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Insulin therapy prevents spontaneous recovery from streptozotocin-induced diabetes in Syrian hamsters

An autoradiographic and immunohistochemical study*

Parviz M. Pour[1,2], William Duckworth[3], Kim Carlson[1], and Kathy Kazakoff[1]

[1] The Eppley Institute, [2] Department of Pathology and Microbiology, [3] Department of Internal Medicine, University of Nebraska Medical Center, Omaha, Nebraska, USA

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Summary. Streptozotocin (Sz) given as a single dose of 50 mg/kg body wt. caused severe diabetes in Syrian hamsters. However, the level of blood glucose decreased gradually after 21 days post-Sz and reached the near normal level at 70 days in 90% of hamsters. The recovery from diabetes was associated with the regeneration of the β-cells of islets and a reduction in the initially increased number of α- and δ-cells. Daily treatment of diabetic hamsters with insulin was associated with the persistence of severe diabetes, lack of or minimal tendency for β-cell regeneration and sustained hyperplasia of α- and δ-cells in 90% of hamsters. Insulin also inhibited DNA synthesis (as measured by incorporation of tritiated thymidine), in ductal, ductular and acinar cells in Sz-pretreated hamsters but not in normoglycemic control hamsters treated with insulin alone. The results demonstrate a deleterious effect of exogenous insulin in the course of Sz-induced diabetes in hamsters.

Key words: Streptozotocin – Diabetes – Syrian hamsters – Autoradiography – Immunohistochemistry

Introduction

Streptozotocin (Sz) is a pancreatic β-cell cytotoxic nitrosourea which produces permanent diabetes in many species, including the rat, mouse, dog, Chinese hamster and monkey (Dulin et al. 1983; Baetens et al. 1978). Syrian hamsters also respond to a single dose of Sz with β-cell necrosis, but in contrast to other species, β-cells regenerate and a high percentage of hamsters subsequently recover spontaneously from their diabetes (Phares 1980). Higher doses of Sz, while increasing acute mortality, do not result in a higher percentage of permanent diabetes in this species. Multiple daily doses of Sz, however, result in permanent diabetes in a large number of hamsters (Rakieten et al. 1976).

To examine the regenerative properties and capacity of β-cells in Sz-treated hamsters, we initiated a study of the effects of this diabetogenic agent, and included a group of insulin-treated hamsters to reduce acute toxicity. In each group, including a group receiving insulin alone and an untreated control group, we examined the glucose levels in the serum and urine, ketones in the urine, the DNA synthesis by autoradiography following treatment of hamsters with tritiated thymidine, and the immunoreactivity of pancreatic cells with antibodies against insulin, glucagon and somatostatin at different intervals.

The results showed that pancreatic islet cells and ductular cells proliferate in Sz-treated hamsters and animals recover from their diabetes spontaneously. However, contrary to our expectation, insulin therapy prevents the spontaneous recovery and causes persistent severe hyperglycemia, obviously by preventing insular and ductular cell proliferation.

Materials and methods

Male Syrian hamsters from the Eppley colony were used. The 8-week-old hamsters with an average initial body wt. of 120 g were housed in groups of 5 in plastic cages on granular cellulose bedding (Bed-O-Cobs, The Anderson Cob Division, Maumee, Ohio), kept under standard laboratory conditions (room temperature 21 ± 3° C; light/dark cycle: 12 h/12 h; relative humidity 40 ± 5%), and given Wayne Lab Blox (Allied Mills, Chicago, Ill.) and water ad libitum.

Animals were divided into four groups of 50 each. They received either citrate buffer (control group), Sz alone (Sz group), Sz plus insulin (Sz + I group), or insulin only (I group). All hamsters were fasted overnight before their treatment.

Hamsters in group 3 were given insulin twice a day during the experimental period to reduce the degree of hyperglycemia. They received 4 units Lente and 1 unit regular insulin/kg body

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Offprint requests to: P.M. Pour, The Eppley Institute for Research in Cancer, University of Nebraska Medical Center, 600 S, 42nd St, Omaha, NE 68198, USA

wt. 2 h before the dark cycle began (at 4.00 p.m.) and then received 4 units Lente and 4 units Ultra Lente/kg body wt. 16 h later (at 8.00 a.m. the following day). Each type of insulin was injected by separate syringes. The insulin dose was determined in a preliminary experiment where we gave different doses of insulin and then tested the hamster plasma for glucose levels, 2, 4, 6, 8, 12 and 24 h later. Hamsters in group 4 were given half the amount of insulin as those in group 3 to prevent the induction of hypoglycemia.

The Sz (Sigma, St. Louis, Mo.) was prepared immediately before use as a 10 mg/ml solution in 0.1 M citrate buffer (pH 4.5) and given once i.p. at a dose of 50 mg/kg body wt.).

Insulin (Lilly, Indianapolis, Ind.) was prepared in normal saline and injected subcutaneously.

Glucose levels in plasma were tested by the glucose oxidase method. Urinary glucose and ketones were tested by diastix and Ketostix (Ames Division, Miles Laboratories, Elkhart, Ind.).

For autoradiographic studies hamsters were killed 7, 14, 21, 42 and 70 days (10 hamsters at each interval) after the treatment was started. Tritiated thymidine (Research Product International, Mount Pleasant, Ill.), with a specific activity of 25 Ci/mmol, was injected s.c. once at a dose of 1.0 μCi/g 1 h after the 12 h dark cycle and 1 h before the hamsters were sacrificed. The pancreas and right kidney were trimmed of all lymph nodes and fat and weighed to the nearest milligram.

Pancreas tissues were fixed in Bouin's fixative for 6 h, then placed in 70% alcohol for 24 h, processed for histological examination by conventional methods, cut into step sections and either processed for autoradiographic examination or for immunohistochemistry.

