Spontaneous Hyperglycemia and Impaired Glucose Tolerance in Athymic Nude BALB/c Mice

ADINA ZEIDLER, CLARA TOSCO, DINESH KUMAR, BERNARD SLAVIN, AND JOHN PARKER

SUMMARY

Basal plasma glucose, glucose tolerance, and insulin secretion were investigated in young and mature athymic nude BALB/c mice and in age-matched controls. Basal plasma glucose levels in male athymic nude mice were similar to those of controls at 1, 3, and 4 wk of age. At 6, 8, and 12 wk of age, male athymic nudes had significantly higher basal plasma glucose levels when compared with controls ($P < 0.01$). Plasma immunoreactive insulin concentrations were similar in athymic nudes and controls at 1 wk of age, but at 3 wk of age and subsequently at 6, 8, and 12 wk athymic nude mice had significantly decreased insulin levels when compared with their age-matched controls ($P < 0.05$).

We found impaired glucose tolerance in male athymic nude mice at all age groups when compared with both female athymic nudes and control BALB/c mice.

The discovery of a spontaneous diabetic syndrome (hyperglycemia, impaired glucose tolerance, and decreased insulin secretion) in a colony of athymic nude mice may provide an excellent model for studying the genetics and interactions between the immune and endocrine systems. DIABETES *31*:821–825, September 1982.

Congenetically athymic nude mice provide a useful model for studies of the immune system and are used as recipients for transplanted allogeneic and xenogeneic tissues.[1] Studies of the endocrine system in athymic mice have demonstrated abnormal spermatogenesis, lack of corpora lutea in the gonads,[2] hypertrophy of the x-zone in the adrenals,[3] abnormal thyroid function,[3] and an abnormal hypothalamic-pituitary-gonadal axis.[4] Restoration of gonadal function has been accomplished by thymic transplantation,[5,6] indicating that the thymus may play a role in the hypothalamic-pituitary-gonadal axis.[4,7]

The role of the immune system in the pathogenesis of diabetes has been studied using athymic nude mice. Buschard and Rygaard have demonstrated that hyperglycemia could be induced with streptozotocin in euthymic heterozygous (+/nu) mice but not in athymic nude (nu/nu) mice.[8] In addition, nude mice injected with spleen cells from streptozotocin-induced diabetic mice developed clinical evidence of diabetes.[9] Studies using encephalomyocarditis virus,[10] which is known to induce diabetes in susceptible mice, show that it could induce hyperglycemia in heterozygous (+/nu) mice but not in nude (nu/nu) mice. The report by Buschard et al.[11] that peripheral lymphocytes from newly diagnosed insulin-dependent diabetic patients induced hyperglycemia in nude mice was interesting; however, these observations indicating the possibility that hyperglycemia in mice is mediated by autoreactive immune lymphocytes were not confirmed in other laboratories.[12–15]

Previous studies by Paik et al.,[16] investigating the role of thymic function in the development of insulin-dependent diabetes, have indicated that the induction of insulin-dependent diabetes in nude mice, with multiple subdiabetogenic doses of streptozotocin, may be thymic-dependent. This further supports the idea that diabetes is mediated by autoimmune T-lymphocytes directed against self-antigens of the beta-cells.[16]

The athymic nude mouse may represent a useful animal model for the study of diabetes, but until now studies of plasma glucose, glucose tolerance, and insulin secretion in these animals have not been conducted.

The purpose of this study was to determine basal plasma glucose levels, immunoreactive insulin concentrations, and glucose response to an intraperitoneal glucose load in athymic nude BALB/c mice and to compare these findings with those of age-matched control BALB/c mice.

MATERIALS AND METHODS

Mice. Athymic nude BALB/c mice (nu/nu), bred and raised in a pathogen-free environment (locked in gnotobiotic isola-

From the Departments of Medicine, Diabetes Section, and Pathology, University of Southern California School of Medicine, Los Angeles, California 90033. Address reprint requests to Dr. A. Zeidler, USC School of Medicine, OCD 120, 2025 Zonal Avenue, Los Angeles, California 90033.

