Pancreatic Islet Cell Tumors Produced by the Combined Action of Streptozotocin and Nicotinamide[1] (35561)

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(Introduced by Robert L. Dixon)

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Streptozotocin [glucopyranose, 2-deoxy-2-(3-methyl-3-nitrosoureido)-D-,], a naturally occurring nitrosourea (1), has been shown to exhibit antibiotic (2), oncolytic (3), oncogenic (4), and diabetogenic (5) activities. Moreover the diabetogenic action of streptozotocin is prevented by nicotinamide in many animal species (6). Because of its selective destruction of pancreatic islet β-cells (5, 7) the antibiotic has been used in the palliative chemotherapy of human pancreatic islet cell carcinoma (8). In the course of a long-term study in male Holtzman rats on repression by nicotinamide of the renal oncogenic action of streptozotocin, pancreatic islet cell tumors (nesidioblastomas) were discovered serendipitously. The present communication presents the incidence, chronology, and characteristics of these tumors.

Material and Methods. Groups of male Holtzman rats were treated on the first day of the study with a single dose of streptozotocin[2] 50 mg/kg iv; with two doses of nicotinamide[3] 350 mg/kg ip at 3-hr intervals; with both streptozotocin, 50 mg/kg IV and nicotinamide, 350 mg/kg ip (10 min before and 180 min after the injection of streptozotocin) or with the streptozotocin vehicle iv.

Results. Pancreatic islet cell tumors occurred in 64% (18/28) of rats treated with both streptozotocin and nicotinamide (Table I). The tumors first became apparent on day 226 of the study but 83% of them were detected in rats sacrificed on days 438–547. Only a single pancreatic islet cell tumor was observed in 26 animals treated with streptozotocin alone, while none were noted in animals treated with nicotinamide alone or the vehicle alone. Sequential measurements of blood sugar (9) (Fig. 1), provided evidence for the development of these pancreatic islet cell tumors. Depression of the blood sugar was progressive from month 12 onward, and the affected animals showed true hypoglycemia (BS <45 mg/100 ml) from month 14 to the termination of the study at 18 months. The nicotinamide controls also showed a decrease in blood sugar on aging (10) but at all times after month 12 the blood sugar values for the animals treated with both streptozotocin and nicotinamide were significantly lower ($p < 0.01$) than those of the animals treated with nicotinamide alone.

The tumors presented as brownish red bodies varying in size from $5 \times 3 \times 2$ mm to $6 \times 3 \times 2$ mm and weighed 50–70 mg. They were first noted in the region of the tail of the pancreas adjacent to the hilus of the spleen. Some, however, were found in the head of the pancreas lying adjacent to the duodenum. Others were embedded in the body of the gland and were seen only on microscopic examination. Microscopically the sections of islet cell tumor showed a well circumscribed neoplasm which in some areas revealed thin encapsulation (Fig. 2A). The tumor was highly vascular and resembled normal islet histologic structure. The islet tumor cells were polyhedral or cuboidal in

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[2] Upjohn Co., Lot 6742-DEG-30-5, 10 mg/ml in 0.025 *M* citric acid in saline, pH 4.0.

[3] 35 mg/ml in saline.

TABLE I. Incidence of Pancreatic Islet Cell Tumors in Male Holtzman Rats Produced by the Combined Action of Streptozotocin and Nicotinamide.

Treatment (mg/kg × no. of Rx)	Route	No. treated	Surviving 8 months or longer		Earliest day tumor detected	Animals with tumors		Day last animal sacrificed
			No.	(%)		No.	(%)	
Streptozotocin, 50 × 1; and nicotinamide, 350 × 2[a]	iv[b] ip	28	26	92	226	18	64	547
Streptozotocin, 50 × 1	iv[b]	26	21	81	543	1	4	547
Nicotinamide, 350 × 2 (q/3 hr)	ip	27	24	89	—	0		550
0.025 *M* Citric acid in saline	iv[b]	26	22	84	—	0		551

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

shape and tended to be arranged as anastamosing short cords forming small glomerular lobules separated by thin vascular bands of connective tissue (Fig. 2C). The tumor cells revealed centrally placed round or oval nuclei which contained no nucleoli and were finely granular. Small strands or groups of pancreatic acinar cells were scattered in the tumor but ductal structures were not seen nor were mitoses observed. The majority of the cells exhibited intense purple cytoplasmic granules (Fig. 2B) after staining according to the aldehyde fuchsin method of Gomori (11). In some areas of the tumor, however, small groups of cells were unstained or only very lightly granular (Fig 2D).

