Neonatal Thymectomy Increases the Incidence of Spontaneous and Methylcholanthrene-Enhanced Thyroiditis in Rats

Abstract. *At 16 weeks of age, 13 percent of untreated Buffalo strain rats showed evidence of autoimmune thyroiditis. Feeding methylcholanthrene increased the incidence to 42 percent. Neonatal thymectomy significantly raised the incidence of disease so that almost all (87 percent) untreated and all methylcholanthrene-treated animals developed severe disease. It is proposed that the thymus exerts a regulatory effect on autosensitization.*

Buffalo strain rats develop autoimmune thyroiditis spontaneously, but the incidence of disease is significantly increased following the administration of 3-methylcholanthrene (MC), a carcinogenic and immunosuppressive agent (*1*). In the course of investigating how MC enhanced the incidence of thyroiditis, neonatal and adult thymectomies were performed on Buffalo strain rats. Neonatal thymectomy was followed by a highly significant increase in the incidence of both spontaneous and MC-enhanced thyroiditis.

Buffalo strain rats were obtained from Simonsen Laboratories, Gilroy, California. Neonatal thymectomies were performed on rats that were always less than 24 hours old and were usually less than 12 hours old. Sham neonatal thymectomy included manipulation of the thymus. Adult thymectomies were performed on 21-day-old rats. We added MC to the diet of treated rats at a concentration of 0.033 percent. Treatment with MC was started when the rats to be treated were 4 weeks old, at which time they were allowed free access to the MC-containing diet. All rats were killed and autopsied at 16 weeks of age. The presence and severity of thyroiditis were determined by histological examination of the thyroid (*2*). The results are given in Table 1.

Treatment with MC increased the incidence of thyroiditis in unoperated Buffalo strain rats ($2\alpha < .0025$, two-tailed chi-square test). Adult thymectomy did not influence the incidence of spontaneous or MC-enhanced thyroiditis. Neonatal thymectomy was followed by a highly significant increase in the incidence of both spontaneous thyroiditis ($2\alpha < .001$) and MC-enhanced thyroiditis ($2\alpha < .001$). The incidence in each neonatally thyroidectomized group also differed significantly from that in the corresponding adult thymectomized group ($2\alpha < .001$).

Rats found at autopsy to have thymus remnants after neonatal thymectomy were designated as partially thymectomized. The extent to which partial neonatal thymectomy was followed by an increased incidence of MC-enhanced and spontaneous thyroiditis seemed to be related to the amount of thymus tissue that could be recovered. Of six partially neonatally thymectomized rats that were not treated with MC and that had less than 65 mg of thymus tissue, three had thyroiditis, whereas none of five with more than 65 mg of thymus tissue had thyroiditis. Of the partially neonatally thymectomized rats that had been treated with MC, four of four with less than 65 mg of thymus tissue had thyroiditis and two of four with more than 65 mg of thymus remnants had thyroiditis. Sham neonatal thymectomy was without effect.

Even though neonatal thymectomy increased the incidence of thyroiditis, it did not greatly affect the severity of disease. When the severity of disease in untreated rats with thyroiditis was compared with that in neonatally thymectomized rats no significant difference was found ($2P > .05$, two-tailed Student's t-test). Likewise, no difference was found when MC-treated rats were compared with rats that were neonatally thymectomized and treated with MC ($2P > .05$). There was also no difference in the severity of thyroiditis between rats that had been neonatally thymectomized and treated with MC and rats that had been neonatally thymectomized only ($2P > .05$). Since the incidence but not the severity of thyroiditis is affected by neonatal thymectomy, the thymus may influence an initial step of autosensitization but not the subsequent development of disease.

Recently, interest in thymus-influenced (T) lymphocytes has expanded to include controlling in addition to helper functions in immunological responsiveness. Gershon *et al.* (*3*) suggested that thymocytes could suppress the response of other thymocytes to antigen. Thymocytes can reduce the immune response to thymus-independent antigens (*4*), the nonspecific mitotic response of non-thymus-influenced (B) cells to lipopolysaccharide endotoxin (*5*), and the plaque-forming B cell response (*6*). Evidence for the inhibitory effect on antibody formation of thymus-derived cells has also been presented by Haskill and Axelrad (*7*). Eidinger and Pross showed that cortisone-resistant thymus cells could suppress antibody formation by spleen cells in vivo and in vitro (*8*). Thymus-derived cells may exert specific feedback control of autoantibody formation (*9*). It is therefore plausible that the thymus is intimately involved in suppression of autosensitization. In the obese strain of chicken, neonatal thymectomy is followed by an increased frequency of thyroiditis (*10*).

