20. Chick lenses were fixed overnight in Carnoy's fixative at 4°C, embedded in paraffin, and sectioned. Sections on glass slides were rehydrated and placed in 0.1*M*, 1,4-piperazinediethanesulfonic acid (Pipes) buffer, *p*H 7.3, containing either 1 m*M* Ca^{2+} or 1 m*M* EGTA for 10 minutes, and then incubated in CaM-RITC (5 μ*M*) for 1 hour at 37°C in buffer containing Ca^{2+} or EGTA; they were then rinsed and mounted in Elvanol. Incubation with nonimmune serum or antiserum (5 mg per milliliter of protein), prior to incubation with CaM-RITC, was performed at 37°C for 1 hour, with the sections being rinsed several times afterwards in Pipes buffer. Sections were viewed and photographed by means of a Leitz Orthoplan microscope equipped for rhodamine epi-illumination fluorescence microscopy. Photographs were made on Kodak Tri-X pan film.
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31. Supported by American Cancer Society institutional grant IN-40T and NIH grants S07RR05383 and EY01855. We thank G. Brewer for facilities used in calmodulin iodination, M. Gnegy for facilities used in calmodulin-rhodamine activity assays (phosphodiesterase activation assays), and M. Johnson and G. Treisman for conducting the activation assays.

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Neonatal Thymectomy Prevents Spontaneous Diabetes Mellitus in the BB/W Rat

Abstract. *Complete neonatal thymectomy reduced the frequency of spontaneous diabetes mellitus in BioBreeding/Worcester rats from 27 to 3 percent. Incomplete thymectomy also significantly reduced the frequency of diabetes (to 9 percent). These findings strengthen the hypothesis that thymus-dependent, cell-mediated autoimmune destruction of pancreatic β cells is responsible for the pathogenesis of diabetes in this experimental animal.*

An acute diabetic syndrome resembling insulin-dependent type 1 diabetes in humans occurs spontaneously in approximately 30 percent of a colony of BioBreeding/Worcester (BB/W) rats. Salient features of the syndrome include genetic predisposition (*1*); abrupt onset of insulin-dependent, ketosis-prone diabetes between 60 and 120 days of age; lymphocytic insulitis with virtually complete destruction of insulin-synthesizing pancreatic β cells (*2*); and occurrence of the syndrome in animals raised in a gnotobiotic environment (*3*).

It has been hypothesized that the BB/W diabetic syndrome is the result of a cell-mediated, autoimmune destruction of pancreatic β cells (*4*). Support for an immune pathogenesis stems from the predominantly lymphocytic nature of the insular infiltrate and the observation that injections of rabbit antiserum to rat lymphocytes frequently normalize plasma glucose levels in acutely diabetic rats and prevent hyperglycemia in susceptible littermates (*4*). Another characteristic of BB/W rats which resembles certain human type I diabetics and lends support to the syndrome's immune pathogenesis is the presence of lymphocytic thyroiditis in approximately 10 to 25 percent of nondiabetic animals and 50 to 60 percent of diabetic animals (*5*).

In this report we present evidence that complete neonatal thymectomy prevents the occurrence of diabetes in virtually all susceptible animals. Inadvertent partial thymectomy also provides significant protection. In contrast, sham-operated rats become diabetic with the expected frequency. The effectiveness of thymectomy in preventing diabetes supports the hypothesis that a thymus-dependent, cell-mediated immune destruction of pancreatic β cells is responsible for diabetes in the BB/W rat (*6*).

We subjected BB/W rats (*7*) to thymectomy or sham surgery within 24 hours of birth (*8*). Surviving animals were tested for glycosuria three times weekly from 60 to 150 days of age. Rats were defined as diabetic if their urine glucose was shown to be 2+ or greater with Testape (Lilly) and if their plasma glucose concentration exceeded 200 mg/dl (*9*). Diabetic animals were killed immediately and nondiabetic rats were killed at 150 days of age. At autopsy, the thoraxes of thymectomized rats were explored for evidence of thymic remnants (*10*). Among sham-operated animals, the presence of an intact thymus was verified by gross or microscopic examination or both. Pancreases were fixed in Bouin's solution, sectioned, stained with hematoxylin and eosin, and examined for evidence of insulitis. Thymus and pancreas slides were studied (by A.A.L.) without knowledge of the operative procedure performed and the physiological status of the animal. The frequencies of diabetes and insulitis among groups were evaluated by chi-square analysis.