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tors) with controlled temperature and illumination, were used for the study. The breeding stock of the nude mice was received at the University of Southern California (USC) in 1978 from the National Cancer Institute (NCI) and derived from the tenth backcross generation in BALB/c mice. The original breeding nucleus at NCI consisted of nude (nu/nu) males and heterozygous (+/nu) females and was a generous gift from Dr. C. W. Friis of the Laboratory Animals Breeding and Research Centre, Bomholtgard Ltd., Denmark, to NCI.

A diet of sterilized Wayne Lab-Blox (F4) mouse chow (Allied Mills, Chicago, Illinois) and sterilized water were supplied constantly to the colony of nude mice. Normal male BALB/c (+/+) mice were used as controls and taken from an established true breeding line of homozygous (+/+) BALB/c mice at USC. The original breeding pairs for the normal BALB/c mice were obtained from the Simonsen Labs, Inc. (Gilroy, California). A similar diet of Wayne Lab-Blox was supplied to the normal BALB/c colony. Heterozygous BALB/c (+/nu) mice had to be removed and killed at an early age (perinatal age) to ensure normal growth and development of the athymic nude mice (nu/nu). The colony of athymic nude BALB/c mice was checked periodically, using random animals, for pathogenic microorganisms. Blood samples and organ tissues were found to be free of any pathogens. Tests on mouse serum were performed at Microbiological Associates (Bethesda, Maryland) for the following viruses: Sendai virus, PVM, LCM virus, polyoma virus, MVM virus, mouse hepatitis virus, GD7 virus, type III reovirus, mouse adenovirus, ectromelia virus, and K virus. The testing for common bacteria (*Pseudomonas aeruginosa, Citrobactor freundii, Salmonella typhi murium, Pasteurella pneumotropicum*) and mycoplasma (*Pulmonis*) was performed at the USC Microbiological Veterinary Laboratories.

On the day before an experiment, all animals 4 wk of age and older were caged separately and fasted for 12 h. Athymic nude and control mice at 1 and 3 wk of age were fasted for only 3 h before an experiment. Because of the small blood volume in the 1- and 3-wk-old mice, the samples were pooled from 3–5 animals in each experiment. Blood samples were collected from the paraorbital venous plexus using heparinized hematocrit capillary tubes (Dade Capilets, American Hospital Supply, Miami, Florida). The samples were immediately centrifuged for plasma glucose (PG) and immunoreactive insulin (IRI) determinations. The plasma for IRI was stored at −20°C until the assay was performed. A Beckman glucose analyzer (Beckman Instruments, Inc., Fullerton, California) was used for the glucose determinations,[17] and measurement of plasma IRI was assayed[18] using purified rat insulin as standard.

Glucose tolerance test. Intraperitoneal glucose tolerance tests (GTT) were performed on nude mice and controls at 0900 h, after a 12-h fast. Food and water were withheld during the tests. The animals were anesthetized with Metafane (2.2 dichloro-1, 1 difluroethyl methyl ether) to reduce excitation, and a baseline blood sample was collected from the paraorbital venous plexus. Each animal was then injected intraperitoneally with an aqueous solution of 20% dextrose (w/v), 2 mg/g body wt.[19] Subsequent blood samples were obtained from the paraorbital sinus at 30, 60, 120, and 180 min after dextrose administration.

Statistical analysis. Statistical evaluation of the results was performed using the Student's *t*-test.[20] Differences were considered to be significant at $P < 0.05$. Results are expressed as mean ± SEM.

RESULTS

Plasma glucose and insulin levels. Basal plasma glucose concentrations in male athymic nude mice and controls are shown in Table 1. At 1, 3, and 4 wk of age, plasma glucose levels were normal and similar in athymic and control mice. The highest levels of plasma glucose in the premature age group were found at 3 wk in both groups (149.0 ± 8.6 and 169.0 ± 10.6 mg/dl, respectively); these levels were higher than those of 1-wk-old animals in the respective groups ($P < 0.05$). The increase in plasma glucose at 3 wk of age in normal BALB/c (+/+) mice was statistically significant when compared with mice at 4 wk of age (169.0 ± 10.6 versus 120.3 ± 4.5 mg/dl, $P < 0.05$). Plasma glucose levels in 6-wk-old athymic mice were significantly increased when compared with those of 6-wk-old normal BALB/c mice (204.5 ± 5.8 versus 137.2 ± 3.7 mg/dl, $P < 0.01$). Plasma glucose levels in these 6-wk-old nude mice were also higher than in 4-wk-old nude mice ($P < 0.05$). Increased basal plasma glucose levels were noted at 8 and 12 wk of age in athymic mice when compared with controls (Table 1). There was no significant difference in body weight between the two groups, but the weights in both groups increased with age (Table 1).

