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Modification of Renal Tumorigenic Effect of Streptozotocin by Nicotinamide: Spontaneous Reversibility of Streptozotocin Diabetes[1] (39209)

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(Introduced by R. L. Dixon)

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Streptozotocin (glucopyranose, 2-deoxy-2-(3-methyl-3-nitrosoureido)-D-), an antibiotic derived from *Streptomyces achromogenes* (1, 2), produces renal adenomas in rats (3, 4). We have previously demonstrated that the diabetogenic action of the compound, mediated through the specific destruction of the pancreatic B cell (5, 6), can be prevented by pretreatment with nicotinamide (7, 8). The present investigation was undertaken to determine whether nicrotinamide would also protect rats against the renal carcinogenic action of streptozotocin and whether long-standing diabetes contributes to its oncogenic action. The scope of the study was broadened when it was found (9) that nicotinamide acted as an apparent cocarcinogen with streptozotocin to produce pancreatic islet cell tumors (nesidioblastomas). Of further interest was the demonstration that streptozotocin diabetes in the rat, as produced by the present dose regimen, was reversible.

Materials and methods. One hundred and seven male Holtzman rats, 106-182 g, were used in this study. Only males were studied since we previously had shown a greater incidence of renal tumors in males treated with streptozotocin than in females (3). The animals were housed individully in suspended cages and were maintained on Hemlock Hollow rat pellets and water, both available *ad libitum*. Animals were separated into four treatment groups so that the average weights of the rats in each group at the initiation of the study were essentially the same. Survivors were followed for periods of 547-551 days at which time the study was terminated.

The following treatment schedules were used. Streptozotocin (Group S): A single dose of 50 mg/kg of streptozotocin was administered intravenously (iv). Nicotinamide + streptozotocin (Group SN): 350 mg/kg nicotinamide were given intraperitoneally (ip) 10 min before the iv injection of a single dose of 50 mg/kg of streptozotocin; 3 hr later a second ip dose of 350 mg/kg of nicotinamide was given. Nicotinamide (Group N): 350 mg/kg of nicotinamide were given ip at time zero. Three hours later a second ip dose of 350 mg/kg was administered. Vehicle Controls (Group VC): 0.025 *M* citric acid in saline was administered as single iv dose in a volume equivalent to the volume dose of streptozotocin. Aseptic technique was used throughout; iv injections were given into the caudal veins. Streptozotocin, Lot No. 6742-DEG-30-5 (Upjohn Co.), was made up in a concentration of 10 mg/ml in 0.02 5 *M* citric acid in normal saline (0.9% NaCl), pH 4.0; nicotinamide (Matheson, Coleman & Bell) was used in a concentration of 35 mg/ml in normal saline. Measurements of blood (10) and urine glucose[2] were performed 3 days post-treatment and serially at 2-month intervals thereafter. The diagnosis of diabetes was established by the occurrence of hyperglycemia, glycosuria, polydipsia, and polyuria.[3]

Complete postmortem examinations, exclusive of the central nervous system, were

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[2] Tes-Tape, Eli Lilly and Co., Indianapolis, Indiana.

[3] Twenty-four-hour samples of urine were collected from animals housed in individual metabolism cages.

performed on all animals that succumbed or were sacrificed. The presence and size of tumors were recorded. Representative sections of all major organs were fixed in 15% neutral formalin and prepared for microscopic examination. All organs and tissues of animals which survived 14 months or longer were studied histologically. Microscopic examination of tissues from animals which were sacrificed or died before this time was restricted to the pancreas, kidney, liver, adrenals, and any other organ which presented gross lesions. Tissues were stained with hematoxylin and eosin, and in addition, sections of pancreas from selected animals were stained by the aldehyde fuchsin method of Gomori (13, 14).

Results. A. Alterations of tumorigenic action of streptozotocin by nicotinamide. As shown in Table I, renal neoplasms occurred in 77% (20/26) of the animals treated with streptozotocin alone (Group S), but only in 18% (5/28) of the animals treated with both streptozotocin and nicotinamide (Group SN). The frequency and size of the tumors appeared to be time-related. In Group S, the first tumor was noted in an animal sacrificed 165 days after treatment, but 80% of all tumors were found in animals sacrificed 268 through 547 days post-treatment. Moreover, tumors were seen in all of the 13 animals sacrificed during the period of 393–547 days. The tumors varied in size from 2 × 2 × 1 mm to 60 × 65 × 35 mm, and in many instances two to three adenomas were noted in either one or both kidneys. A typical renal adenoma found in an animal sacrificed 511 days post-treatment is presented in Figs. 1 and 2. In contrast to the rats treated with streptozotocin alone (Group S), renal tumors occurred in only 18% (5/28) of the animals that received the combination of nicotinamide and streptozotocin (Group SN). These adenomas were generally smaller than those noted in the animals treated with streptozotocin alone: one was found only on microscopic examination and the other four varied in size from 2 × 2 × 1 mm to 12 × 12 × 12 mm. In addition, the tumors in the Group SN developed later, the first tumor having been noted 281 days post-treatment. No renal tumors were seen in either of the control and vehicle treated animals (Groups N & VC).

