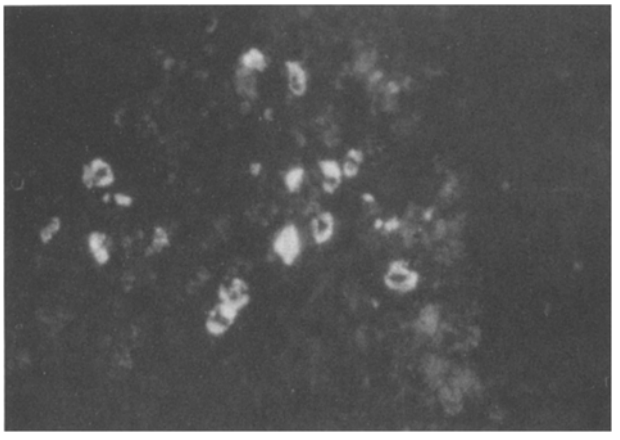

FITC-insulin staining of frozen thymus from a cortisol-treated rat. × 400.

mained FITC-insulin-positive in the fixed sections, indicating that the staining reaction was not simply a result of phagocytosis of the FITC-insulin.

Staining of alternating thymic sections for FITC-insulin binding and ANAE activity produced labeling patterns which showed that FITC-insulin bound cells were also ANAE positive. As with FITC-insulin binding, ANAE-staining of the thymic sections was heaviest at the cortico-medullary junction and was markedly increased following cortisol administration.

Discussion. It is known that insulin receptors are located on splenic and cultured macrophages [7], and on monocytes isolated from peripheral blood [7, 8]. A mixture of thymic cells was shown to bind ^{125}I-insulin, but there has been no direct evidence to indicate which thymic cell contains the insulin receptors. Mature nonstimulated T-cells do not bind insulin [9], suggesting that the thymic cells with insulin receptors are either immature thymocytes or members of the non-lymphoid population of cells. We have shown that FITC-insulin-staining cells are also ANAE positive. Preincubation with an excess of unlabeled insulin suppressed FITC-insulin binding, indicating that the binding observed is to cells that have specific binding sites. FITC-insulin has also been shown to be useful as a probe for insulin receptors on blood monocytes [5]. Cortisol treatment of the rats, which is known to augment the thymic macrophage population [10], increased both FITC-insulin and ANAE staining which were heaviest in normal thymus at the cortico-medullary juncture. Unique nonspecific-esterase positive macrophages have been identified in this area of the thymus [11, 12].

Thymic macrophages are believed to function in T-cell maturation [13], and it has been postulated that the macrophages involved in this process are located at the cortico-medullary juncture [11]. Interleukin 1 and prostaglandin E_2, which may influence thymocyte proliferation, have been shown to be produced by thymic macrophages [14]. Furthermore these cells are believed to regulate intrathymic T-cell development [15]. The fact that diabetic animals have abnormal T-cell function [1], maturation impairment [2], and depressed thymic proliferation [16, 17] may be due to aberrant function of insulin-requiring macrophages at the thymic cortico-medullary juncture.

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Effect of niacin/nicotinamide deficiency on the diabetogenic effect of streptozotocin

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Summary. Weanling CD-1 mice were fed either a control diet or a diet deficient in niacin/nicotinamide for one month and then injected i.v. with 60, 80, 100, 120, 140, or 160 mg/kg streptozotocin. Mice on the deficient diet developed a higher incidence of diabetes and more severe hyperglycemia than those on the control diet.
Key words. Streptozotocin; diabetes mellitus; NAD; niacin; nicotinamide; tryptophan; CD-1 mice.

Streptozotocin (STZ), a methylnitrosourea with a 2-substituted glucose, is a drug which has been widely used to induce experimental diabetes mellitus [1, 2]. However, the mechanism by which STZ exerts its diabetogenic effect has not been totally elucidated. Over the past 20 years, numerous investigators have demonstrated that STZ decreases nicotinamide

adenine dinucleotide (NAD) levels within islets and that nicotinamide, a precursor of NAD, will prevent the onset of diabetes if administered before or simultaneously with STZ[3-10].

Clearly, STZ acts as an alkylating agent and damages the DNA of pancreatic beta cells[11, 12]. Okamoto and co-workers have demonstrated that STZ-induced DNA damage activates poly(ADP-ribose) synthetase, an enzyme involved in the DNA repair process that utilizes NAD as its substrate[13]. Okamoto hypothesizes that activation of poly(ADP-ribose) synthetase critically depletes the beta cell of NAD causing widespread beta cell necrosis and diabetes. This hypothesis is supported by the observation that poly(ADP-ribose) synthetase inhibitors prevent diabetes[14, 15].

Based on the experimental data showing that supplemental nicotinamide protects against STZ toxicity and that STZ damages islets by NAD depletion, one might predict that severe niacin/nicotinamide deficiency (these nutrients are metabolically interconvertible) would enhance the diabetogenicity of STZ. The present study demonstrates that niacin/nicotinamide deficiency potentiates the beta cell toxicity of STZ in CD-1 mice.