Basal plasma immunoreactive insulin levels at 1 wk of age were normal in male athymic mice and not significantly different from those of control BALB/c mice (14.4 ± 2.2 versus 24.5 ± 2.2 μU/ml, P = NS). Plasma insulin levels at 3, 6, 8, and 12 wk of age in male athymic mice were significantly decreased when compared with those of normal BALB/c mice (Table 1). In addition, when considered in relation to the higher basal plasma glucose levels at 6, 8, and 12 wk of age, the plasma insulin levels in athymic mice were significantly decreased.

TABLE 1
Basal plasma glucose and immunoreactive insulin (IRI) levels at different ages in male athymic nude (nu/nu) and control BALB c (+/+) mice

Gene type	Age (wk)	Body weight (g)	Plasma glucose (mg/dl)	IRI (μU/ml)
Nude BALB/c (nu/nu)	1 (15)	4.5 ± 0.5	95.5 ± 5.6	14.4 ± 2.2
	3 (15)	7.3 ± 1.2	149.0 ± 8.6	4.6 ± 0.8†
	4 (5)	12.1 ± 1.5	127.0 ± 5.0	—
	6 (6)	19.6 ± 1.6	204.5 ± 5.8*	12.8 ± 2.1†
	8 (15)	25.4 ± 1.3	192.5 ± 4.9*	7.4 ± 0.8†
	12 (10)	27.5 ± 1.6	205.3 ± 4.5*	5.9 ± 0.9†
BALB/c (+/+)	1 (10)	4.3 ± 0.5	97.7 ± 2.7	24.5 ± 2.2
	3 (11)	11.8 ± 1.3	169.0 ± 10.6	15.4 ± 1.8
	4 (8)	13.0 ± 1.5	120.3 ± 4.5	—
	6 (10)	17.0 ± 1.5	137.2 ± 3.7	24.8 ± 2.5
	8 (11)	23.2 ± 1.8	124.1 ± 3.9	20.0 ± 1.7
	12 (11)	24.3 ± 0.6	156.4 ± 3.7	11.3 ± 1.1

The numbers in parentheses indicate numbers of animals studied.
* $P < 0.01$ (indicates significance when compared with control BALB/c mice).
† $P < 0.05$ (indicates significance when compared with control BALB/c mice).

TABLE 2
Plasma glucose concentrations after intraperitoneal glucose tolerance test at different age groups in male athymic nude (nu/nu) mice and control (+/+) BALB/c mice (mg/dl)

Gene type	Age (wk)	Body weight (g)	Plasma glucose (mg/dl) 0 min	30 min	60 min	120 min	180 min
Nude BALB/c (nu/nu)	1 (16)	4.6 ± 0.3	104.0 ± 2.6*	373.9 ± 4.9	365.1 ± 4.9†	280.0 ± 8.3†	102.8 ± 5.8*
	6 (6)	19.6 ± 1.6	204.5 ± 5.8†	322.6 ± 7.3†	306.8 ± 7.1†	231.8 ± 6.2†	217.0 ± 6.0†
	8 (15)	25.4 ± 1.3	192.2 ± 4.9†	320.2 ± 5.2†	289.7 ± 5.7†	272.4 ± 4.9†	230.1 ± 4.6†
	12 (10)	27.5 ± 1.6	205.3 ± 4.5†	336.5 ± 5.8†	345.0 ± 5.9†	282.2 ± 5.3†	242.3 ± 5.8†
BALB/c (+/+)	1 (18)	4.3 ± 0.5	96.7 ± 2.7	348.5 ± 5.2	190.5 ± 3.5	93.7 ± 3.6	94.5 ± 8.0
	6 (10)	17.0 ± 1.5	137.2 ± 3.7	178.6 ± 0.3	138.2 ± 3.5	123.4 ± 3.5	101.6 ± 3.2
	8 (11)	23.2 ± 1.8	124.1 ± 3.9	195.1 ± 4.2	174.3 ± 3.9	149.4 ± 3.7	140.2 ± 3.7
	12 (11)	24.3 ± 0.6	156.4 ± 3.7	210.3 ± 4.4	190.0 ± 4.1	150.2 ± 3.7	146.8 ± 3.6

The numbers in parentheses indicate the number of animals studied.
* $P < 0.001$ (indicates significance compared with athymic nudes (nu/nu) at older age groups).
† $P < 0.05$ (indicates significance compared with age-matched control BALB/c mice).