Two groups of rats were subjected to the experimental procedures. The first group was studied in the summer of 1980 when the incidence of diabetes in the colony was approximately 20 percent. The second group was studied from January to June 1981 when the frequency of diabetes had reached approximately 40 percent (*11*). The data for both experimental groups are combined for the sake of brevity. This does not alter the results or the statistical evaluation of the data.

Complete thymectomy proved difficult to achieve. It was verified microscopically in 63 of 296 (21 percent) of the animals in which it was attempted (Fig. 1).

As shown in Table 1, 27 percent of the sham-operated rats became diabetic while only 3 percent of the completely thymectomized and 9 percent of the incompletely thymectomized animals evidenced diabetes. The severity of diabetes and the age of onset, however, were similar among the three groups. Insulitis with normoglycemia was present in 15 percent of the sham-operated rats, 11 percent of the completely thymectomized rats, and 20 percent of the incompletely thymectomized animals. Although these results are not significantly different, the incidence of combined diabetes and normoglycemic insulitis was significantly greater among sham-operated rats than among thymectomized animals (Table 1).

Table 1. Incidence of diabetes and insulitis in BB/W rats given complete thymectomy, incomplete thymectomy, or sham surgery as neonates. Numbers in parentheses are percentages.

Treatment	Number of rats showing disease		
	Diabetes	Insulitis with normoglycemia	Diabetes and insulitis
Sham surgery	39 of 144 (27)	16 of 105 (15)	55 of 144 (38)
Complete thymectomy	2 of 63 (3)*	7 of 61 (11)	9 of 63 (14)†
Incomplete thymectomy	21 of 233 (9)*	42 of 212 (20)	63 of 233 (27)‡

*Significantly different from corresponding control value at $P < .0005$ (chi-square test). †Significantly different from control value at $P < .001$ and from incomplete thymectomy value at $P < .05$. ‡Significantly different from control value at $P < .025$.

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The occurrence of insulitis and diabetes in rats without microscopically identifiable thymic tissue might be explained by the presence of ectopic (*12*) or residual thymic tissue overlooked at the time of initial surgery and at autopsy. Alternatively, immunologically competent cells may have left the thymus prior to thymectomy.

The observation that partial thymectomy also protects against diabetes may be without precedent. Although data concerning the effects of incomplete neonatal thymectomy are not readily gleaned from the early thymectomy literature, Martinez *et al.* (*13*) reported that mice subjected as neonates to 90 to 95 percent thymectomy did not accept skin allografts across the H-2 histocompatibility barrier, while complete thymectomy prevented rejection in almost all the animals. In another study, the presence of residual thymic tissue in mice afforded, at best, partial protection against successful transplantation of polyoma virus–induced tumors (*14*).

It is well known that neonatal thymectomy induces profound lymphopenia in rats and mice and is responsible for impaired cellular immune responses such as experimental allergic encephalitis, tuberculin skin reactions, and rejection of skin allografts (*15*). Antiserum to rat lymphocytes also reduces the number of peripheral blood lymphocytes and impairs or abolishes the same cell-mediated immune responses (*15*). We previously reported that rabbit antiserum to rat lymphocytes protects against diabetes in the BB/W rat (*4*). It is, therefore, reasonable to expect that partial as well as complete thymectomy would induce a state of relative immunologic incompetence in which the mass of effector lymphocytes is reduced below a certain threshold level of effectiveness. Under these circumstances, the susceptible BB/W rat may be unable to consummate or even initiate the destruction of pancreatic β-cells necessary for the induction of diabetes. The present results, therefore, are not consistent with the widely held assumption that incomplete neonatal thymectomy affords no protection in immunologic processes of the cell-mediated or delayed type.

Functional assays (mixed lymphocyte culture or mitogen-induced T cell replication) of surviving T cells were not employed to evaluate the completeness of thymectomy. In retrospect, measurements of this type might have been useful because partial thymectomy protected against diabetes. It is not certain, however, whether available in vivo or in vitro functional assays are sufficiently

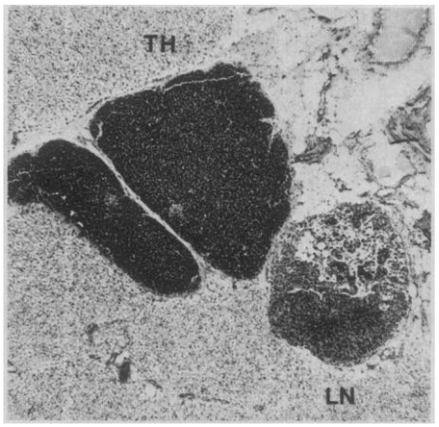

Fig. 1. Photomicrograph of a very small thymic remnant (*TH*) embedded in mediastinal adipose tissue (×35). The compact aggregate of thymic lymphocytes is adjacent to a small lymph node (*LN*). The remnant was not macroscopically recognizable (stain: hematoxylin and eosin).