NTB-2 Tracking Emulsion (Eastman Kodak) was diluted: 10 ml with 10 ml deionized water at 50° C and poured into Copeland jars. The deparaffinized slides were dipped slowly into emulsion 3 times, with care taken not to touch the sides of the jar. They then were placed in lightproof boxes overnight to completely drain off excess emulsion. The procedure was performed in total darkness.

On the next morning the slides were placed in Copeland jars that were wrapped in aluminium foil and contained an organic-based scintillation fluid (e.g., Omnifluor). The jars were sealed, covered with a second aluminum foil layer, and placed in a deep-freeze for 5 days.

For development, the slides were removed from the Copeland jars, again in complete darkness, and washed several times by dipping them in xylene, 100% alcohol, 95% alcohol and deionized water. The washed slides were then taken through the following solutions: Developer D-19 (5 min), deionized water (5 min), fixer (5 min) and deionized water (30 min). The slides were counterstained with 0.25% aqueous toluidine blue.

This technique has been found in our laboratory to show little background grain.

The labelling of islet, acinar, ductular (intralobular and interlobular ducts) and ductal cells was determined at 1000× magnification. For evaluation of acinar cell and ductular cell labelling, five different but assigned regions of each hamster pancreas were screened. In each field about 200 acinar cells and 20 ductular cells, including centroacinar cells, were visible. In each area, at least 10 fields (2000 acinar cells, 200 ductular cells) were evaluated so that the acinar and ductular cells screened in each pancreas numbered at least 10000 and 1000, respectively. For the evaluation of ductal cells, which may or may not have been found in these regions, the entire tissue was screened at low magnification and, after their localization in any area, the number of labelled cells was determined by counting at least 500 ductal cells. Areas of inflammation (primarily peri-ductal), in which the labelling of cells was found to be increased, were disregarded. For acinar, ductal and ductular cells the labelling index (LI) was determined by dividing the number of labelled cells by the total number of cells counted.

Since Sz causes β-cell necrosis, the number of labelled cells (NLC) per islet, rather than the percentage of the labelled cells, was evaluated. In each pancreas, as many visible islets as possible (usually between 20 and 50) were screened, and in each islet the NLC located in the center or periphery was determined separately (Fig. 1). In the islet periphery, two-cell layers in the most outer zone of islets, corresponding to the location of glucagon and somatostatin cells in hamsters (Pour 1978), were considered as peripheral cells. Also, the number of labelled acinar cells immediately surrounding islets (peri-insular acinar cells) was determined as the NLC per islet. A zone of three rows of acinar glands around the islets was considered as peri-insular acini. These areas of acinar cells were excluded from the areas for determination of LI of acinar cells remote from islets (tele-insular acinar cells) (Fig. 1).

The minimum criterion for a cell to be considered labelled was five or more grains over background over the nuclei. The standard error of this method in our laboratory is less than 1%.

Evaluation of cell labelling was performed by two independent observers. The average of the two values was considered as representative if differences found did not exceeded 20%. Otherwise, the slides were re-evaluated by the two observers and, in some cases, also by a third observer. The mean of two values closer to each other were regraded as representative.

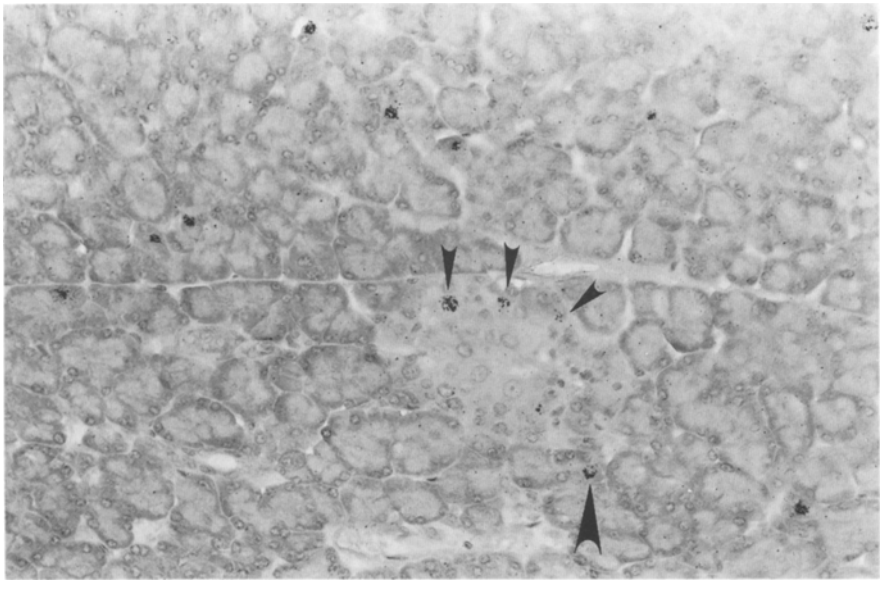

Fig. 1. Labelled nuclei of islet cells in islet periphery (*small arrowheads*), in peri-insular acinar cells (*large arrowhead*) and in tele-insular acinar cells (remaining labelled cells). Pancreas of streptozotocin (Sz)-treated hamsters, day 14. Tritiated thymidine, ×200

For immunohistochemical studies, deparaffinized slides were incubated with antibodies against insulin, glucagon and somatostatin using MAXXUM/Omnitag (Lipshaw, Detroit, Mich.). The slides were stained by the avidin/biotin method (Vector, Vector Lab., Burlingame, Calif.). For maximum specificity, the slides were incubated overnight at 4° C with the primary antibody.