Neonatal thymectomy enhances thyroiditis in Buffalo strain rats. We propose that a restraining effect is normally exerted by the thymus on the development of autoimmune thyroiditis, that this thymic regulatory effect is removed by neonatal thymectomy but not by adult thymectomy, and that it is imperfectly expressed in the intact Buffalo strain rat as evidenced by the eventual occurrence of spontaneous autoimmune thyroiditis in 13 percent of 16-week-old animals. It has recently been reported that older Buffalo strain rats frequently develop thymomas simi-

Table 1. Effect of neonatal and adult thymectomy in spontaneous and methylcholanthrene-enhanced thyroiditis in Buffalo strain rats.

Treatment	Number with thyroiditis/ total	Incidence (%)	Mean severity in positive rats (*2*)
None	3/23	13	0.25
Methylcholanthrene	13/31	42	2.0
Adult thymectomy	1/11	9	0.5
Adult thymectomy + methylcholanthrene	3/8	37	1.5
Neonatal thymectomy	13/15	87	1.5
Neonatal thymectomy + methylcholanthrene	14/14	100	1.5
Partial neonatal thymectomy	3/11	27	2.0
Partial neonatal thymectomy + methylcholanthrene	6/8	75	1.5
Sham neonatal thymectomy	0/5	0	0

lar in histology to human thymomas, which are often associated with various autoimmune disorders (*11*). One action of MC may be to interfere with the thymic regulatory effect.

Of equal interest is the rising occurrence of autoimmune thyroiditis in the Buffalo strain rat with increasing age. It suggests diminishing thymic control during aging. Many human autoimmune disorders such as chronic thyroiditis seem to increase with advancing age. On the other hand, immunological potential generally is thought to decline with time. In a separate study, MC was found to depress the delayed hypersensitivity response to Bacillus Calmette-Guérin in Buffalo strain rats, although it did not significantly affect the immunoglobulin M or immunoglobulin G antibody response to sheep red blood cells. It would be useful to titrate thymus-dependent and thymus-independent immune reactivities during aging.

Another animal in which autoimmune disease occurs frequently, NZB mice, has also been reported to show an increased evidence of disease following neonatal thymectomy (*12*). However, the major autoimmune disorders of NZB mice, such as hemolytic anemia and immune complex renal disease, are the types most frequently associated with the products of B cells, that is, antibodies. In the rat, autoimmune thyroiditis has been transferred between histocompatible animals by viable lymph node cells but not by serum (*13*). It must presently be associated, therefore, with the cell-mediated immunological responses. The results presented in this report may require a reassessment of the roles of T and B cells in autoimmune tissue damage.

DAVID A. SILVERMAN*
NOEL R. ROSE†
Center for Immunology and
Department of Microbiology,
State University of New York at Buffalo, Buffalo 14214

References and Notes

1. D. A. Silverman and N. R. Rose, *Proc. Soc. Exp. Biol. Med.* **138**, 579 (1971).
2. The thyroid glands were scored as follows, in respect to percent infiltration: score 0.5, 10 to 19 percent; 1.0, 20 to 29 percent; 1.5, 30 to 39 percent; 2.0, 40 to 49 percent; 2.5, 50 to 59 percent; 3.0, 60 to 69 percent; 3.5, 70 to 79 percent; 4.0, 80 to 89 percent; 4.5, 90 to 99 percent; 5.0, 100 percent.
3. R. K. Gershon, P. Cohen, R. Hencin, S. A. Liebhaber, *J. Immunol.* **108**, 586 (1972).
4. P. J. Baker, P. W. Stashak, D. F. Amsbaugh, B. Prescott, R. F. Barth, *ibid.* **105**, 1581 (1970).
5. M. Yoshinaga, A. Yoshinaga, B. H. Waksman, *J. Exp. Med.* **136**, 956 (1972).
6. R. R. Rich and C. W. Pierce, *ibid.* **137**, 649 (1973).
7. J. S. Haskill and M. A. Axelrad, *Nat. New Biol.* **237**, 251 (1972).
8. D. Eidinger and H. F. Pross, *Scand. J. Immunol.* **1**, 193 (1972).
9. A. C. Allison, A. M. Denman, R. D. Barnes, *Lancet* **1971-II**, 135 (1971).
10. G. Wick, J. H. Kite, E. Witebsky, *J. Immunol.* **104**, 54 (1970); P. Welch, N. R. Rose, J. H. Kite, *ibid.* **110**, 575 (1973).
11. S. Yamada, K. Masuko, M. Ito, T. Nagayo, *Gann Jap. J. Cancer Res.* **64**, 287 (1973).
12. J. B. Howie and B. J. Helyer, *Adv. Immunol.* **9**, 215 (1968).
13. F. J. Twarog and N. R. Rose, *J. Immunol.* **104**, 1467 (1970); N. R. Rose, M.-F. Molotchnikoff, F. J. Twarog, *Immunology* **24**, 859 (1973).
14. Presented in part at the annual meeting of the American Society for Experimental Pathology in Atlantic City, New Jersey, April 1973. Supported by NIH research grant CA 02357 and NIH training grant 1 TO1 Al 11442. This is publication No. 76 from the Center for Immunology.
* Present address: Department of Tumor Biology, Karolinska Institute, Stockholm 60, Sweden.
† Present address: Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, Michigan 48201.
21 November 1973