Glucose tolerance test. Plasma glucose concentrations in athymic nude and control mice at different ages after an intraperitoneal glucose load are given in Table 2. At 1 wk of age, athymic and control mice had similar glucose levels at 30 min (373.9 ± 4.9 and 348.5 ± 5.2 mg/dl, respectively), but at 60 and 120 min male athymic mice demonstrated significantly higher glucose levels when compared with control mice (365.1 ± 4.9 versus 190.5 ± 3.5 mg/dl, $P < 0.001$, and 280.0 ± 8.3 versus 93.7 ± 3.6 mg/dl, $P < 0.001$). At 6, 8, and 12 wk of age male athymic mice had significantly higher plasma glucose levels at 30, 60, 90, and 120 min when compared with control mice (Table 2). Female athymic nude mice were also studied and fasting plasma glucose levels at 6, 8, and 12 wk of age were obtained as shown in Table 3. These results demonstrated lower basal plasma glucose levels in female nude mice when compared with male athymic mice; however, the differences at 6 and 8 wk were not significant (181.3 ± 6.9 versus 204.0 ± 5.8 and 187.0 ± 6.8 versus 192.5 ± 4.9 mg/dl, P = NS). At 12 wk of age, female athymic mice showed significantly lower basal plasma glucose levels when compared with age-matched male nudes (165.8 ± 5.2 versus 205.3 ± 4.5 mg/dl, $P < 0.05$). The body weight of female nude mice was slightly lower than that of male nude mice in the age-matched groups. Female athymic mice at 6, 8, and 12 wk of age had a normal response to the intraperitoneal glucose load when compared with age-matched male athymic BALB/c mice (Table 3).

DISCUSSION

Sequential plasma glucose levels, glucose tolerance, and basal insulin concentrations were studied in athymic nude (nu/nu) BALB/c mice. As far as we know, such studies have not been previously described. We found that male athymic nude mice at 1 and 4 wk of age had normal basal plasma glucose levels when compared with normal BALB/c mice. At 3 wk of age there was an increase in basal plasma glucose levels in both athymic nude and normal BALB/c mice. The increase of plasma glucose at 3 wk of age had been described previously[21] and can be attributed to the transition at weaning from a restricted milk diet to a solid diet richer in carbohydrate and available ad libitum. From 6 wk to 12 wk of age, athymic nude mice had increased basal plasma glucose levels, which were significantly higher than those seen in control BALB/c mice (Table 1). Although basal plasma insulin concentrations at 1 wk of age were normal in athymic nude mice, they became significantly lower in all the older age groups of nude mice studied. Athymic nude mice are known to be lean, and their body weights were comparable to those of normal BALB/c mice. In addition, the rate of increase in body weight was constant during their growth so that the spontaneous hyperglycemia, starting at 6 wk of age, could not be attributed to obesity.

An intraperitoneal glucose challenge evoked an abnormal response in 1-wk-old athymic nude mice at all time points measured, but only at 30 min after glucose administration in control mice. An impaired glucose tolerance in 1-

TABLE 3
Comparison of plasma glucose concentration after a glucose tolerance test in male and female athymic nude BALB/c mice

Age (wk)	Sex	Body weight (g)	Plasma glucose (mg/dl) 0 min	30 min	60 min	120 min	180 min
6 (8)	M	19.6 ± 1.6	204.5 ± 5.8	322.6 ± 7.3*	306.8 ± 7.1*	231.8 ± 6.2*	217.0 ± 6.0*
6 (13)	F	15.5 ± 1.0	181.3 ± 6.8	241.3 ± 4.3	190.7 ± 8.3	146.5 ± 7.4	148.1 ± 7.1
8 (15)	M	25.4 ± 1.3	192.2 ± 4.9	320.2 ± 5.2*	289.7 ± 5.7*	272.4 ± 4.9*	230.1 ± 4.6*
8 (4)	F	19.8 ± 1.6	187.7 ± 6.8	196.5 ± 9.9	170.0 ± 9.2	149.5 ± 8.6	131.0 ± 8.0
12 (10)	M	27.5 ± 1.4	205.3 ± 4.5*	336.5 ± 5.8*	345.0 ± 5.9*	282.2 ± 5.3*	242.3 ± 5.8*
12 (6)	F	23.2 ± 1.4	165.8 ± 5.2	231.6 ± 6.2	222.7 ± 6.1	218.8 ± 6.0	174.2 ± 4.2