In each slide as many islets as possible were evaluated. The cells immunoreactive with glucagon and somatostatin were counted per islet and the mean value with standard errors was regarded as the representative value. The reduction of islet size caused by Sz occurs because of β-cell necrosis in a pattern indistinguishable from extra-insular islet cell aggregates (nesidioblastosis). Therefore, only islets of a size of 100 μm or larger were evaluated. In non-Sz-treated hamsters, β-cells were evaluated by a grading system. Staining of most β-cells, comparable with that seen in control hamsters, was considered as ++++, reactivity of 30–70% of the cells as +++, staining of 10–30% as ++, staining of up to 5% as +, and no reactivity as 0.

Data between the groups and within the group were evaluated statistically by the ANOVA method using the Scheffe test.

Results

In the pilot study, different levels of several types of insulin were tested in groups of 5 hamsters at different times of day after Sz treatment. Because hamsters are nocturnal animals, the dose (given per kg body wt.) and the time of the insulin application had to be adjusted according to their eating habits. It was found that glucose levels were high 2 h after the dark cycle began. Therefore, regular insulin was given at this time plus Lente insulin to provide adequate insulin for the remaining 8 h. The combination of 4 units Lente and 4 units Ultra Lente was found to be the lowest possible dose to suppress overt hyperglycemia for the next 16 h.

Blood glucose was determined daily before scheduled insulin treatment.

Table 1 lists data relative to the weights of the body, the pancreases and kidneys of hamsters at autopsy, the average level of glucose in the plasma and the urine ketone levels.

In the Sz and Sz+I groups, hamsters gained less weight than those in the control and I groups. Hamsters in the Sz+I group gained relatively less weight than those in the Sz group. Moreover, in contrast to the Sz-treated group that regained weight after 42 weeks, the body weights of hamsters in the Sz+I group remained below the control level. The untreated control and insulin-treated hamsters (I group) showed no differences in the body weight gain.

There were no remarkable differences in the weights of pancreases among the different groups. However, the weights of kidneys were higher in the Sz group and highest in the Sz+I group, compared with the control or insulin groups. Macroscopically, the kidneys of hamsters in the Sz and Sz+I groups were swollen and pale.

The blood glucose levels of control hamsters showed variations at each interval, ranging between 90 and 151 mg/dl (Table 1). Insulin treatment did not alter this level. In Sz-treated hamsters, the initially high glucose level gradually decreased and reached the near control level after 42 weeks. In hamsters of the Sz+I group, however, no such decrease was seen and the glucose concentration was generally above 400 mg/dl at each interval. In terms of individual animals at both 42 and 70 day, 9 of 10

Table 1. Data on the weight of the body, pancreas and kidney as well as the level of glucose and ketones in untreated hamsters and in those treated with Sz, Sz+I or I[a]

Treatment	Day	No. of animals	Body weight (g)	Plasma glucose (mg/dl)	Urine glucose	Urine ketones (mg/dl)	Pancreas weight (g)	Kidney weight (g)
0 (control)	7	10	129±4	107±24	0	0	0.224±0.01	ND[b]
	14	10	141±4	163±25	0	0	0.208±0.01	0.385±0.03
	21	7	151±5	116±29	0	0	0.204±0.01	0.356±0.03
	42	7	141±5	117±30	0	0	0.235±0.01	0.394±0.03
	70	7	151±5	90±29	0	0	0.213±0.01	0.381±0.03
Sz	7	10	124±6	369±32*	+	147± 6	0.221±0.01	0.550±0.04
	14	10	128±7	330±39**	++	78±19	0.268±0.02	0.612±0.04
	21	10	117±6	299±35*	+	29±18	0.226±0.01	0.626±0.04
	42	10	138±4	160±24	+	0	0.271±0.05	0.469±0.03
	70	10	135±4	159±25	+	0	0.242±0.03	0.586±0.03
Sz+I	7	10	108±5	368±24*	+	114±12	0.210±0.01	ND[b]
	14	10	108±5	520±24**,***	++	136±13	0.256±0.01	0.688±0.03
	21	10	114±5	560±25**,***	++	113±14	0.233±0.01	0.628±0.02
	42	10	130±4	486±23**,***	+++	130±12	0.246±0.01	0.650±0.02
	70	10	120±5	532±28**,***	+++	101±14	0.273±0.01	0.819±0.03
I	7	10	132±4	151±24	0	0	0.224±0.01	0.370±0.04
	14	10	143±4	183±25	0	0	0.224±0.01	0.389±0.03
	21	10	149±4	118±26	0	0	0.216±0.01	0.365±0.03
	42	10	148±4	107±25	0	0	0.210±0.01	0.385±0.03
	70	10	154±4	109±25	0	0	0.223±0.01	0.438±0.03

[a] Data are given as mean±standard error
[b] Not determined
* $P<0.005$ compared with group 0
** $P<0.0001$ compared with group 0
*** $P<0.0001$ compared with group Sz

hamsters in the Sz group had blood glucose values less than 200 mg/dl (102–163 mg/dl at 42 days and 119–184 mg/dl at 70 days) with only 1 animal in each group clearly diabetic (372 mg/dl at 42 days and 304 mg/dl at 70 days). The opposite was seen in the Sz+I group with only 1 non-diabetic animal at day 42 (103 mg/dl) and all of the hamsters at day 70 were diabetic.

Urinary glucose and ketone were found only in the Sz and Sz+I groups. Their levels corresponded to the level of plasma glucose and were highest and most persistent in hamsters of the Sz+I group, whereas in the Sz group only traces of glucose, but not of ketone, were found at and after 42 days (Table 1).

The autoradiographic labelling of different pancreatic cells in different groups of hamsters are summarized in Graphs 1–4. In general, the labelling of cells varied greatly between animals of the same group and even between different areas of the pancreas in the same animal. This variation particularly was pronounced in acinar cells, which showed random distribution of groups of labelled cells. The following data are based on 4 consecutive screenings by the two observers.

Islet cells (Fig. 2). The pattern of islets, their number and size were histologically similar in control and I groups and were in accord with our previous findings in untreated hamsters (Pour 1978). In these hamsters, there were no remarkable changes in histocytological patterns of islets at different intervals.