Pineal Gland: 24-Hour Rhythm in Norepinephrine Turnover

Abstract. *There is a 24-hour rhythm in the turnover of norepinephrine in sympathetic nerves innervating the pineal gland. This rhythm persists in blinded animals but is suppressed in normal rats by light. The rhythm in norepinephrine turnover generates the rhythms in pineal indoleamines and* N*-acetyltransferase.*

There are 24-hour cycles in the concentrations of serotonin (*1*), *N*-acetylserotonin (NAS) (*2*), and melatonin (*3*) in the pineal gland of the rat. The levels of NAS, the immediate precursor of melatonin, and of melatonin itself are higher at night than during the day, while the level of serotonin is higher during the day than at night. The reciprocal relation between the levels of serotonin and NAS is the consequence of a 24-hour cycle in the activity of serotonin *N*-acetyltransferase (NAT) (*4*), the enzyme that converts serotonin to NAS. At night, when the activity of this enzyme is 25 to 60 times higher than it is by day, serotonin is rapidly converted to NAS; thus, the former falls and the latter increases (*5, 6*). The cycles in NAT and serotonin are endogenous in that they persist in animals placed in constant darkness and in blinded animals (*4, 7*), although the phase of the cycles gradually shifts in such animals (*8*).

Sympathetic (norepinephrine-containing) nerves that arise in the superior cervical ganglia provide the major, if not the sole, source of innervation to the pineal gland (*9*). Denervation of the pineal gland by removal of the superior cervical ganglia abolishes the serotonin (*10*) and NAT (*11*) rhythms. Physiological or pharmacological stimulation (*12–14*) of the pineal β-adrenergic receptors during the day markedly increases the level of NAT. Conversely, the nighttime rise of NAT and NAS and the fall of serotonin are prevented or reversed by the administration of the β-adrenergic receptor blocking agent, propranolol (*5, 13*). These observations indicate that norepinephrine released from the sympathetic nerve terminals regulates circadian rhythms of indoleamines by stimulating β-adrenergic receptors on pineal cells. A 24-hour rhythm in norepinephrine in the rat pineal has been reported (*15*). Paradoxically, the norepinephrine rhythm in the pineal was not endogenous since it was abolished by blinding rats whereas the serotonin and NAT cycles were not (*16*). To provide a link between release of the sympathetic neurotransmitter and circadian rhythms, we studied norepinephrine turnover in the pineal gland at night and during the day in normal and blinded rats.

Osborne-Mendel rats (NIH strain) weighing 180 to 200 g were housed under diurnal lighting conditions (lights on from 6 a.m. to 6 p.m. and off from

Table 1. Daily rhythm in norepinephrine turnover in the rat pineal in normal and blinded animals. Half-lives ($t_{1/2}$) were calculated by multiplying the slope of the regression line by $\log_{10} 2$. The rates of flux were obtained by multiplying the slopes of the regression line by the steady-state level of norepinephrine. Data for norepinephrine content are means ± S.E.M.

Group	$t_{1/2}$ (min)	Norepinephrine per pineal (pg)	Norepinephrine efflux (pg/min)
Day	111	2450 ± 610	15.3
Day, blinded animals	117*		
Night	43	2660 ± 270	42.9
Night (lights on)	115		
Night, blinded animals (lights on)	43		

* This $t_{1/2}$ value in blinded animals during the day agrees well with the value of 113 minutes determined by measuring the rate of fall of norepinephrine after treatment with α-methyl-*p*-tyrosine (*22*).