B. Reversibility of Streptozotocin Diabetes. Only those animals treated with streptozotocin alone (Group S) became diabetic, and this condition was first noted 3-days post-treatment, at which time the mean blood glucose of the animals was 280 ± 10.9 mg% (Fig. 3). These animals also developed the other classical signs of diabetes: glycosuria, polydipsia, and polyuria. In addition, 61% (16/26) developed cataracts between the 72nd and the 317th day of the study. As shown in Fig. 4, the diabetic animals showed smaller weight increments that the animals in the other treatment groups. None of the animals treated with both nicotinamide and streptozotocin (Group SN), nicotinamide

TABLE I. INCIDENCE OF RENAL ADENOMAS IN MALE HOLTZMAN RATS TREATED WITH STREPTOZOTOCIN (NSC 85998) OR STREPTOZOTOCIN AND NICOTINAMIDE AND CONTROLS.

Group	Treatment (mg/kg × No. Rx)	Route	Number treated	Number surviving 8 months or more	Earliest day tumor detected	Animals with tumors	Day last animal sacrificed
S	Streptozotocin 50 × 1	iv[a]	26	21 (81%)	165	20(77%)	547
SN	Streptozotocin 50 × 1[a]	iv[a]	28	26 (92%)	281	5(18%)	547
	Nicotinamide 350 × 2[b]	ip					
N	Nicotinamide 350 × 2 every 3 hr	ip	27	24 (89%)		0	551
VC	0.025 *M* Citric acid in saline	iv[a]	26	22 (84%)		0	550

[a] Caudal veins used.
[b] Nicotinamide administered 10 min before and 180 min after Streptozotocin.
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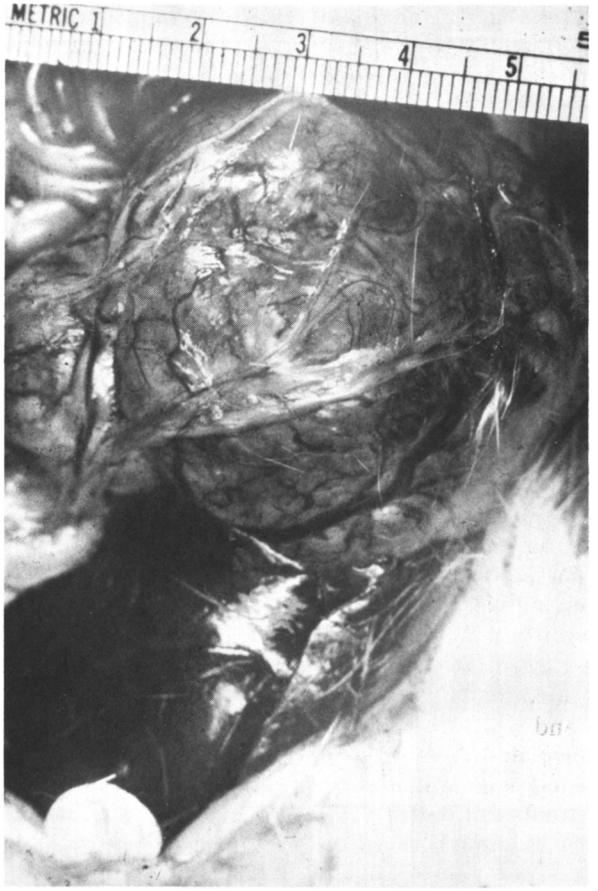

FIG. 1. Renal adenocarcinoma in rat sacrificed 511 days post 50 mg/kg × 1 of streptozotocin.

alone (Group N), or with the streptozotocin vehicle (Group VC) demonstrated any signs of diabetes. As the study progressed, it became apparent that streptozotocin diabetes at the 50 mg/kg dose level was not permanent. While at 6 months, all of the Group S animals were hyperglycemic (Fig. 3), between 8 and 10 months the majority of the animals showed reversal of these signs of polyuria and polydipsia, and at 10 months, only 5 of the 16 survivors had a markedly elevated blood glucose. After 16 months observation, none of the rats were hyperglycemic. As reported previously (9), 64% of the Group SN animals developed islet cell adenomas and were hypoglycemic from Month 12 (blood glucose 45 ± 2.2 mg%) through Month 18 (blood glucose 30 ± 2.6 mg%).