The patterns of NLC in the central and peripheral regions of islets in different groups are summarized in Fig. 2a and b, respectively. Significant differences are also given when found.

In Sz-treated hamsters the number and size of islets were reduced. Most islets contained degenerated and ballooned cells and macrophages, a finding that was more pronounced at day 14. In general, the number of altered cells decreased after day 21. However, there were

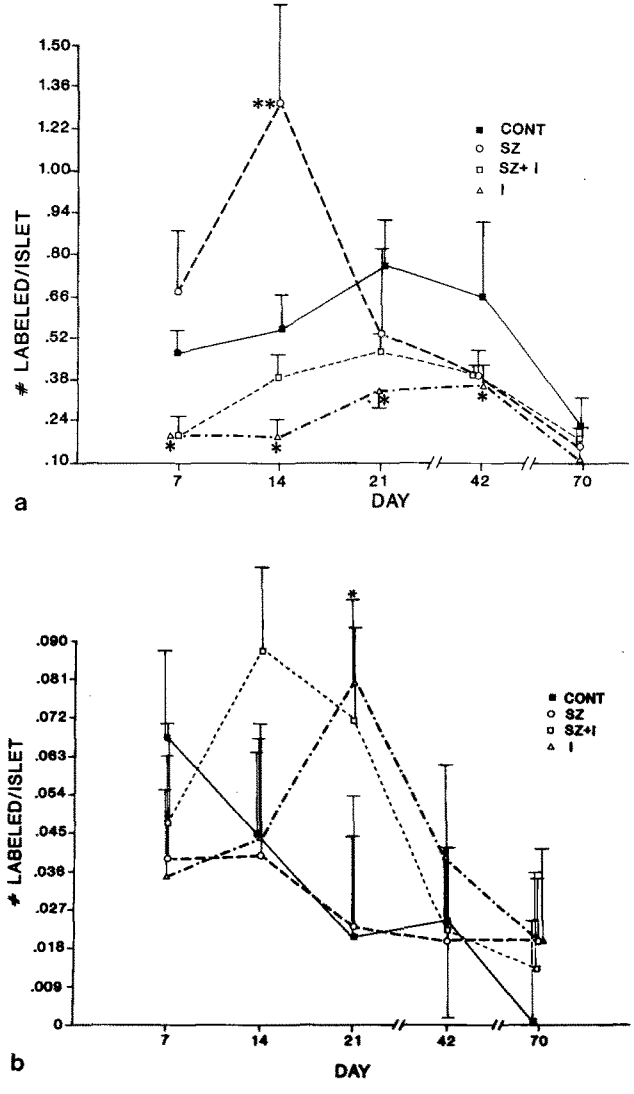

Fig. 2. Labelling index (LI) of islet cells in the central (**a**) and peripheral regions (**b**) of islets in untreated control hamsters (cont) and in those treated with Sz, Sz+insulin (Sz+I) or insulin (I). * $P<0.05$, ** $P<0.005$ as compared with controls

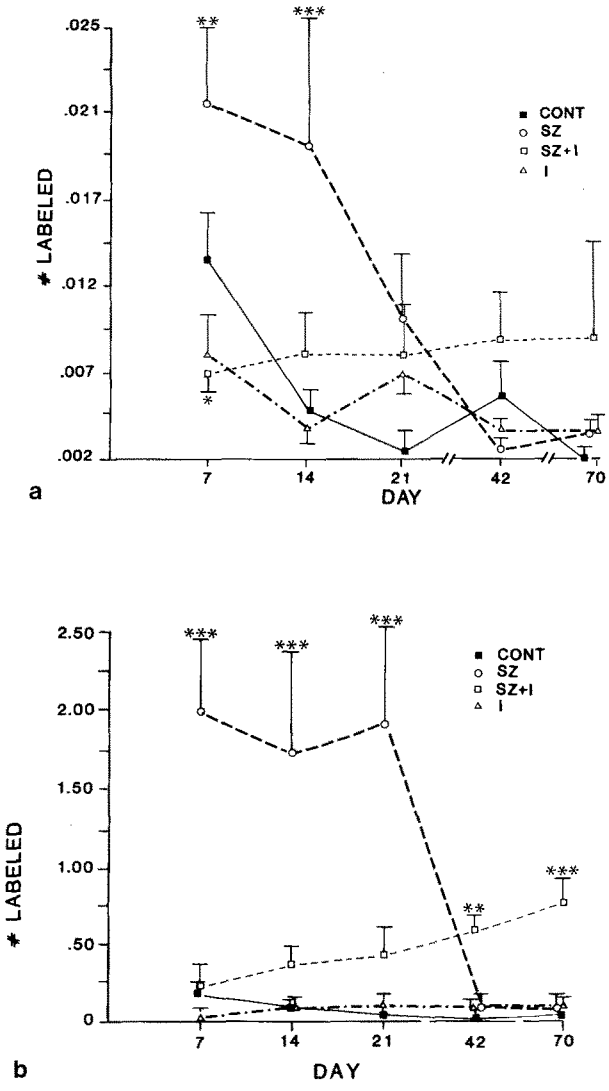

Fig. 3. LI of acinar cells in tele-insular (**a**) and tele-insular pancreatic regions (**b**) in untreated control hamsters (cont) and in those treated with Sz, Sz+I or I, * $P<0.05$, ** $P<0.005$ as compared with controls

remarkable individual variations in the extent of cell damage. Although in some hamsters, islet cells seemed to have regenerated at and after 21 days, others showed a number of ballooned cells even at day 70. In general, the degree of cell alteration in each animal correlated with the level of hyperglycemia. The NLC varied greatly in the islets of this group of hamsters than in other groups as reflected in Fig. 3.