The numbers in parentheses indicate the number of animals studied.
* $P < 0.05$ (indicates significance when compared with age-matched female nude mice).

wk-old mice has previously been described and may reflect a continuing influence of the metabolic bias to prevent hypoglycemia in early life.[22] Glucose tolerance was significantly impaired in mature athymic male mice when compared with normal BALB/c mice. A prolonged fast of 24 h or longer is known to reduce glucose tolerance and suppress plasma insulin response to glucose in lean mice at all ages.[23–25] Since our experiments were undertaken after a 12-h fast, and the glucose tolerance was not impaired in the control mice, the fasting period does not explain our findings in the athymic mice. In contrast, glucose tolerance in female athymic nude mice was found to be normal when compared with that of age-matched male athymic nude BALB/c mice. Previous studies have shown that sex (maleness) is an important factor in modifying the diabetogenic effect of streptozotocin (SZ).[26,27] In addition, the thymectomized C57BL/Ks female mice were more resistant to SZ-induced hyperglycemia when compared with thymectomized male mice.[28] It has been proposed that the high testosterone level in male mice may account for the greater male to female sensitivity to SZ-induced diabetes.[26,27] Whether the testosterone levels in male athymic nude mice or a protective effect of estrogens in the females explains this difference in glucose intolerance in our colony is unclear. The precise effect of testosterone on the beta-cell is unknown; however, testosterone is known to exert its anabolic effect by increasing RNA polymerase activity, by producing specific RNA and protein, and by increasing intracellular aldose reductase.[27] The role of sex hormones in the pathogenesis of experimental and spontaneous diabetes in animals needs further elaboration.

The experiments presented in this report furnish evidence that male athymic nude BALB/c mice (nu/nu) in our colony develop spontaneous hyperglycemia, decreased basal insulin secretion, and impaired glucose tolerance. Spontaneous hyperglycemia and decreased insulin levels have been reported in different strains of mice.[29–31] However, these abnormalities have not been previously described in athymic nude mice. The possibility of environmental and/or genetic factors should be considered. Hyperglycemia due to infection seems unlikely, since these mice were isolated and serologically screened and cultured for mycoplasma, common bacteria, and viral pathogens.

Histologic examination of pancreatic islets in selected pancreata showed no discernible differences between athymic nude and control mice, as described in our previous study, but morphometric differences have been observed.[32] Rossini et al.[33] proposed that the generation of lymphocytes directed against the beta-cells might involve the participation of beta-cell-trophic type C viruses induced by streptozotocin. Electron microscopic examination of thin sections of the pancreatic islets from nude mice and controls in our colony revealed no evidence that type C viruses were present in these cells (unpublished data).

Our studies suggest that the athymic nude mice in our colony develop a diabetic syndrome, and therefore differ from the colony of athymic nude mice described by Paik et al.[16] While the primary origin of the breeding stock of athymic nude BALB/c was similar in both colonies, our nude BALB/c mice were derived after a longer period of backcross generations in BALB/c mice when compared with the colony of Paik et al. Therefore, one may speculate that, following a mutation, a diabetogenic gene occurred before our receipt of the breeding stock after establishment of our colony. Whether the lack of the thymus plays a role in the development of the diabetic syndrome in athymic nude mice in our colony, as previously described for gonadal malfunction,[5,6] is unknown. The lack of diabetes in our female athymic mice argues against it, but the importance of interactions between sex hormones and the thymus in this population has yet to be explored.