Discussion. Streptozotocin produces many diverse biological activities. It is antibiotic (2), oncolytic (2), carcinogenic (3, 4, 9), and diabetogenic (5), and when administered with nicotinamide, produces islet cell adenomas.

Streptozotocin is composed of 1-methyl-1-nitrosourea (MNU), a known carcinogen, attached to a glucosamine carrier. The diabetogenic activity of this drug has been correlated with its uptake into pancreatic islets and inhibition of pyridine nucleotide synthesis (16, 17). Pharmacologic doses of nicotinamide not only prevent the acute reduction in islet nicotinamide adenine dinucleotide content, but also protect the beta cell from necrosis. However, the DNA of such beta cells are still subject to methylation by the MNU moiety of the streptozotocin molecule, and the resultant carcinogenic activity is expressed with the development of a functioning islet cell adenoma.

Streptozotocin is known to concentrate in

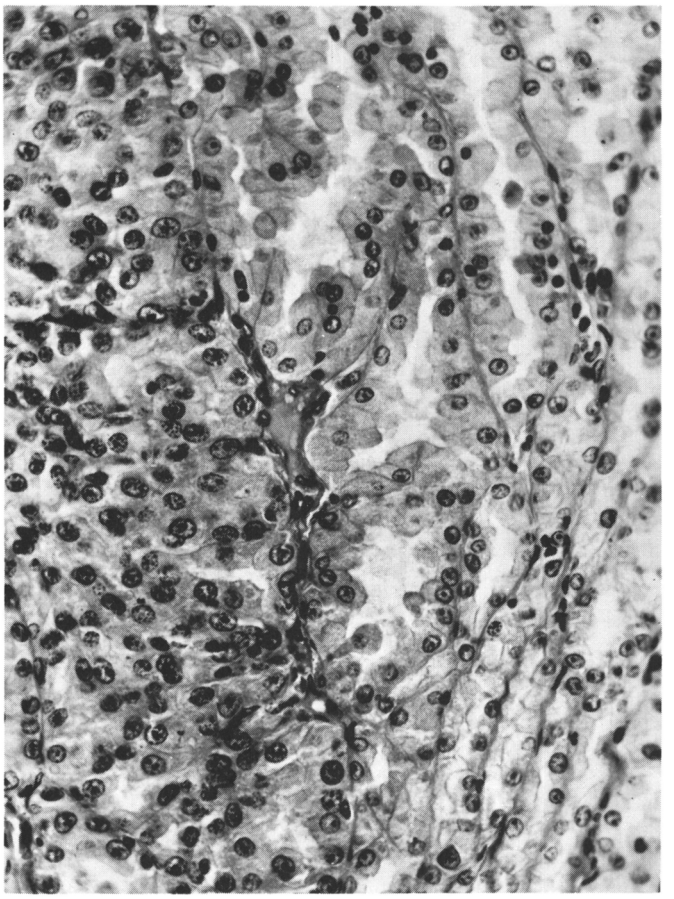

FIG. 2. Usual structure of renal nodules consisting of glandular structures with irregular papillations supported on a finely branching framework composed of connective tissue and capillary vascular supply (×100 slightly reduced).

the rodent kidney (17), and the evidence of renal tumor induction was to be expected. In the present study, nicotinamide has been shown to reduce the renal carcinogenic action of streptozotocin significantly. The fact that some renal adenomas appeared in the combination treatment group (SN), suggests that doses of nicotinamide are fully adequate to protect against diabetes, were only partially effective in neutralizing the tumorigenic actions of the antibiotic. It is possible that the administration of a larger dose of the vitamin would offer complete protection against this oncogenic activity. We had, in fact, intended to explore such a dose-response effect, until it became clear that nicotinamide given with streptozotocin was promoting the development of insulin-secreting islet cell adenomas, at the same time and under the same circumstances as it was preventing the development of renal adenomas. Certainly the reasons for these diametrically opposite actions are unclear at the present time. It is apparent from the present finding that its long-term diabetes plays only a minor adjunctive role, if any, in the induction of renal adenomas since similar tumor were demonstrated in some of the Group SN animals which never developed diabetes. Further studies will be initiated to determine whether nicotinamide blocks the uptake of streptozotocin into the renal tubular cell.

