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Streptozotocin-Induced Pancreatic Insulitis: New Model of Diabetes Mellitus

Abstract. *Multiple small injections of streptozotocin in mice produce pancreatic insulitis, with progression to nearly complete beta cell destruction and diabetes mellitus. The timing and appearance of the inflammatory islet lesions suggest but do not prove that streptozotocin acts by initiating a cell-mediated immune reaction. Ultrastructural evidence of abundant type C viruses within beta cells of treated mice suggests that streptozotocin may activate murine leukemia virus in vivo in susceptible hosts.*

Streptozotocin (SZ) is a broad-spectrum antibiotic possessing antitumor (*1*), oncogenic (*2*), and diabetogenic (*3*) properties. The last action is mediated by pancreatic beta cell destruction and is widely used as a method for induction of diabetes in experimental animals and for clinical treatment of malignant beta cell tumors. For induction of experimental diabetes, SZ is conventionally administered as a single injection (*3*). After rapid clearance of SZ from the bloodstream [serum half-life is 15 minutes (*4*)], light microscopic evidence of beta cell necrosis is apparent within 24 hours (*5*). Beta cell necrosis, however, is detected by conventional ultrastructural examination after 2 to 4 hours (*5*), and intramembranous particle depletion of beta cell plasma membranes is observed within 45 minutes in freeze-fracture studies (*6*). Dissolution and phagocytosis of necrotic cells is rapid, with virtually no evidence of debris or inflammation visible after 3 days (*3, 5*). In a correspondingly rapid manner, blood glucose values peak 1 to 2 days after SZ administration and remain elevated if the appropriate quantity of the agent is given (*7*).

We present evidence here that SZ, given intravenously or intraperitoneally to laboratory mice in multiple subdiabetogenic doses, the usual method of clinical administration (*1, 4*), induces (i) pronounced pancreatic insulitis, with eventual destruction of insulin-secreting beta cells and diabetes mellitus, and (ii) enhanced replication of type C virus particles within pancreatic beta cells. The timing and appearance of the inflammatory islet lesions suggest but do not prove that SZ may initiate a cell-mediated immune reaction directed against the beta cells. The relevance, if any, of the increased number of type C virus particles to the inflammation and beta cell destruction is unknown.

Forty adult male mice (Charles River CD-1 strain), allowed free access to food and water, received five daily intravenous or intraperitoneal injections of SZ (40 mg per kilogram of body weight) (*8*) dissolved in a citrate buffer, *p*H 4.2, just before injection. A total of 43 uninjected mice and animals receiving equal volumes of citrate buffer were controls. Blood samples for glucose determination were collected in heparin-treated pipettes by orbital sinus puncture and assayed as described (*9*). Samples were obtained before each of the five daily injections and at frequent intervals afterward until animals were killed 6 days after the last injection. Four mice were also killed 12, 16, and 25 days after the completion of the SZ injections. The unpaired *t*-test (*10*) was used in statistical analyses. Pancreatic tissue obtained at death was fixed for light and electron microscopic study as described (*11*).

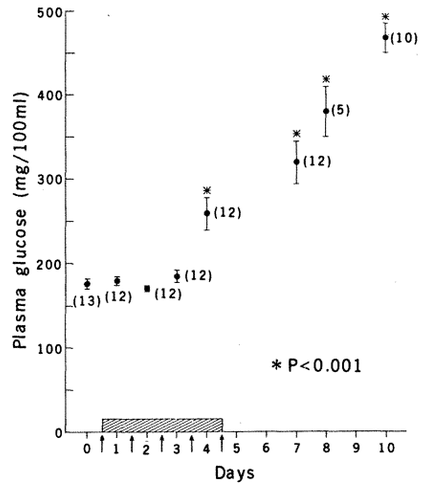

Fig. 1. Plasma glucose response in Charles River CD-1 mice after five intraperitoneal injections of SZ (40 mg/kg). The mean glucose value was significantly increased over the preinjection value (day 0) after the fourth dose of SZ and continued to increase progressively until termination of the experiment. Virtually identical results were obtained in 24 mice given intravenous injections of SZ (data not shown).