sensitive to detect the low level of T cell competence that might result from the presence of microscopic thymic remnants. In preliminary studies, peripheral blood lymphocytes from sham-operated BB/W rats were compared with lymphocytes from athymic nude rats and completely or incompletely thymectomized BB/W animals. Monoclonal antibodies (Pel-Freeze Biologicals) were used to enumerate total T cells (W3/13), nonhelper T cells (OX8), and helper T cells (W3/25) (*16*). Only cells reacting with W3/25 were consistently depressed in nude rats and in most completely thymectomized BB/W rats. Many BB/W rats with microscopic thymic remnants also evidenced a marked reduction in the number of W3/25-labeled lymphocytes.

The observation that the predisposition of BB rats to diabetes may be linked to the major histocompatibility complex (*17, 18*) and the report that neonatal BB rats inoculated with Wistar-Furth bone marrow evidence a decreased incidence of diabetes (*19*) provide additional and persuasive support for an immunologic pathogenesis of the syndrome. The recent reports of generalized lymphopenia among diabetic and diabetes-susceptible rats (*20, 21*) and of reduced numbers of helper T cells (*22*) are enigmatic and difficult to reconcile with the protection afforded by neonatal thymectomy and injections of antiserum to rat lymphocytes (*4*). Detailed studies—including selective depletion and cell-transfer studies—of the known T cell subsets, other lymphocyte groups, and macrophages are required to clarify the pathogenic significance of lymphopenia in these animals.

The report of lymphocytic thyroiditis among diabetic and nondiabetic BB/W rats (*5*) and the frequent presence of autoantibodies to smooth muscle, thyroid colloid, and other tissue components (*22, 23*) are also consistent with an autoimmune pathogenesis of the BB/W syndrome. These data suggest that more than one cell type or antigenic determinant are under immunologic attack and that the predisposing defect in these animals is more likely to be an abnormal immune response than an antigenically altered target cell or tissue.

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8. Newborn pups were cooled on ice for anesthesia. The skin and sternal notch were incised and the thymus was removed by gentle suction. Sham-operated animals were subjected to the same operative procedure but the thymus was not removed.
9. Samples of tail blood were collected in heparin-treated pipettes and assayed for plasma glucose as described by A. A. Rossini, M. Berger, J. Shadden, and G. F. Cahill, Jr. [*Science* **183**, 424 (1974)].
10. Mediastinal fat, lymph nodes, and great vessels were removed en bloc, fixed in Bouin's solution, and embedded in paraffin for sectioning at 15 to 20 levels (200-μm intervals). At each 200-μm interval, three to five sections were stained with hematoxylin and eosin and studied microscopically to detect fragments of thymic tissue.
11. Because of a pneumonia epidemic in the BB/W colony in early 1980, a large number of animals were mated and the pups were removed from the dams by cesarean section. Since morbidity was more frequent among diabetic rats, litters from phenotypically normal mating pairs were more frequently cesarean-derived. The incidence of diabetes, therefore, was only 20 percent among the first experimental animals. In the absence of pneumonia, selective breeding for the diabetes phenotype was again possible and the incidence of diabetes in the second experimental group was 40 percent.
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High Angiotensin-Converting Enzyme Activity in the Neurohypophysis of Brattleboro Rats

Abstract. *The activity of angiotensin-converting enzyme is significantly higher in the intermediate and posterior pituitary lobes of Brattleboro rats than in Long-Evans control rats. The high activity level was reversed by vasopressin treatment. Conversely, angiotensin-converting enzyme activity was significantly lower in the anterior pituitary of Brattleboro rats than in Long-Evans rats, and this activity level was not affected by vasopressin. These findings suggest an inverse relation between vasopressin and angiotensin systems in the posterior and intermediate lobes of the pituitary gland.*

Two hormones, angiotensin II and vasopressin, regulate water balance in mammals (*1*). Angiotensin II increases fluid intake, whereas vasopressin is antidiuretic. An interaction between the renin-angiotensin and vasopressin systems has been established (*2*). After its synthesis and transport in the hypothalamo-neurohypophyseal system and its release into the general circulation, vasopressin is able to control the release of renin from the kidney (*3*). Angiotensin and vasopressin have also been postulated to interact at the neurohypophyseal level. Administration of angiotensin II increases the release of vasopressin from the neurohypophysis (*4*). The localization of the precursor of angiotensin II, angiotensin I (*5*), and the existence of a highly active angiotensin-converting enzyme (ACE) (E.C. 3.4.15.1) in the neurohypophysis (*6*) suggest an interaction between renin-angiotensin and vasopressin systems in this organ.