Methods and materials. Weanling male CD-1 mice (21 days old) were purchased from Charles River (Wilmington, MA) and fed either standard laboratory animal chow (Purina # 5001, St Louis, MO) or a special chow deficient in niacin (nicotinic acid) and nicotinamide (nicotinic acid amide) (Purina # 5821 Niacin Deficient Purified Diet, Richmond, IN) for 1 month. Purina # 5821 chow is a vitamin-free casein hydrolysate (20%) supplemented with dextrin (44.55%), sucrose (15%), lard (5%), corn oil (5%), minerals (5%), fiber (3%), vitamins (2%), choline chloride (0.20%), DL-methionine (0.15%), and L-tryptophan (0.10%); it is a nutritionally balanced niacin-deficient (0.8 mg niacin/kg chow) diet providing 4.10 kcal of energy/g. The standard laboratory animal chow contains 23.4% protein (including 0.10% tryptophan), 49% carbohydrate, 4.5% fat, 5% fiber, 7.3% minerals, and vitamins (including niacin, 95 ppm); it provides 4.25 kcal of energy/g. Cages holding deficient animals were checked daily to assure that they were never contaminated with control chow or fecal material from control animals.

An absolute deficiency of niacin/nicotinamide is not possible. A nicotinamide/niacin deficient diet must contain a low concentration of the essential amino acid tryptophan, a portion of which may be converted to niacin via a tryptophan-niacin pathway[16]. Furthermore, certain microorganisms in the gut are capable of making small quantities. A relative nicotinamide/niacin deficiency in rodents can be induced by feeding weanlings a deficient diet for several weeks; the deficient state is characterized by stunted growth relative to control animals (pers. commun., J. Van Eys, M. D. Anderson Hospital, Houston, TX).

Streptozotocin (STZ lot No. 0070 C), kindly provided by the Upjohn Co. (Kalamazoo, MI), was dissolved in citrate buffer and immediately injected i.v. via the tail vein. Control and experimental mice received STZ at doses of 60, 80, 100, 120, 140, or 160 mg/kg.

All animals were tested for hyperglycemia 1, 2, and 5 days after treatment; animals that were clearly diabetic (non-fasted plasma glucose values repeatedly > 200 mg/dl) were sacrificed after day 5. Animals that were not diabetic were given a glucose tolerance test on day 8. Glucose tolerance tests were performed as follows. Mice were fasted overnight and

Incidence and severity of diabetes in niacin/nicatinamide deficient and control mice following intravenous doses of STZ

Dose (mg/kg)	Diet	Day 1 Incidence	Day 1 Plasma glucose Mean (SD) (mg/dl)	Day 2 Incidence	Day 2 Plasma glucose Mean (SD) (mg/dl)	Day 5 Incidence	Day 5 Plasma glucose Mean (SD) (mg/dl)	Day 8 Abnormal G.T.T[a]
0	Ndef.	0/9	149.1 (17.7)	–	–	–	–	–
	Cont.	0/15	154.9 (16.7)	–	–	–	–	–
60	Ndef.	0/4	nd 136.0 (35.2) d –	1/4	nd 136.3 (41.7) d 288.0	1/4	nd 183.0 (20.0) d 301.0	1/4
	Cont.	0/5	nd 140.2 (14.2) d –	0/5	nd 156.0 (25.2) d –	0/5	nd 168.0 (20.6) d –	0/5
80	Ndef.	0/5	nd 111.2 (26.3) d –	1/5	nd 146.8 (40.3) d 203.0	0/5	nd 159.4 (13.6) d –	4/5
	Cont.	0/6	nd 142.0 (19.5) d –	0/6	nd 146.8 (8.8) d –	0/6	nd 167.0 (9.7) d –	0/6
100	Ndef.	0/5	nd 133.8 (11.5) d –	3/5	nd 156.0 (33.9) d 222.0 (27.4)	4/5	nd 161.0 d 332.0 (50.0)	1/1
	Cont.	0/5	nd 134.0 (11.9) d –	0/5	nd 162.4 (22.5) d –	0/5	nd 166.0 (21.2) d –	1/5
120	Ndef.	0/5	nd 129.0 (16.7) d –	4/5	nd 169.0 d 332.5 (111.8)	5/5	nd – d 450.8 (147.9)	–
	Cont.	0/5	nd 129.8 (34.8) d –	0/5	nd 162.6 (24.2) d –	3/5	nd 168.5 (12.0) d 254.0 (19.2)	2/5
140	Ndef.	1/5	nd 138.8 (19.5) d 232.0	5/5	nd – d 533.8 (46.7)	5/5	nd – d 611.4 (57.2)	–
	Cont.	0/5	nd 140.8 (15.2) d –	2/5	nd 176.7 (5.1) d 372.5 (36.1)	4/5	nd 179.0 d 317.3 (121.7)	3/3
160	Ndef.	2/5	nd 136.0 (8.5) d 285.5 (55.9)	5/5	nd – d 531.2 (39.9)	2/2[b]	nd – d 514.0 (15.6)	–
	Cont.	0/5	nd 98.7 (20.6) d –	4/5	nd 144.0 d 343.0 (77.4)	4/5	nd 183.0 d 393.0 (74.5)	–
Total	Ndef.	3/29	n.s.	19/29	X = 11.31 df = 1	17/26	X = 3.93 df = 1	
	Cont.	0/31		6/31	p < 0.001	11/31	p < 0.05	