In the Sz+I-treated group, the numbers and size of islets were small. Ballooned cells were present in the central portion of the islet at all times. As in the Sz group, some islets showed distended vascular spaces containing inflammatory cells. However, in contrast with the findings in the Sz group, β-cell regeneration was not evident. In fact, the number of ballooned cells was greatest at day 42, and was detectable in most animals at day 70. There was a large number of mast cells in the interstitium of many hamsters, not seen in any Sz-treated group, particularly at day 42. In the central region of islets (Fig. 2a) the NLC was significantly lower than the Sz group at days 7 ($P<0.0006$) and 14 ($P<0.0001$), but not different at other intervals. In the islet periphery (Fig. 2b) more labelled cells were found at day 14 when compared with the data in the Sz group ($P<0.02$); it remained elevated at day 21 but fell steeply thereafter.

Acinar cells (Fig. 3). Histologically, the structure of acinar cells did not vary among the groups. The LI of acinar cells in the tele-insular region and the NLC in the peri-insular region in each group are given in Fig. 3a and b, respectively. There was remarkable focality of labelled acinar cells in tele-insular regions, a situation which was responsible for the individual variations found in the LI.

In the Sz+I group, the LI was significantly lower than that in the Sz group at days 7 ($P<0.0001$) and 14 ($P<0.01$), but not at any other times. In the peri-insular region (Fig. 3b), the NLC was significantly higher than that in the Sz group at day 42 ($P<0.002$) and 70 ($P<0.0004$).

Ductal/ductular cells (Fig. 4). The LI of ductal and ductular cells are presented in Fig. 4a and b, respectively.

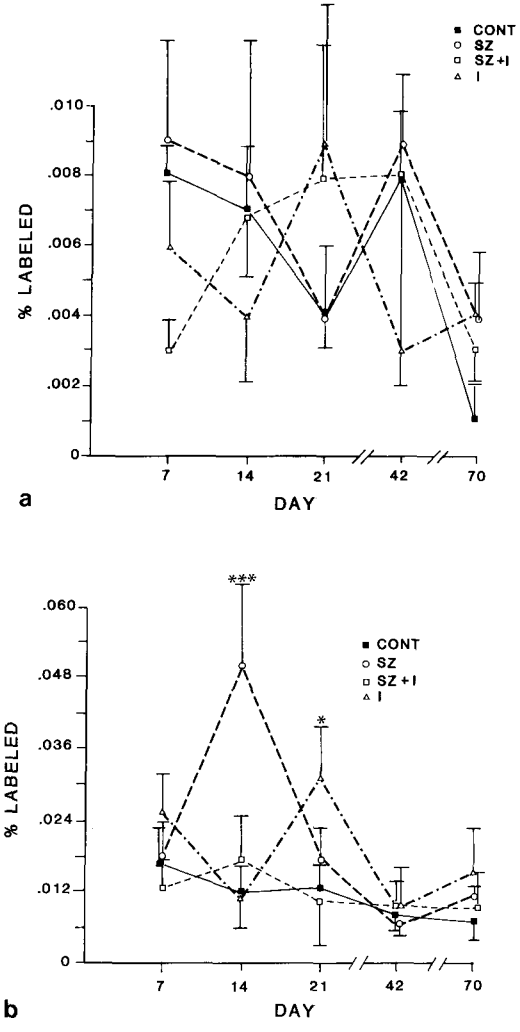

Fig. 4. LI of ductal (**a**) and ductular cells (**b**) in untreated control hamsters (cont) and in those treated with Sz, Sz+I or I. * $P<0.05$, ** $P<0.005$ as compared with controls

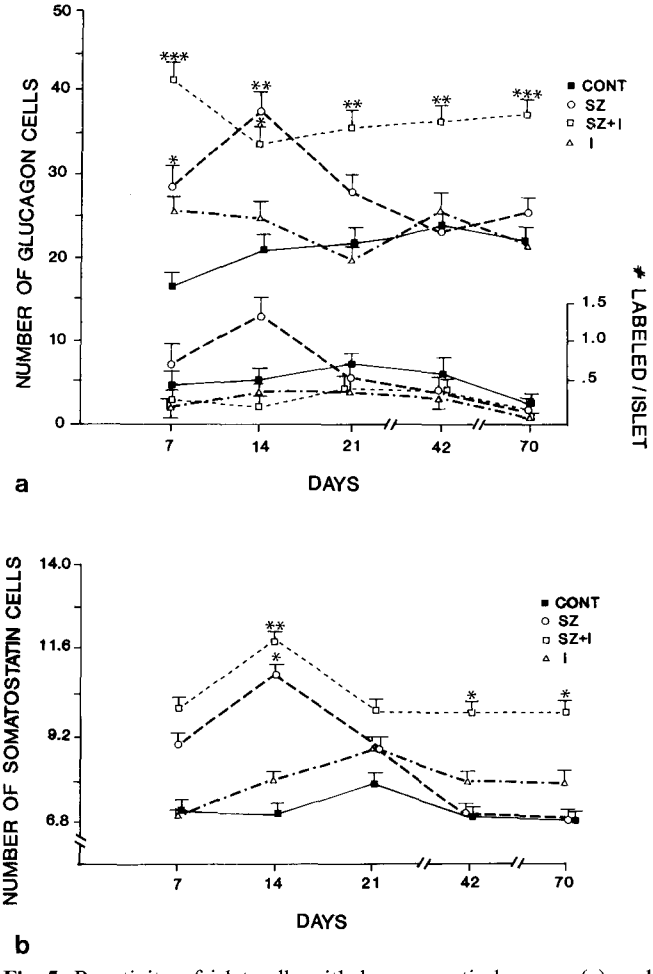

Fig. 5. Reactivity of islet cells with human anti-glucagon (**a**) and anti-somatostatin (**b**) in untreated control hamsters (cont) and in those treated with Sz, Sz+I or I. *Ordinate* refers to the number of immunoreactive cells per islet. * $P<0.05$, ** $P<0.005$, *** $P<0.0005$ as compared with controls