The interaction can be advantageously studied in homozygous Brattleboro rats (*7*). In these animals, diabetes insipidus is associated with high plasma renin activity and angiotensin II concentration (*8*) without changes in renin substrate (*9*). The high plasma renin activity can be corrected by treatment with vasopressin (*10*), but vasopressin is ineffective against the high isorenin activity in the adrenal gland and hypothalamus of these rats (*10*).

Homozygous Brattleboro rats lack angiotensin II binding sites in the neurohypophysis (*11*). To determine whether there are other abnormalities in the renin-angiotensin system in the neurohypophysis of Brattleboro rats and whether these changes can be influenced by vasopressin, we studied ACE activity in the posterior, intermediate, and anterior pituitary lobes of Long-Evans rats and age-matched, heterozygous and homozygous male Brattleboro rats (*12*).

The animals were decapitated between 9:00 and 11:00 a.m. and blood samples were taken from the trunks, poured into ice-chilled tubes containing heparin, and centrifuged. A piece of lung was removed and the pituitary glands were separated into anterior, posterior, and intermediate lobes under a dissecting microscope. These tissues were homogenized in cold 0.1*M* tris buffer (*p*H 7.4) containing 1 m*M* parachloromercuriphenylsulfonic acid, and portions of the homogenate were analyzed for protein content (*13*) and ACE activity (*14*). ACE activity was also measured in duplicate 10-μl samples of plasma.

There were significant differences between the Brattleboro and Long-Evans rats in ACE activity in the three lobes of the pituitary gland. ACE activity in the

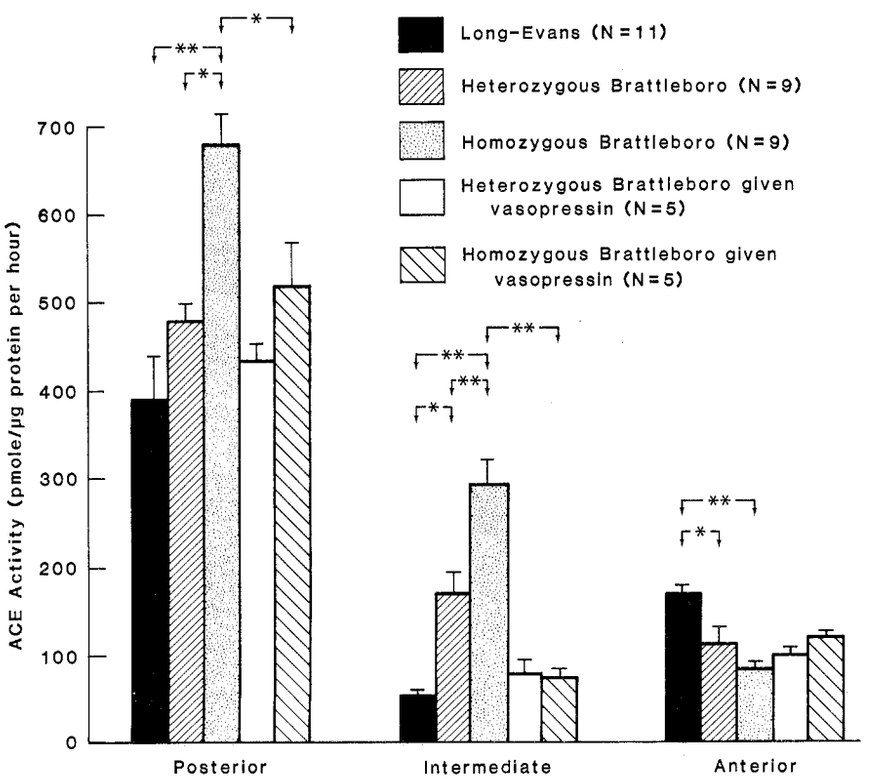

Fig. 1. Angiotensin-converting enzyme activity in the three lobes of pituitary glands from Long-Evans, heterozygous Brattleboro, and homozygous Brattleboro rats and the effect of vasopressin. The data are means ± standard errors. Single asterisks indicate significant differences at $P < .05$; double asterisks indicate $P < .01$ (analysis of variance followed by multiple comparisons among individual means).

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