The tumors were insulin secreting as evidenced by: (i) hypoglycemia as demonstrated in Fig. 1; (ii) intensely basophilic cytoplasmic granulation (11); and (iii) positive immunoreactive insulin (12) concentrations of 230-350 units/g of tumor.

Discussion. Under the conditions of this chronic experiment, nicotinamide exerted two diametrically opposite effects: the vitamin appeared to act as a cocarcinogen in the

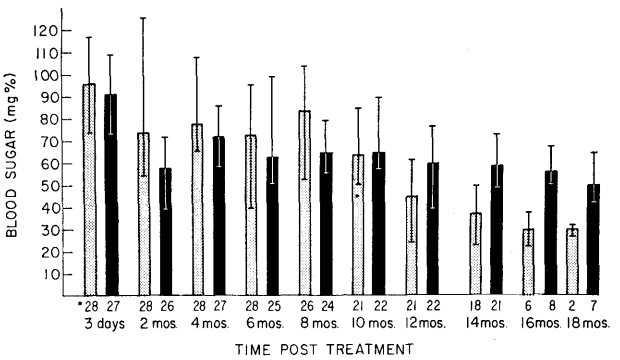

FIG. 1. Sequential measurements of the blood sugar of rats protected against streptozotocin diabetes with nicotinamide (cross-hatched bars) the mean nonfasted blood sugars (± range) of rats treated intravenously on day 0 with streptozotocin (50 mg/kg × 1) and with intraperitoneal nicotinamide (350 mg/kg) 10 min before and 180 min after streptozotocin (black bars) the mean nonfasted blood sugars (± range) of rats receiving only nicotinamide (350 mg/kg ×2) q/3 hr ip. Sugars were measured enzymatically with glucose oxidase (9) on blood taken from the caudal vein.
* Number of animals studied.