Previous studies indicate that abnormalities in the secretory role of the endocrine thymus are associated with autoimmune and immune deficiency diseases.[34] In animals, neonatal thymectomy[35,36] induces not only severe impairment of immunologic responsiveness but also a wasting syndrome and other endocrine abnormalities.[5,7,37] Studies involving thymus implantation into athymic nude mice have demonstrated reconstitution of gonadal function but not of thyroid function.[7] In addition, administration of bovine thymosin to neonatally thymectomized mice was shown not only to restore cell-mediated immunity to normal, but to reduce the incidence of the wasting syndrome.[34] Whether or not the development of the normal endocrine system, including the endocrine pancreas, in athymic nude BALB/c mice may be partly dependent on the intact thymus or on certain thymic hormones is not known, but the mice described here offer a model for such studies.

REFERENCES

[1] Rygaard, J.: Thymus and Self: Immunobiology of the Mouse Mutant Nude. London, John Wiley and Sons, 1973.

[2] Shire, J. G. M., and Pantelouris, E. M.: Comparison of endocrine function in normal and genetically athymic mice. J. Biochem. Physiol. *47*:93–100. 1974.

[3] Pierpaoli, W., and Sorkin, E.: Alterations of adrenal cortex and thyroid in mice with congenital absence of the thymus. Nature *238*:282–85, 1972.

[4] Weinstein, Y.: Impairment of the hypothalamo-pituitary-ovarian axis of the athymic "nude" mouse. Mech. Ageing Dev. *8*:63–68, 1978.

[5] Sakakura, T., and Nishizuka, Y.: Thymic control mechanism in ovarian development: reconstitution of ovarian dysgenesis in thymectomized mice by replacement with thymic and other lymphoid tissues. Endocrinology *90*:431–37, 1972.

[6] Rebar, R. W., Morandini, I. C., Benirschke, K., and Petze, J. E.: Reduced gonadotropins in athymic mice: prevention by thymic implants. The Endocrine Society *88*:256, 1980. Abstract.

[7] Pierpaoli, W., and Besedovsky, H. O.: Role of the thymus in programming of neuro-endocrine functions. Clin. Exp. Immunol. *20*:323–38, 1975.

[8] Buschard, K., and Rygaard, J.: Is the diabetogenic effect of streptozotocin in part thymus dependent? Acta Pathol. Microbiol. Scand. [C] *86*:23–27, 1978.

[9] Buschard, K., and Rygaard, J.: Passive transfer of streptozotocin-induced diabets mellitus with spleen cells. Acta Pathol. Microbiol. Scand. [C] *85*:469–72, 1977.

[10] Buschard, K., Rygaard, J., and Lund, E.: The inability of a diabetogenic virus to induce diabetes mellitus in athymic (nude) mice. Acta Pathol. Microbiol. Scand. [C] *84*:299–303, 1976.

[11] Buschard, K., Madsbad, S., and Rygaard, J.: Passive transfer of diabetes mellitus from man to mouse. Lancet *1*:900–910, 1978.

[12] Lipstick, J., Beattie, G., Osler, A. G., and Kaplan, N. O.: Letter: Passive transfer of lymphocytes from diabetic man to athymic mouse. Lancet *1*:1290–91, 1979.

[13] Thurneyssen, O., Jansen, F. K., Vialettes, B., Vague, P. H., Selam, J. L., and Mirouze, J.: Letter: Passive transfer of lymphocytes from diabetic man to athymic mouse. Lancet *1*:1291–92, 1979.

[14] Sitges-Serra, A., Farndon, J. R., Shenton, B. K., and Johnson, I. D. A.: Letter: Passive transfer of lymphocytes from diabetic man to male WAG rats. Lancet *1*:1292, 1979.

[15] Neufeld, M., McLaughlin, J., Maclaren, N. K., Rosenbloom, E., and Donnelly, W.: Failure to transfer diabetes mellitus from man to mouse. N. Engl. J. Med. *301*:665, 1979.

[16] Paik, S. -G., Fleischer, N., and Shin, S.-I.: Insulin-dependent diabetes mellitus induced by subdiabetogenic doses of streptozotocin: obligatory

role of cell-mediated autoimmune processes. Proc. Natl. Acad. Sci. USA 77:6129–33, 1980.

[17] Alpert, N. L.: Glucose analyzer and BUN analyzer. Lab World *24,12*:40–44, 1973. Instrument Series Report 14.

