G.I. (1981): The weight of the thymus gland in a population of wild bank voles, *Clethrionomys glareolus*, from Wicken Fen, Cambridgeshire. J. Zool. 194: 323–339.

LEE, A.K. and MCDONALD, I.R. (1985): Stress and population regulation in small mammals. In: Oxford reviews of reproductive biology. J.R. Clarke (ed.). Oxford Univervsity Press, New York. 261–304

LOCHMILLER, R.L., VESTEY, M.R. and MCMURRY, S.T. (1993): Development of altered immunity in adult cotton rats (*Sigmodon hispidus*) during moderate dietary restriction. Comp. Biochem. Physiol. 104: 593–599.

MIHOK, S. and SCHWARTZ, B. (1989): Anemia at the onset of winter in the meadow vole (Microtus pennsylvanicus). Comp. Biochem. Physiol. 94A: 289–304.

MIHOLJCIC, B., RADIC, L.J. and ALECKOVIC, Z. (1990): Stereological analysis of rat thymus after treatment with different steroids. Acta Vet. 40: 269–278.

MILLER, J.F. (1990): The thymus and its role in immunity. Chem. Immunol. 49: 51–68.

MONEY, W.L., FAGER, J. and RAWSON, R.W. (1952): The comparative effects of various steroids on lymphoid tissue of the rat. Cancer Res. 12: 206–210.

MORRISON, W.I., LALOR, P.A., CHRISTENSEN, A.K. and WEBSTER, P. (1986): Cellular constituents and structural organization of the bovine thymus and lymph node. In: The ruminant immune system in health and disease. W.I. Morrison (ed.). Cambridge Univ. Press, New York, pp. 220–251.

MYERS, K., HALE, C.S., MYKYTOWYCZ, R. and HUGHES, R.L. (1971): The effects of varying density and space on sociality and health in animals. In: Behavior and environment. A.H. Esser (ed.). Plenum Press, New York, pp. 148–184

ODUM, E.P. (1955): An eleven year history of a *Sigmodon* population. J. Mammal. 36: 368–378.

OZOGA, J.J. and VERME, L.J. (1978): The thymus gland as a nutritional status indicator in deer. J. Wildl. Managem. 42: 791–798.

REILLY, R.W., THOMPSON, J.S., BIELSKI, R.K. and SEVERSON, C.D. (1967): Estradiol-induced wasting syndrome in neonatal mice. J. Immunol. 98: 321–330.

SAS INSTITUTE, INC. (1988): SAS/Stat user's Guide; release 6.03 edition. Statistics. SAS Institute, Inc., Cary, North Carolina, 1028 pp.

SCHETTER, T. (1996): Examination of the nitrogen limitation hypothesis in populations of cotton rats (*Sigmodon hispidus*). M.S. Thesis, Oklahoma State Univ., 95 pp.

SEALANDER, J.A. and BICKERSTAFF, L.K. (1967): Seasonal changes in reticulocyte number and in relative weights of the spleen, thymus, and kidneys in the northern red-backed mouse. Can. J. Zool. 45: 253–260.

THOMPSON, E.A. (1981): The effects of estradiol upon the thymus of the sexually immature female mouse. J. Steroid Biochem. 14: 167–174.

TWIGG, G. I. and HARRIS, S. (1982): Seasonal and age changes in the thymus gland of the red fox, *Vulpes vulpes*. J. Zool. 196: 355–370.

WEAVER, J.A. (1955): Changes induced in the thymus and lymph nodes of the rat by the administration of cortisone and sex hormones and by other procedures. J. Pathol. Bacteriol. 69: 133–139.