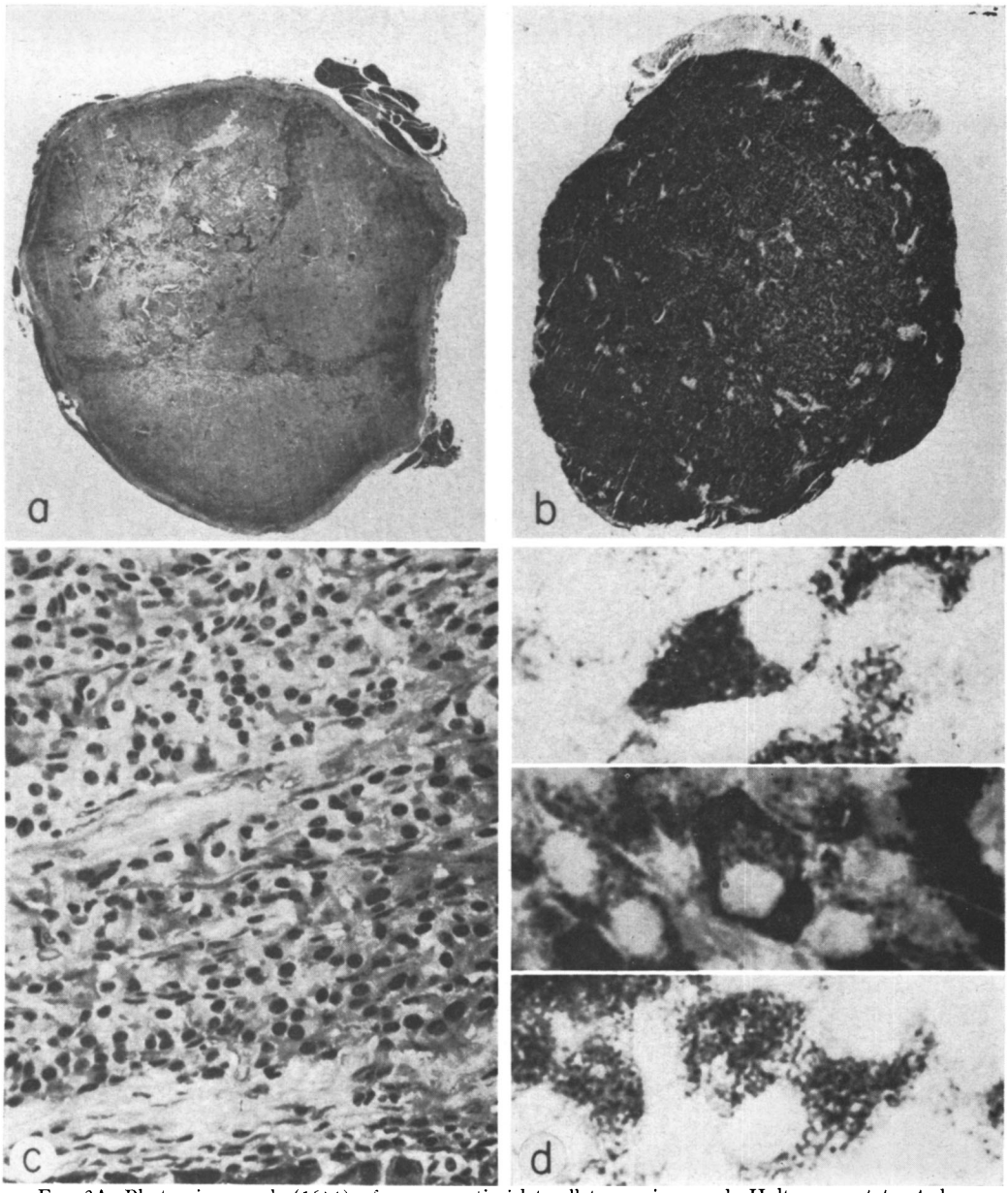

FIG. 2A. Photomicrograph (16×) of a pancreatic islet cell tumor in a male Holtzman rat treated with both streptozotocin and nicotinamide, stained with hematoxylin and eosin. The tumor was found at sacrifice on day 527, measured 5 × 3 × 2 mm, weighed approximately 70 mg and contained 230 U of insulin/g of tumor. Exocrine pancreatic tissue is shown adhering to the capsule of the tumor. In addition, dark strands of compressed exocrine epithelium persist in the body of the tumor. (B) Photomicrograph (16×) of a second pancreatic islet cell tumor produced by the conjoint action of streptozotocin and nicotinamide. The section has been stained with the aldehyde fuchsin method of Gomori (11). In this black and white photograph the intense purple coloration of the section is indicated by the dark granular appearance of the majority of the cells. (C) Islet tumor cells showing cord-like arrangement and vascularity (hematoxylin and eosin 400×). (D) Unstained or very lightly stained granular cells intermingled with intensely stained granular cells seen in a few areas of the tumor shown in Fig. 2B (Gomori aldehyde fuchsin 1000×).

production of the pancreatic islet cell tumors, and concomitantly as an inhibitor of the renal oncogenic action of streptozotocin as will be described in a forthcoming publication. Although other techniques for the production of pancreatic islet cell tumors have been described (13, 14), the present study suggests that the combined action of streptozotocin and nicotinamide in male Holtzman rats provides a facile method for producing such tumors by chemical means. Studies now in progress in our laboratory are designed to determine, by periodic serum insulin immunoassays and necropsy, the detailed chronology of the development of these nesidioblastomas. Moreover, since streptozotocin has been reported to be effective in the therapy of human islet cell carcinoma, it is planned to treat these streptozotocin–nicotinamide-induced tumors with streptozotocin itself.

Summary. Pancreatic islet cell tumors (nesidioblastomas) were produced in 64% (18/28) of the male Holtzman rats treated with both streptozotocin and nicotinamide, while only one tumor was noted in 26 male Holtzman rats treated with streptozotocin alone, and none in rats treated with nicotinamide alone or with the streptozotocin vehicle. The majority of these neoplasms occurred in animals sacrificed 14–18 months after treatment. Hypoglycemia, intensely basophilic cytoplasmic granulation in the islet tumors, and the presence of immunoreactive insulin in tumor extracts indicate that the adenomas secreted insulin.

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