Streptozotocin Diabetes in Monkeys and Dogs, and Its Prevention by Nicotinamide (37355)

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(Introduced by D. P. Rall)

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The antibiotic and antitumor agent, streptozotocin, produces a permanent diabetic state in rodents, dogs and monkeys through a direct destruction of the pancreatic beta cell (1, 2). This observation has been exploited clinically in the treatment of malignant insulinoma in man (3). In previous studies, we have demonstrated that pharmacologic doses of nicotinamide prevent the diabetogenic action of streptozotocin in mice and rats without loss of antitumor activity against the L-1210 mouse ascitic leukemia system (4, 5). In contrast to the diabetogenic activity which is mediated through a depression in pyridine nucleotide concentrations, the antileukemic activity of streptozotocin has been related to the blockade of cells in the G_2 phase of the cell cycle and an inhibition of DNA synthesis (6).

In preparation for the possible use of the nicotinamide–streptozotocin combination for treatment of human malignancies other than malignant insulinoma, these studies were extended to the beagle dog and rhesus monkey.

Methods. Rhesus monkeys (Macaca mulatta) weighing 3.14–5.37 kg and beagle dogs, 6.8–11.3 kg, were used in the study and maintained on Purina monkey chow or Gaines dog meal. Streptozotocin, NSC-85998, was prepared fresh daily in 0.05 *M* citrate buffer, pH 4.0 and administered by rapid intravenous injection. Nicotinamide (Matheson, Coleman, and Bell) was dissolved in normal saline for intravenous injection and gavage in monkeys, and placed in gelatin capsules for oral administration to dogs. The ocular fundi of long-term diabetic monkeys were followed with ophthalmoscopy. All animals underwent autopsy with complete gross and microscopic examination of all organs and tissues listed in the Cancer Chemotherapy National Service Center (CCNSC) protocol (7). The retinae of diabetic monkeys, sacrificed after an extended observation period, were digested with trypsin and examined histologically for signs of diabetic retinopathy.

Portions of the pancreas from monkeys were obtained immediately after sacrifice for mincing and fixation in 3% gluteraldehyde in sodium phosphate buffer, pH 7.4, and rinsed in buffer. They were then postfixed in 2% osmium tetroxide in phosphate buffer, dehydrated in graded acetone, and embedded in epoxy resin. Ultrathin sections of the tissue, unstained or stained by Venable's lead citrate method, were examined, using an RCA EMU 3H electron microscope (8).

Results. Streptozotocin produced diabetes in monkeys. A single intravenous injection of streptozotocin, 50–55 mg/kg, was diabetogenic in all three test monkeys. However, the degree of glucose intolerance produced, varied greatly among the individual animals. (Table I). The most marked response appeared in Monkey 2 which was found to have a blood

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TABLE I. Fasting Blood Glucose of Rhesus Monkeys after Treatment with Streptozotocin and Nicotinamide.

Monkey	Treatment[a]	Day	Blood glucose (mg/100 ml)
1	S, 50 mg/kg × 1, iv	0	80
		243	208
		691	212
2	S, 50 mg/kg × 1, iv	0	122
		5	720 (died in ketoacidosis)
3	S, 55 mg/kg × 1, iv	0	100
		165	550
		336	550
4	S, 12 mg/kg × 14 days, iv	0	120
		8	270
		15	120
5	S, 15 mg/kg/day × 4 days, iv	0	100
		7	14
		15	104
6	N, 500 mg/kg, iv 15 min prior to S, 50 mg/kg, iv	0	98
		25	108
		109	92
7	N, 250 mg/kg, iv 15 min prior to S, 50 mg/kg, iv	0	96
		25	100
		88	96
8	N, 500 mg/kg, PO 1 hr before and 4 hr after S, 50 mg/kg, iv	0	118
		24	85
		80	104
9	N, 100 mg/kg, PO 1 hr before and repeated 4 and 8 hr after S, 50 mg/kg, iv	0	92
		20	100
		38	84

[a] S, Streptozotocin; N, Nicotinamide.

sugar concentration of 760 mg/100 ml on Day 4 after treatment, and was moribund with ketoacidosis on Day 6. The remaining two animals survived the initial posttreatment period and were placed on long-term observation for complications attributable to their diabetes. They were sacrificed in a persistent hyperglycemic state at 336 and 691 days, respectively. During this time, ophthalmologic examination failed to show signs of diabetic retinopathy or cataract formation. Monkey 3 developed persistent elevations of serum glutamic–pyruvate transaminase (SGPT) and BSP retention. Neither animal demonstrated any abnormality in renal function.

Histological examination of the pancreatic islets showed virtually complete absence of beta cells on staining with chrome alum hematoxylin phloxine, while alpha and delta cells appeared qualitatively and quantitatively normal. There was marked fatty change in the liver of Monkey 2 which died in ketoacidosis, but hepatic histology of the two long-term animals was normal. The kidneys of Monkey 1 demonstrated areas of chronic pyelonephritis, but there was no evidence of renal tubular pathology or renal adenomas in any of the specimens. Histologic examination of the retinae showed no evidence of microaneurysms. The major blood vessels and hearts were essentially free from atherosclerotic changes.

