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Comparison of Streptozotocin and Alloxan-Induced Diabetes in the Rat, Including Volumetric Quantitation of the Pancreatic Islets*

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Summary. Diabetes was induced in rats with equal molar dosages of either streptozotocin or alloxan. The clinical course of the diabetes (mortality, hyperglycemia, weight loss, polydipsia, hyperphagia, polyuria, glycosuria and diabetic indices) was recorded for six weeks before the animals were sacrificed for volumetric quantitation of the pancreatic islets. No significant differences in the pancreas (islet volumes of pancreas; beta, alpha and non-granular cell volumes and vessel volumes of both islet and total pancreas) were seen between the two groups, although differences in the clinical parameters were observed. The diabetic index at three and four weeks post injection was the clinical parameter which best reflected the terminal pancreatic beta cell volume. Analysis of the scanning data adds further empirical support for the accuracy of the linear scan method of quantitation.

Key words: Streptozotocin, alloxan, diabetes, quantitation, islet volume, beta cell volume, alpha cell volume, vessel volume.

Streptozotocin is becoming increasingly popular as a diabetogenic agent [1—23]. Although the early workers reported only degranulation of the beta cells of the pancreatic islets without necrosis after streptozotocin administration [1, 14], streptozotocin probably produces diabetes by causing a selective necrosis of the pancreatic beta cells [2, 8, 9, 13, 21, 23, 24]. Streptozotocin made available and used prior to 1965 may have been contaminated with an impurity [5, 8] which could explain the conflicting reports on the effects of streptozotocin on the beta cells of the pancreatic islets.

A number of authors have made comparisons of the effects of streptozotocin with those of alloxan, the "classical" diabetogenic agent [1, 4, 5, 8, 9, 18, 21]. However, in only a few studies [7, 11, 16, 17, 23, 25] have comparisons been made directly, using both agents simultaneously in a controlled study.

Our study was designed so that direct comparisons of the effects of streptozotocin could be made with those of alloxan. An attempt was made to standardize streptozotocin, a comparatively new diabetogen, using alloxan, the classical diabetogenic agent, as the standard. Quantitation of the volume of the pancreatic islets was selected as one of the parameters for comparison of streptozotocin- and alloxan-induced diabetes.

Materials and Methods

Animals and Agents

Sixty male albino rats (Sprague-Dawley strain from Holtzman Laboratories), weighing 100—150 g after a 24 h fast, were used in this study. Equal molar dosages of either streptozotocin[1] (65 mg/kg) or alloxan (40 mg/kg) were administered intravenously (tail vein). The ratio, 0.604, of the molecular weights of alloxan, 160.08, and streptozotocin, 265.00, is approximately equal to the ratio, 0.615, of the dosages of the agents used in this study. Both agents were dissolved in saline previously adjusted to pH 4.3 with 0.05 M citric acid. There were 25 streptozotocin-diabetic rats (SDR) and 24 alloxan-diabetic rats (ADR). Eleven control rats (CR) received equal volumes of acidified saline. Immediately after they were injected, the rats were supplied with Purina Rat Chow and water *ad libitum* throughout the study, except when food was withheld prior to glucose tolerance testing. The animals were followed clinically for six weeks to determine the course of the resulting diabetes.

Clinical Studies

Blood glucose levels were determined on the Auto Analyzer using a modification of Hoffman [26]. Body weight, as well as food and water consumption and urine excretion within a 24 h period, was recorded at weekly intervals on alternate subgroups throughout the study period. The Somogyi method [27] was used to estimate the amounts of glucose excreted in the urine during the 24 h periods. Glucose tolerance tests (GTT) were carried out at one and three weeks or at two and four weeks post injection on alternate subgroups. Animals, fasted for 18 h, received 30% glucose (3 g/kg body weight) intravenously; blood samples were drawn from the tail at one-half, one and 2 h post injection for determination of blood sugar (BS) levels (in milligram per cent). A diabetic index was calculated from the results of the GTT using the following formula [28]:

$$I_D = \frac{\text{1 h experimental BS}}{\text{av. normal 1 h BS}} \times \frac{\text{2 h experimental blood sugar}}{\text{av. normal 2 h blood sugar}}$$

The average normal blood sugar values, determined from standardized values obtained from a large group of normal rats in our department, were 200 and 107 milligrams per cent (mg %), respectively.