Plasma glucose values for SZ-injected mice were significantly elevated after the fourth injection (*12*) and increased substantially during the subsequent 6 days (Fig. 1) (*13*). Examination by light microscopy (Fig. 2) revealed large numbers of lymphocytes, moderate numbers of macrophages, and rare neutrophils surrounding and permeating the islets of Langerhans, with distortion of architecture and beta cell necrosis. Surviving beta cells were variably degranulated and the islets were generally smaller. Islet inflammation gradually diminished in animals killed 12, 16, and 25 days after the completion of injections [mean plasma glucose values of 396 mg/100 ml (N = 12), 525 mg/100 ml (N = 7), and 657 mg/100 ml (N = 7), respectively], and the remaining islets were small and composed almost exclusively of alpha and delta cells.

Ultrastructural studies of the islets in ten mice killed 6 days after the completion of injections revealed occasional necrotic beta cells and numerous infiltrating lymphocytes and macrophages. Unexpected, however, was the presence of large numbers of type C virus particles (*14*) within many intact, partially degranulated beta cells (Fig. 3B). Alpha and delta cells were normal. The pancreatic islets of uninjected mice and mice injected with citrate buffer appeared normal when examined by light and electron microscopy. Only an occasional virus particle was observed within the usually well-granulated beta cells (Fig. 3A). Quantitative morphometric studies of the number of virus particles per cell and the frequency of cells harboring viruses were not performed. Viruses were observed neither in the alpha and delta cells nor in the inflammatory cells.

In an effort to ascertain the timing of the appearance of the inflammatory cells in and around the pancreatic islets, animals were killed at daily intervals after having received one, two, or three intraperitoneal injections of SZ (40 mg/kg).

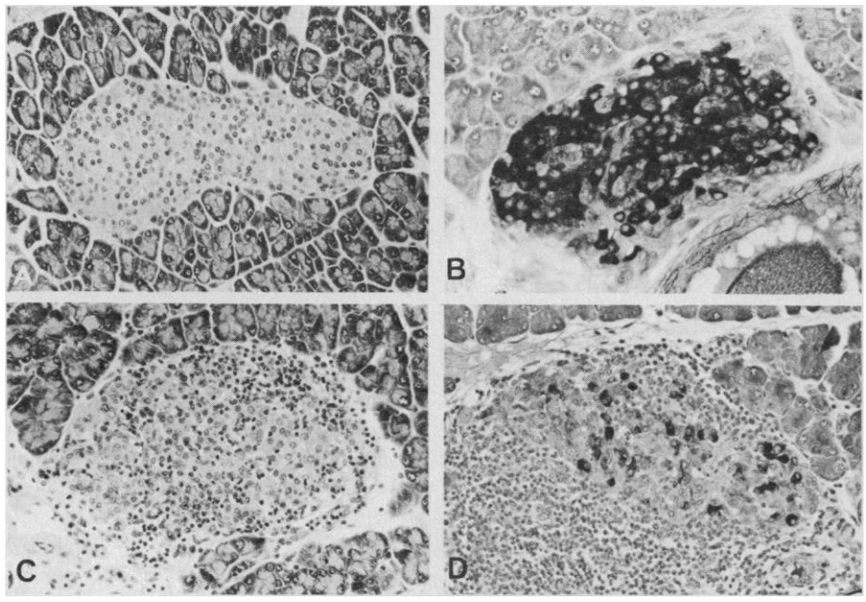

Fig. 2. (A and B) Light microscopic photomicrographs of pancreatic islets from mouse injected with citrate buffer. The well-delineated islets are enmeshed in the surrounding acinar cells of the exocrine pancreas. Well-granulated beta cells stain intensely with aldehyde fuchsin (black in photograph) indicating an abundance of stored insulin. (A) Hematoxylin and eosin, × 144; (B) aldehyde fuchsin, × 222. (C and D) Inflamed islets from mouse killed 6 days after receiving five injections of SZ. The interior and periphery of the islets are permeated with large numbers of mononuclear inflammatory cells (identified as lymphocytes and macrophages by electron microscopy), which distort the islet architecture and extend into the adjacent exocrine tissue. Substantial beta cell degranulation is evident (D) and is consistent with the presence of hyperglycemia. (C) Hematoxylin and eosin; (D) aldehyde fuchsin; both × 144.