Electron microscopy studies were performed on the islets of Langerhans of long-term diabetic Monkeys 1 and 3. The islets were populated by normal alpha and delta cells, while cells containing typical beta cells granules

could not be found. However, interspersed in the islet tissue were cells that appeared essentially agranular while possessing a prominent endoplasmic reticulum and Golgi apparatus, swollen mitochondria, and occasional very dense granules (Fig. 1).

A single male monkey was placed on a daily schedule of administration, at a dose of 12 mg/kg/day for 14 consecutive days (total dose, 168 mg/kg, Table I). Except for a transient rise in blood glucose concentration on Day 8, there was no overall lasting effect; the fasting blood glucose returned to and remained at pretreatment levels, despite the uninterrupted administration of six additional doses. Histology of the pancreas of this animal demonstrated positive staining for beta cell granules.

An additional monkey was administered a daily dose of 15 mg/kg for 4 consecutive days for a total dose of 45 mg/kg (Table I). There was a transient increase in blood glucose concentration to 140 mg/100 ml three days after completion of treatment, but this mild degree of glucose intolerance was reversible. Pancreatic islets and beta cell granules were well preserved at time of sacrifice.

Monkeys protected with nicotinamide. Monkeys received intravenous and oral nicotinamide prior to streptozotocin using the four different treatment regimens presented in Table I. Although there was a transient period of hyperglycemia during the early posttreatment phase in Monkey 7, all animals were protected against diabetes. Fasting blood sugars remained normal throughout the observation period which extended from 38 to 109 days.

Light and electron microscope studies of the islets of Langerhans of all animals were comparable to those of untreated monkeys, with normal numbers and appearance of beta, alpha, and delta cells (Fig. 2).

Streptozotocin-induced diabetes in beagle dogs. Both dogs which received a single intravenous dose of streptozotocin, 35 mg/kg, developed diabetes which persisted throughout the 24- and 49-day observation (Table II). Beta cell granules could not be identified in the cells of the islets of Langerhans of either animal by light microscopic methods.

Beagle dogs protected with nicotinamide. Intravenous nicotinamide was tested against a single diabetogenic dose of streptozotocin. Oral nicotinamide was chosen to protect against a regimen of two diabetogenic doses administered one week apart. In both animals, protection against diabetes was demonstrated

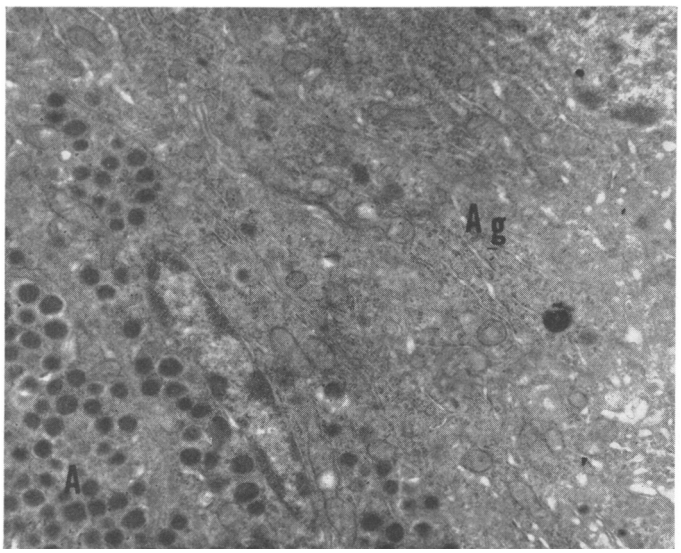

FIG. 1. Portion of an islet of Langerhans showing normal alpha cells (A) and essentially agranular cells (Ag) presumed to represent surviving altered beta cells. Animal No. 100 which received a single intravenous dose of streptozotocin at 55 mg/kg. ×8700.