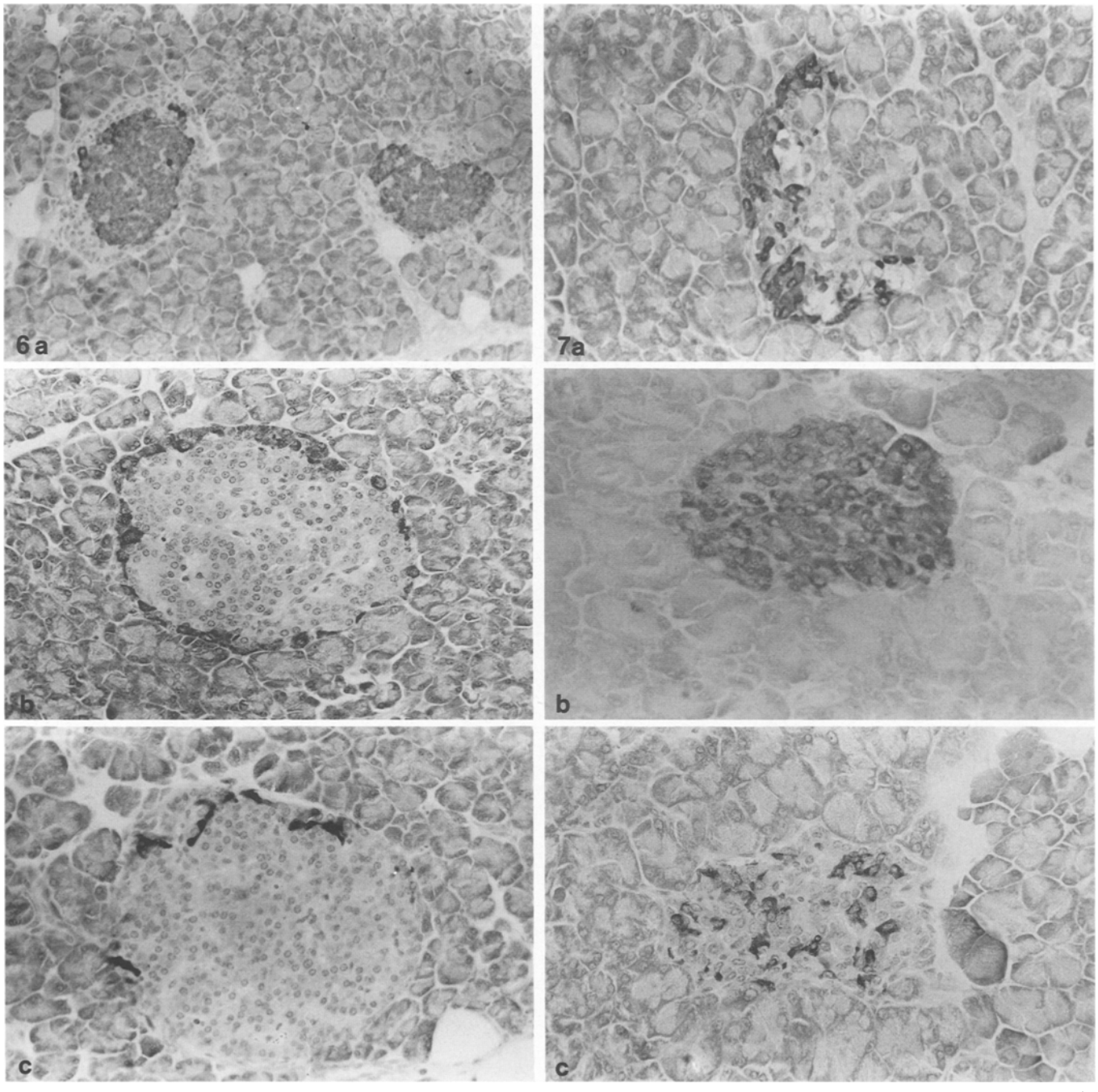

Fig. 6. Islets of an untreated hamster showing cells immunoreactive with anti-insulin (**a**), -glucagon (**b**) and -somatostatin (**c**). ×200

Fig. 7. a Degenerated cells in an islet of a hamster treated with Sz+I. The intact cells are immunoreactive with anti-glucagon (black in photo), ×200. **b** An islet of a hamster treated with Sz+I. Note that the islet is composed entirely of α-cells. Day 42; anti-glucagon, ×200. **c** Islet of a Sz-treated hamster after 14 daily insulin treatments. There are a large number of cells immunoreactive with anti-somatostatin (black in photo) with irregular arrangement. ×200

Focality of labelling similar to that in acinar cells was found for ductal cells (Fig. 4a) and ductular cells (Fig. 4b) in control hamsters, again explaining the great individual variations seen in the LI. In general, the LI of ductal and ductular cells decreased by age.

In the Sz group, a significant increase ($P<0.0001$) of the LI was observed in ductular cells (Fig. 4b) at day 14 compared with the values in other groups. This increase coincide with the significant increase of the labelled cells in the islets (Fig. 2a).