Ndef., niacin/nicatinamide deficient; Cont., control; nd, nondiabetic; d, diabetic; G.T.T., glucose tolerance test; [a] G.T.T. were only performed on nondiabetic or weakly diabetic rats; [b] 3 mice died before day 5.

fasting plasma glucose levels were determined the next morning. Mice were then injected i.p. with 2 g/kg of dextrose (Abbott Labs, North Chicago, IL) and plasma glucose levels were determined 1 and 2 h later. Mice were considered to demonstrate abnormal glucose tolerance when at least two of three plasma glucose values (i.e., 0, 1, and 2 h) exceeded the respective mean values obtained from control animals by greater than 3 standard deviations. In all instances, blood samples were collected using heparinized capillary tubes via the retro-orbital plexus. Plasma glucose concentrations were analyzed by the glucose-oxidase method using a glucose analyzer (Beckman Instruments, Fullerton, CA).

Statistical analyses were by chi-square test using the Yates' correction. Probability values of 0.05 or less were considered significant.

All animals were sacrificed after the last plasma glucose determination and the pancreata were fixed in Bouin's solution and processed for light microscopy. Sections were stained with hematoxylin and eosin or aldehyde fuchsin and were examined. The histologic appearance of islets was consistent with plasma glucose levels. Severely diabetic mice, regardless of type of diet, had islets with degranulated and necrotic beta cells while islets from non-diabetic mice had minimal or less severe damage.

Results and discussion. The table shows the mean plasma glucose values for deficient and control mice 1, 2, and 5 days after STZ injection. Untreated mice on the control and deficient diet had nearly identical mean plasma glucose values. However, the incidence of diabetes on both days 2 ($p < 0.001$) and 5 ($p < 0.05$) was higher in STZ-treated mice receiving the deficient diet than in those receiving the control diet. At every dose, the degree of hyperglycemia (i.e., mean plasma glucose level) in the diabetic mice on the deficient diet exceeded that of the mice on the control diet. Furthermore, abnormal glucose tolerance was more frequent in the deficient group. These data demonstrate that niacin/nicotinamide deficient CD-1 mice show a markedly enhanced susceptibility to the diabetogenic effect of STZ. Both STZ[3-10, 14, 15] and dietary niacin deficiency[17] are known to decrease NAD levels in islets. If STZ induces beta cell necrosis and diabetes by critically depleting the beta cells of NAD, it is not surprising that these two treatments have an additive or synergistic effect. Finally, niacin/nicotinamide deficiency may also lower STZ's LD-50. Three of five mice on the deficient diet receiving 160 mg/kg died; the LD-50 for mice on the control diet exceeded 200 mg/kg (data not shown).

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Thermogenic effects of thyrotrophin-releasing hormone and its analogues in the rat

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Summary. Acute or chronic injection of RX 77 368 (a TRH analogue; 1 mg/kg s.c.) stimulated oxygen consumption (VO_2) and brown adipose tissue activity in the rat, and decreased weight gain. Other TRH analogues (CG 3509, RGH 2202) and TRH itself also stimulated VO_2. These thermogenic actions are probably mediated centrally by stimulation of sympathetic outflow to brown fat.
Key words. TRH; TRH analogues; thermogenesis; brown adipose tissue.

Thyrotrophin releasing hormone (TRH) was first identified as a hypothalamic hormone releasing thyroid stimulating hormone (TSH) from the pituitary gland[1]. In common with many other peptide hormones, TRH has additionally been ascribed a role as a central neurotransmitter or neuromodulator with a variety of physiological actions[1, 2]. For example, TRH affects thermoregulation in many species[3-7], although the responses may be somewhat varied. Peripheral administration of TRH usually induces hyperthermia[6, 7], but this may be at least partly dependent on release of TSH and a subsequent rise in circulating thyroid hormone levels[7, 8]. Central administration of TRH can also induce hyperthermia and reverse experimentally-induced hypothermia[9, 10], but in some studies a reduction in body temperature has been observed[9, 11].

Increases in body temperature can be achieved either by reductions in heat loss or by stimulation of heat production. In small mammals, non-shivering thermogenesis which al-