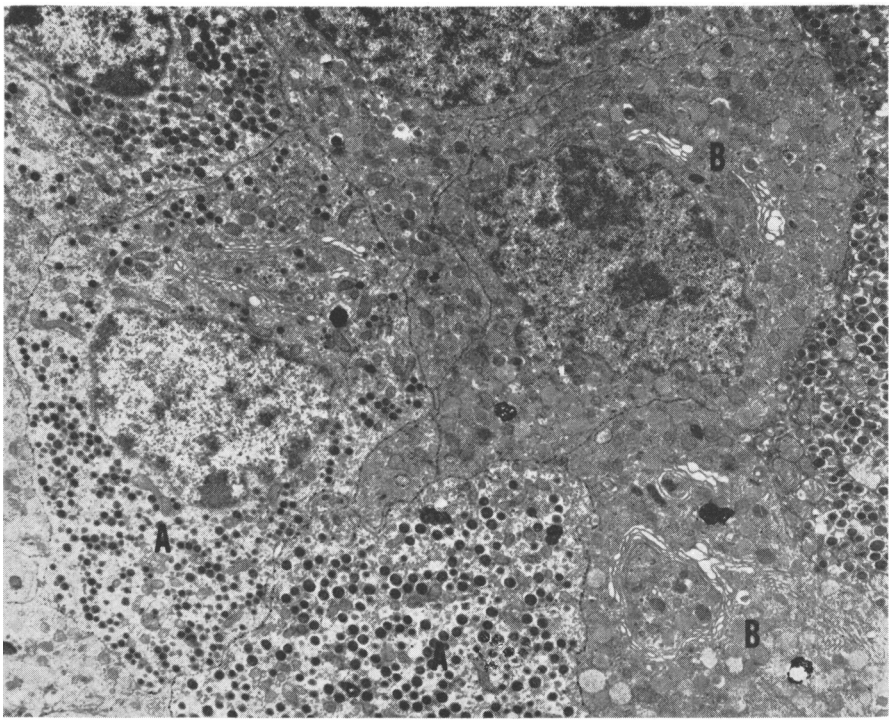

FIG. 2. Portion of an islet of Langerhans showing normal appearing alpha (A) and beta (B) cells. The latter appeared to have a full complement of β type granules. From animal 126 which had received 500 mg/kg nicotinamide 15 min before a single dose of streptozotocin at 50 mg/kg and remained normoglycemic for two months before sacrifice. ×4800.

both biochemically (Table II) and histologically, with preservation of the pancreatic beta cells.

TABLE II. Blood Glucose in Beagle Dogs after Treatment with Streptozotocin and Nicotinamide.

Dog	Treatment[a]	Day	Blood glucose (mg/100 ml)
1	S, 35 mg/kg × 1, iv	0	70
		14	272
		27	291
2	S, 35 mg/kg × 1, iv	0	107
		10	480
		78	565
3	N, 500 mg/kg, iv 15 min prior to S, 35 mg/kg, iv	0	116
		35	105
		80	92
4	N, 50 mg/kg 1 hr before and repeated 3 and 7 hr after S, 35 mg/kg/week × 2, iv	0	100
		17	81
		41	127

[a] S, Streptozotocin; N, Nicotinamide.

Discussion. The purpose of the present study was to examine the protective role of nicotinamide in the monkey and dog and determine the long term toxic effects of streptozotocin in these specimens. The regimens of nicotinamide administration employed were based on the following information obtained in studies in rodents: oral nicotinamide can prevent the diabetes of intravenous streptozotocin; a schedule of small divided doses of nicotinamide administered over the first 24 hr were more effective than the large single dose given just prior to streptozotocin (9). As in the rodents, oral and intravenous nicotinamide protects the pancreatic beta cell of monkeys and dogs from the diabetogenic action of streptozotocin.

Of interest was the schedule-dependence of treatment of the diabetogenesis in the monkey. Administration of total doses of 45 and 168 mg/kg over a 4–14 day period failed to produce significant beta cell toxicity. This is in contrast to the major and apparently ir-

reversible diabetic state produced when the same or smaller total doses are administered as a single injection. This observation would suggest that a single intermittent high-dose therapy may be the most effective schedule of treatment for malignant insulinoma, the regimen employed in the majority of successfully treated case reports (3).

The permanence of the diabetic state produced by streptozotocin was demonstrated in the two monkeys kept on observation for 1–2 yr. Typical beta cells in islets of these animals could not be identified by electron microscopy but scattered degenerated cells were found. These probably represent the few remaining insulin-producing cells that have maintained their integrity, but whose morphology has become distorted by the previous action of the drug and/or the constant, intense stimulation of the persistent hyperglycemia.

A histologic study of the retinae, kidneys and blood vessels for changes which could be related to the long-term diabetic state failed to demonstrate the complications recognized in human diabetes mellitus. It is probable that observation periods longer than 2 yr are required, or that the rhesus monkey may not be the most suitable species for this type of investigation.

The observation of normal renal function in animals has particular interest since renal tubular damage has been the most significant toxicity encountered with the human use of the compound (10), while this toxicity was not observed in either the monkey or dog. Renal adenomas have been demonstrated in diabetic rats kept on observation for one year (11). This carcinogenic activity has been related to the methylating action of the 1-methyl-1-nitrosourea cytotoxic moiety, a known tumor-producing agent. Renal tumors were not observed in the two long-term diabetic monkeys.

Summary. Streptozotocin produces a permanent diabetic state in monkeys and dogs which can be prevented by the use of oral or intravenous nicotinamide. A total dose that is diabetogenic in the monkey when administered by a single injection is ineffective in producing a diabetic state when administered over a 4–14 day period. This observation may have relevance in regard to the schedule employed in the treatment of malignant insulinoma in man.

Rhesus monkeys observed in a persistent diabetic state for up to two years failed to demonstrate any of the known extra pancreatic complications of human diabetes mellitus. There was no evidence of renal tumor induction in these animals, as had previously been reported in rats.

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