Immunohistochemistry (Fig. 5). In untreated hamsters the β-cells comprised about 70–80% of the total islet cells (grade ++++ reactivity, Fig. 6a), about 15–20% of α-cells (17–22 cells/islet, Fig. 6b) and 5–10% of δ-cells (4–8/islet, Fig. 6c). The β-cells occupied the central por-

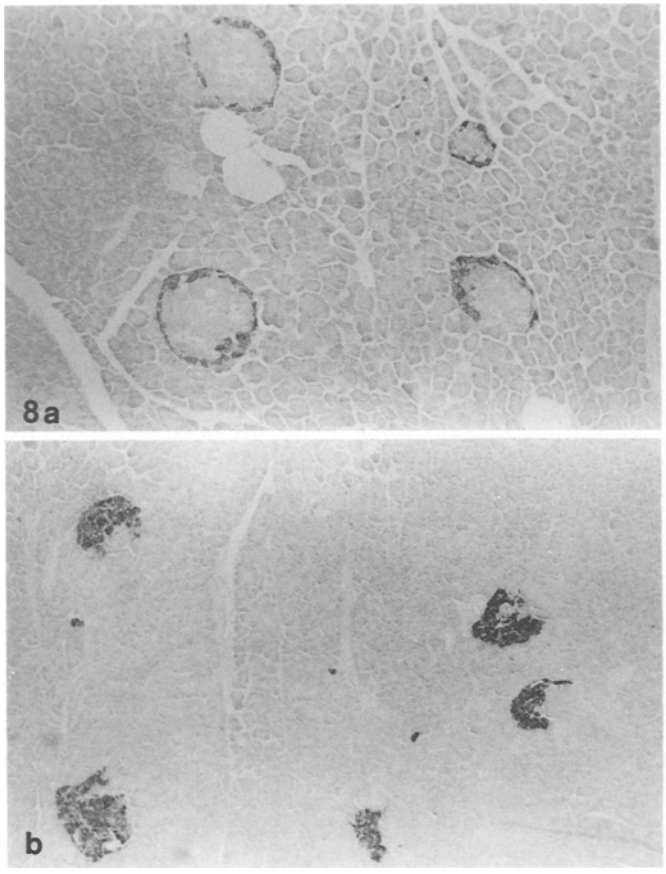

Fig. 8. a Pancreas of a control hamster treated with anti-glucagon. Note regular arrangements of α-cells in the islet periphery. **b** Pancreas of an aged-matched Sz-treated hamster who received daily insulin for 21 days. Note that almost all islets are composed to cells immunoreactive with anti-glucagon. Small black spots between the islets represent small islet cell aggregates. × 78

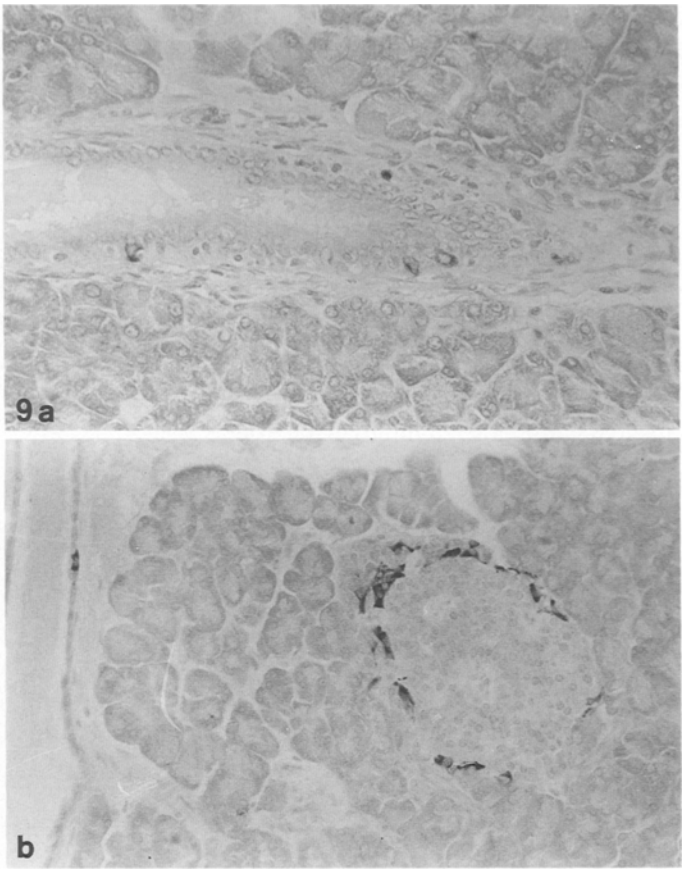

Fig. 9. Presence of α- (**a**) and δ-cells (**b**) in ductal epithelium. Sz treatment; day 21, × 200

tion of the islet, whereas α- and δ-cells had a fairly strict peripheral location (one to two cell layers). There were no regional differences in the distribution of β-, α- and δ-cells in different regions of the pancreatic lobes examined (gastric and splenic lobes).

In control and insulin-treated hamsters, the number of β-, α- and δ-cells did not change significantly during the study period and were comparable in both groups.

In Sz-treated hamsters, only a few (grade +) β-cells were found at days 7–21, and more (++) at days 42 and 70. Conversely, the number of both α- and δ-cells increased at days 7–21 when compared with the control values. The difference was significant at day 7 ($P<0.02$) and at day 14 ($P<0.001$) for α (Fig. 5a) and only at day 14 ($P<0.02$) for δ (Fig. 5b). At day 14 the number of both α- and δ-cells peaked.

In the Sz+I group a highly significant increase in the number of α and δ cells were found throughout the observation period (Fig. 7). Unlike the situation in the Sz group, the number of α-cells did not decrease in this group after day 14 and was higher at days 21–70 than in the Sz group ($P<0.05$–0.006). Many islets were composed primarily of α-cells (Figs. 7b, 8b). The situation was similar for δ cells (Fig. 3c) except that their number decreased at day 21 but remained steady and higher than values in the Sz-group ($P<0.005$).

The number of immunoreactive cells compared with labelled cells (Figs. 2a, b, 5a) showed a close correlation between the number of α- (and δ-) cells and NLC in the central islets in the Sz group. Such a correlation was not seen for α- (or δ-) cells in the Sz+I group. However, in the islet periphery the number of δ-cells correlated with the NLC in the Sz+I group but not in the Sz group. For α-cells, there was a reverse correlation between their number and the NLC in the islet periphery.