The demonstration that streptozotocin diabetes produced by a single iv dose of 50 mg/kg was reversible after 8–10 months was not an unexpected finding. In a previous study it was demonstrated that while the 50

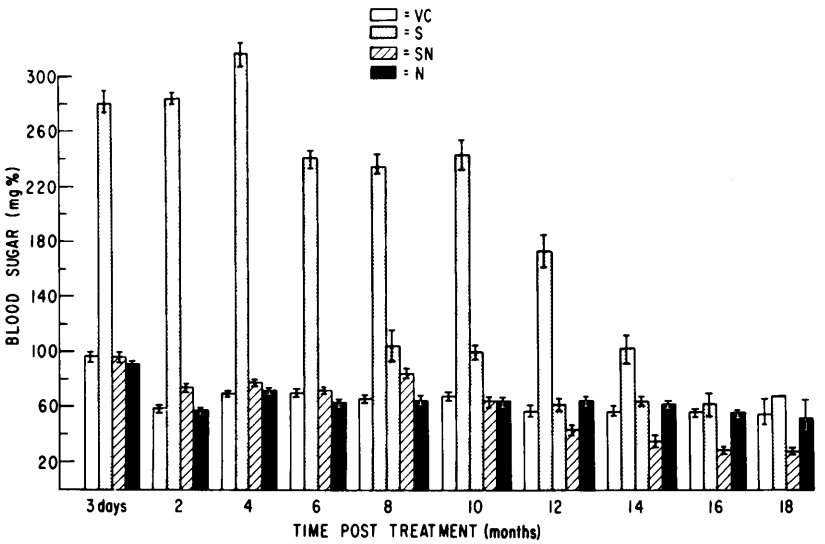

FIG. 3. Sequential measurements of the average blood sugars, with standard errors, of male Holtzman rats treated on Day 0 with streptozotocin 50 mg/kg × 1, iv (S); with streptozotocin 50 mg/kg × 1, iv, and with nicotinamide 350 mg/kg, ip, 10 min before and 180 min after streptozotocin (SN); with nicotinamide 350 mg/kg × 2, every 3 hr ip (N), and with 0.025 *M* citric acid in saline, pH 4.0, iv × 1 (VC).

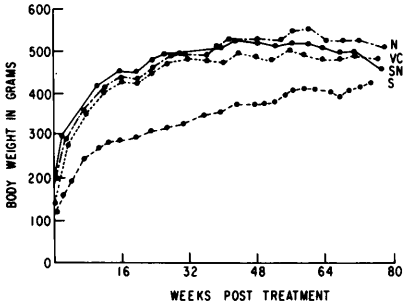

FIG. 4. Growth curves of male Holtzman rats treated on Day 0 with streptozotocin 50 mg/kg × 1 iv (S); with streptozotocin 50 mg/kg × 1 iv and nicotinamide 350 mg/kg ip, 10 min before and 180 min after streptozotocin (SN); with nicotinamide 350 mg/kg × 2 every 3 hr ip (N), and with 0.025 *M* citric acid saline, pH 4.0, iv × 1 (VC).

mg/kg dose was clearly diabetogenic, larger doses of streptozotocin produced a significantly greater diabetic state, including the development of ketoacidosis (18). Moreover, evidence of B cell regeneration in rats treated with 50 mg/kg × 1 of the compound has been reported (19). However, we have produced a diabetic state in Rhesus monkeys lasting up to 2 yr with single doses of streptozotocin (20).

Summary. The renal oncogenic activity of streptozotocin in male Holtzman rats was significantly decreased by nicotinamide. Adenomas of the kidney were noted in 77% (21/28) of the animals treated with single iv dose of the streptozotocin, 50 mg/kg, while only 18% (5/28) of animals given nicotinamide ip, 350 mg/kg, 10 min before and 180 min after the same dose of streptozotocin had demonstrable renal tumors. Moreover, the renal adenomas induced by streptozotocin alone occurred sooner and were generally larger when compared with those in the animals treated with the nicotinamide-streptozotocin combination.

The 50 mg/kg dose of streptozotocin was diabetogenic in all rats, but the diabetic state was not permanent. Spontaneous recovery from the diabetes was first noted after 8 and 10 months of followup, and after 16 months none of the surviving rats were diabetic.

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