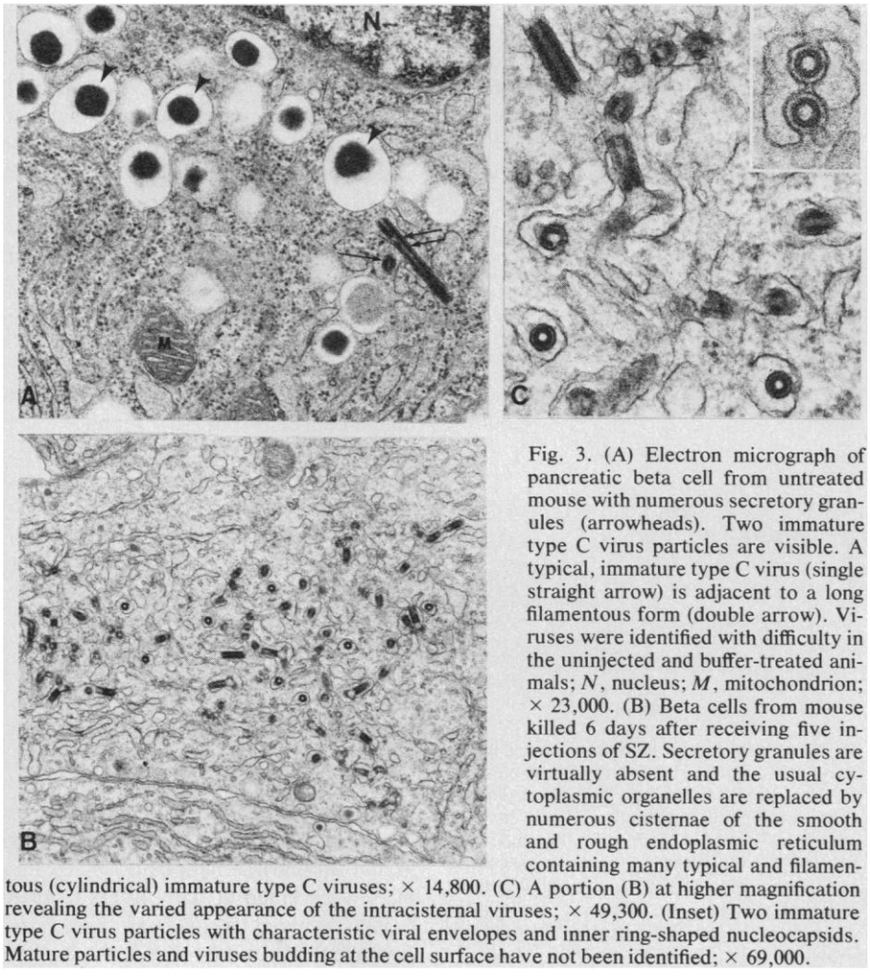

Fig. 3. (A) Electron micrograph of pancreatic beta cell from untreated mouse with numerous secretory granules (arrowheads). Two immature type C virus particles are visible. A typical, immature type C virus (single straight arrow) is adjacent to a long filamentous form (double arrow). Viruses were identified with difficulty in the uninjected and buffer-treated animals; *N*, nucleus; *M*, mitochondrion; × 23,000. (B) Beta cells from mouse killed 6 days after receiving five injections of SZ. Secretory granules are virtually absent and the usual cytoplasmic organelles are replaced by numerous cisternae of the smooth and rough endoplasmic reticulum containing many typical and filamentous (cylindrical) immature type C viruses; × 14,800. (C) A portion (B) at higher magnification revealing the varied appearance of the intracisternal viruses; × 49,300. (Inset) Two immature type C virus particles with characteristic viral envelopes and inner ring-shaped nucleocapsids. Mature particles and viruses budding at the cell surface have not been identified; × 69,000.

One injection of SZ induced transient, mild beta cell degranulation; insulitis and plasma glucose elevation were absent. The islets of mice receiving two and three SZ injections had moderate beta cell degranulation during the initial 4 to 5 days after the last injection. Insulitis was first recognized 6 and 5 days after the last injection in the mice that received two and three injections, respectively. Inflammation was more pronounced after three injections, and these animals also evidenced mild hyperglycemia (mean plasma glucose, 214 mg/100 ml) 7 days after completion of injections.

The same dose schedule (*8*), routes of administration, and timing of killing failed to produce similar lesions in 21 Charles River rats despite the fact that these animals become markedly hyperglycemic after a single large injection of SZ.