In both Sz and Sz+I groups there was a derangement of both α- and δ-cells in that they were present also in the center of islets (Fig. 7). The numbers of extrainsular α- and δ-cells (between the acinar cells and in the ductal/ductular cell epithelium) were increased in the Sz group (Fig. 9a, 5b) compared with the control data.

Discussion

The pancreatic β-cell cytotoxic nitrosourea, Sz, produces permanent diabetes in many species including the rat,

mouse, dog, Chinese hamster and monkey (Baetens et al. 1978; Dulin et al. 1983). Syrian hamsters also respond to a single dose of Sz with β-cell necrosis, but in contrast with other species, β-cells regenerate and a high percentage of hamsters subsequently recover spontaneously from their diabetes (Phares 1980). Higher doses of Sz, while increasing acute mortality, do not result in a higher percentage of permanent diabetes in this species. Daily treatment with 50 mg/kg body wt. Sz for 3 days induced permanent diabetes in 81% of hamsters, but lower dose (40 mg/kg body wt.) given daily for 5 days caused diabetes in 54% (Phares 1980; Bell and Strayer 1983). Hamsters also recover spontaneously from alloxan-induced diabetes (House and Tassoni 1957) and only 37% of surviving animals remain diabetic after 8 weeks.

Recovery from Sz-induced diabetes has also been reported in rats (Rakieten et al. 1976). However, recovery required 8–18 months in rats and the induced diabetes was relatively mild, blood glucose was less than 300 mg/dl, and animals were non-ketonic (Rakieten et al. 1976).

In the present study, we have found that a single dose of Sz induces diabetes in all hamsters, causes degeneration and depletion of β-cells and proliferation of glucagon and somatostatin cells and their derangement within the islets. Similar findings have also been observed in other species (Cantenys et al. 1981; Dutrillaux et al. 1982; Karunanayake et al. 1976; Katsilambros et al. 1970; Logothetopoulos and Brosky 1968; Patel et al. 1978; Patel and Weir 1976; Steiner et al. 1970). We also confirmed that most of the hamsters treated with a single dose of 50 mg/kg body wt. Sz recover from their diabetes and only 10% of the animals remain diabetic (blood glucose 300 mg/dl). Recovery was accompanied by evidence of β-cell regeneration as manifested by an increase of both the cell labelling and the number of immunreactive cells in these animals. With regard to β-cell regeneration, there was considerable variability among animals. Some animals showed cell alteration even at late times. Also similar to other species (Hashimoto et al. 1988), the β-cell regeneration was accompanied by a decrease in the number of initially increased α- and δ-cells. We did not examine the serum level of either hormone, nor did we the tissue content of these peptides. However, based on the positive correlation found between the immunohistochemical and serological findings in other laboratory animals in response to Sz or alloxan (McEvoy and Herge 1977; Patel et al. 1978), it can be assumed that the serum glucagon and somatostatin were increased initially in Sz-treated hamsters.

In our study, the β-cell degeneration was associated with a significant increase of DNA synthesis in the central portion of islets (with a negligible increase in the peripheral islet cells as well) at day 14, followed by a rapid fall at day 21 to reach the control level thereafter. Based on the location of the labelled cells in the islets, this finding could indicate that the new β-cells arise from the surviving β-cells. However, the increased labelling of islet cells also correlated well with the increased numbers of α- and δ-cells, which, in contrast with the situation in untreated hamsters, were also located in the central portion of islets. However, concomitant significant increase in the LI of ductular cells, as well as the increased number of extra-insular islet cells within the ductal and ductular epithelium, is consistent with the view that new islet cells, at least in part, derive from ductal/ductular cells (Cantenys et al. 1981; Dutrillaux et al. 1982; Like and Chick 1970). Our experiment does not allow us to conclude whether the replication of β-cells occurred primarily from (surviving) β-cells, ductal/ductular cells or other endocrine cells (Gotoh et al. 1987; Hashimoto et al. 1988).

The purpose of the study was to determine the cell replication rate of hamsters' pancreatic cells after Sz treatment and to see if insulin therapy inhibits the cell replication by correcting hyperglycemia, the assumed stimulant for β-cell regeneration (Brockenbrough et al. 1988; Chick 1973; Haist and Best 1940; Logothetopoulos et al. 1983). Indeed, a significant increase of pancreatic islet and ductular cells occurred in diabetic hamsters with the peak 14 days after Sz treatment. Insulin therapy prevented cell proliferation and this prevention was associated with permanent hyperglycemia. Thus, the results argue against the notion that hyperglycemia stimulates β-cell regeneration. Studies are required to elucidate the mechanisms by which exogenous insulin inhibits β-cell regeneration. It is also of interest to know whether this effect is specific for Syrian hamsters.

The temporary significant increase of LI of acinar cell labelling, particularly in the peri-insular areas during the 7–21 days after Sz treatment, is difficult to explain. If the DNA synthesis of peri-insular acinar cells were caused by a direct local (paracrine) effect of insulin (Bendayan and Grégoire 1987), an increase of the LI would be expected during the first few days after Sz because of the leakage of insulin from the dying cells, but not at the later days when the number of β-cells is significantly reduced. Nevertheless, the sustained increase of DNA synthesis in both peri- and tele-insular acinar cells in Sz-treated hamsters receiving insulin points to the contributing role of insulin in the replication of acinar cells.

Remarkably, in every hamster, regardless of the treatment, the LI of acinar cells was strictly random and occurred in a group of cells. This may be related to the hemisynchrony in the function and cell replication of acinar cells (Hirsch 1932). Nevertheless, our observation calls for attention in examining the LI of pancreatic cells, and highlights the requirement for screening of large pancreatic areas to obtain a closer estimation on the pancreatic cell replication.

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