[18] Morgan, C. R., and Lazarow, A.: Immunoassay of insulin: two antibody system, plasma insulin levels of normal, subdiabetic and diabetic rats. Diabetes *12*:115–26, 1963.

[19] Lavine, R. L., Chick, W. L., Like, A. A., and Makdisi, T. W.: Glucose tolerance and insulin secretion in neonatal and adult mice. Diabetes *20*:134–39, 1971.

[20] Afifi, A. A., and Azen, S. P.: Statistical Analysis. Application of a Factorial Program to Other Models. A Computer Approach. New York, Academic Press, 1979, pp. 241–44.

[21] Dubuc, P. U.: The development of obesity hyperinsulinemia and hyperglycemia in ob/ob mice. Metabolism *25*:1567–74, 1976.

[22] Adam, P. A. J.: Control of glucose metabolism in the human fetus and newborn infant. Adv. Metab. Disord. 5:183–275, 1971.

[23] Grey, N. J., Goldring, S., and Kipnis, D. M.: The effect of fasting, diet and actinomycin D on insulin secretion in the rat. J. Clin. Invest. *49*:881–89, 1970.

[24] Malaisse, W. J.: Hormonal and environmental modification of islet activity. *In* Handbook and Physiology, Section 7, Vol. 1. Greep, R. O., Astwood, E. B., Freinkel, N., Steiner, D., and Geiger, S. R., Eds. Washington, American Physiological Society, 1972, pp. 237–60.

[25] Hediskov, C. J., and Capito, K.: The effect of starvation on insulin secretion and glucose metabolism in mouse pancreatic islets. Biochem. J. *140*:423–33, 1974.

[26] Rossini, A. A., Williams, R. M., Appel, M. C., and Like, A. A.: Sex differences in the multiple-dose streptozotocin model of diabetes. Endocrinology *103*:1518–20, 1978.

[27] MacLaren, N. K., Neufeld, M., McLaughlin, J. V., and Taylor, G.: Androgen sensitization of streptozotocin-induced diabetes in mice. Diabetes *29*:710–16, 1980.

[28] Leiter, E. H.: Multiple low dose streptozotocin induced hyperglycemia and insulitis in C57BL mice: influence of inbred background, sex and thymus. Proc. Natl. Acad. Sci. USA 79:630–34, 1982.

[29] Cameron, D. P., Stauffacher, W., and Renold, A. E.: Spontaneous hyperglycemia and obesity in laboratory rodents. The endocrine pancreas. *In* Handbook of Physiology, Section 7. Vol. 1. Greep, R. O., Astwood, E. B., Freinkel, N., Steiner, D., and Geiger, S. R., Eds. Washington, American Physiological Society, 1972, pp. 611–25.

[30] Like, A. A.: Spontaneous diabetes in animals. *In* The Diabetic Pancreas. Volk, B. W., and Wellman, K. F., Eds. New York, Plenum Press, 1977, pp. 381–423.

[31] Herberg, L.: Spontaneously hyperglycemic laboratory animals—models of human diabetes syndrome? Horm. Metab. Res. *11*:323–31, 1979.

[32] Zeidler, A., Tosco, C., Kumar, D., Mahan, E., Parker, J., and Slavin, B.: Pancreatic islet cell studies in athymic nude mice BALB/c. The Endocrine Society *149*:266, 1981. Abstract.

[33] Rossini, A. A., Like, A. A., Chick, W. L., Appel, M. C., and Cahill, G. F., Jr.: Studies of streptozotocin-induced insulitis and diabetes. Proc. Natl. Acad. Sci. USA 74:2485–89, 1977.

[34] Shulof, R. S., and Goldstein, A. L.: Thymosin and the endocrine thymus. Adv. Intern. Med. 22:121–43, 1977.

[35] Miller, J. F. A. P.: Immunological function of the thymus. Lancet *2*:748–49, 1961.

[36] Good, R. A., and Gabrielson, A. E., Eds.: The Thymus in Immunobiology: Structure, Function and Role in Disease. New York, Hoeber-Harper, 1964.

[37] Taguchi, O., Nishizuka, Y., Sakakura, T., and Kojima, A.: Autoimmune oophoritis in thymectomized mice: detection of circulating antibodies against oocytes. Clin. Exp. Immunol. *40*:540–53, 1980.