In contrast with the one-injection technique of SZ-induced diabetes, wherein beta cell necrosis occurs within 4 hours and hyperglycemia is achieved rapidly, multiple subdiabetogenic injections of SZ induced gradual elevation of plasma glucose in all animals tested, with maximum values realized 1 week or longer after the last injection. Microscopic examination revealed mononuclear inflammatory cells, including large numbers of lymphocytes, in and around the pancreatic islets in all mice studied, in contrast with the virtually inflammation-free islet lesions observed after a single large injection. The time required for the first appearance of inflammatory cells (5 to 6 days after the last injection) is compatible with a cell-mediated immune reaction conceivably directed against beta cells modified by the administration of SZ. A nonspecific inflammatory response to low-grade beta cell injury induced by SZ has not been excluded, however. In further support for an immunologic pathogenesis, the reduction in beta cell numbers and plasma glucose elevation become progressively more pronounced during the 10 to 25 days after the last injection, long after SZ is cleared from the bloodstream (*4*) and the short-lived SZ beta cytotoxic action completed. It is therefore tempting to speculate that the mononuclear inflammatory cells (lymphocytes and macrophages) are responsible for the progressive beta cell destruction and the resulting increasingly severe hyperglycemia.

The possibility of an immunological role of the type C virus particles within the beta cells of SZ-treated mice should be considered. Virus particles were not observed budding at the beta cell plasma membranes, and the cells in which they

were located did not usually manifest degenerative changes. It is conceivable, nevertheless, that interactions between virus and cell membrane may have induced the formation of accessible, abnormal beta cell immunogenic proteins responsible for the initiation of a cell-mediated immune response (*15*).

Pancreatic insulitis was reported in experimental virus-induced diabetes (*16*), in juvenile diabetics autopsied shortly after onset of clinical symptoms (*17*), and in rare examples of maturity-onset diabetes (*18*). A chronic inflammatory cell infiltrate within the islets of Langerhans was also described after unsuccessful attempts to produce an immune-type diabetes (*19*).

Of independent significance, however, is the observation that SZ, a beta cell toxin, is apparently responsible for the activation of virus replication in mouse beta cells. The genome of the mouse type C virus is present in most if not all strains of laboratory and wild mice (*20*). Furthermore, structures resembling type C virus particles have been observed in the beta cells of certain inbred mice (*21*). 5′-Bromodeoxyuridine and 5′-iododeoxyuridine induce viral genome activation in vitro (*22*), and activation of mouse leukemia virus is induced in vivo by x-irradiation, chemical carcinogens, and steroid hormones (*23*). This report of virus activation in vivo by SZ is important because of the drug's occasional clinical use in the treatment of neoplastic disease. This finding is of more than theoretical importance when one considers the oncogenic potential of SZ (*2*).

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12. Results of intravenous and intraperitoneal injections were essentially equivalent. Only the data from animals given intraperitoneal injections are presented.
13. Mean plasma glucose values for mice receiving citrate buffer alone were 175 to 185 mg/100 ml; those for mice receiving a single injection of SZ (200 mg/kg) were greater than 500 mg/100 ml within 48 hours.
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Alternative Transformation Behavior in Sulfides: Direct Observations by Transmission Electron Microscopy

Abstract. *Structural phase transformations in Ni_7S_6 and Cu_7S_4 have been observed dynamically by in situ experiments in a transmission electron microscope. In this way it is possible to demonstrate the possibility of two fundamentally different types of behavior: (i) the ideal transformation from the stable high-temperature form to the stable low-temperature form and vice versa and (ii) alternative metastable processes which operate when the formation of the low-temperature state is impeded.*

The process of inversion from a high-temperature to a low-temperature form is of considerable importance for an understanding of the behavior of crystalline phases. In particular, the situation can arise when the kinetics of the process are such that the formation of the low-temperature form is impeded and hysteresis is introduced, or in the extreme case when the low-temperature form is inaccessible. Although the existence of a metastable, high-temperature polymorph is not uncommon, in some cases processes may operate to reduce the free energy of the system by the formation of some modification of the high-temperature form. These alternative processes lead to structural forms which do not have a stability field at any temperature and whose modes of behavior are determined by the kinetics of the processes involved rather than by thermodynamic considerations alone. Such alternative states may persist for long periods of time and have been shown to be fundamental to a description of the transformation behavior of a number of silicate systems (*1*). In this report I describe experiments in which both the ideal and the alternative behavior can be observed dynamically and the course of the transformations can be directly controlled by variations in the experimental conditions.

Structural phase transformations in a number of sulfides occur at rates which make direct observation possible in a transmission electron microscope (TEM). The transformations are induced by electron beam heating, and the temperature of an individual crystal fragment is controlled by focusing and defocusing or by lateral shifts of the beam, although the temperature cannot be directly measured in this way. Further details of the experimental method are described elsewhere (*2*). The ability to observe these transformations directly makes it possible to examine the ideal and the alternative behavior separately and to study the conditions operative in each case independently. Such information is not readily obtainable by other means, and the method leads to a rigorous definition of the transformation be-