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Tissue Preparation

Six weeks following injection, the animals were sacrificed by decapitation. The total pancreas was removed and fixed in Bouin's fixative for 4 h. After routine dehydration, the tissue was double infiltrated with celloidin and paraplast. Sequential sections, four microns thick, at 400 micron intervals (every 100 th section) from the entire pancreas, were mounted on glass slides and stained with Carpenter's modification of Gomori's aldehyde fuchsin [29] and counterstained with ponceau.

Quantitation

Aldehyde fuchsin-ponceau stained sections from randomly selected pancreases were scanned using the micrometer component quantitator [30, 31]. Islet volume, expressed as per cent of the total pancreas, was determined at low power (10X objective) with traverse intervals of one millimeter (mm). The total linear scan for each determination was 500 mm or more. The relative volumes of the various components of the islets, expressed as per cent of total islet, were measured at high magnification, using an oil immersion objective, with traverse intervals of 20 micra. Five mm of total linear scan were completed for each animal. The components of the islet also were expressed as per cent of the total pancreas by multiplying the volume per cent of the islet of each component by the islet volume of the total pancreas.

Identification of Islet Components

The classification of cells of the islet was patterned after that of Carpenter and Lazarow [32]. Color plates 1—3 on page 495 of their article should be seen for further details. In aldehyde fuchsin stained material, beta cells, containing varying numbers of granules, were classified on a scale from 1+ to 4+ granulation. Cells without granules, but with pale blue cytoplasm, were seen, especially in the experimental animals; these cells were identified as non-granular cells in this study. The finely granular cytoplasm of the alpha cells stained varying tints of pink with ponceau. Thus, the distinction between alpha cells and the non-granular cells could be made easily.

The component identified as vessel included the lumen and the endothelial lining of the capillaries within and surrounding the islet.

Because the number of micrometers limited the number of components that could be quantitated per scan to five, the designations were: 1) 3—4+ beta cells (beta cells containing more than half of the full complement of granules), 2) 1—2+ beta cells (beta cells containing less than half of the full complement of granules), 3) non-granular cells, 4) alpha cells, and 5) vessels.

Results

Clinical Study

All the experimental animals became diabetic after a single i.v. injection of either of the two diabetogens.

Mortality was highest in the ADR (Table 1). 42% of all the mortalities (eight out of 19) occurred during the GTT procedure, usually immediately following the glucose injection. Death of ten of the ADR, but only one SDR was due to factors other than the stress of the GTT. The clinical results include data only from the rats that survived the six week study period.

The level of hyperglycemia was consistently lower among the SDR than among the ADR (Fig. 1). The mean blood sugar level of the SDR was 408 ± 75[1] mg% after the first week; during the last five weeks, the average blood glucose levels were between 450 and 500 mg%. The ADR showed an average blood sugar level of 493 ± 70 mg% the first week which increased to 620 ± 240 mg% the second week and to 730 ± 203 mg% the third week; during the last three weeks, the average blood glucose levels were between 680 and 722 mg%. The CR maintained average blood sugar levels below 100 mg% at all times tested.

Table 1. *Survival and mortality of rats. Six weeks after injection. The CR groups represents the control rats, while the SDR and ADR groups represent the streptozotocin diabetic rats and the alloxan-diabetic rats respectively*

Group	Number of Rats injected	Rats alive at the end of six weeks Number	Per cent
CR	11	10	90.9
SDR	25	22	88.0
ADR	24	9	37.5

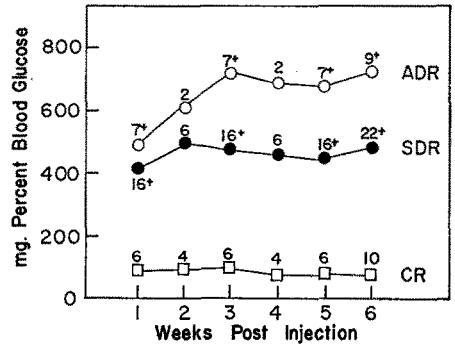

Fig. 1. Mean blood sugar values. The mean values are represented by open squares (□—□) for the control rats (CR), by filled circles (●—●) for the streptozotocin-diabetic rats (SDR), and by open circles (○—○) for the alloxan-diabetic rats (ADR). The numbers indicate the number of animals used for determination of the mean. The + symbol indicates a significant difference of the means at the $p < 0.01$ level

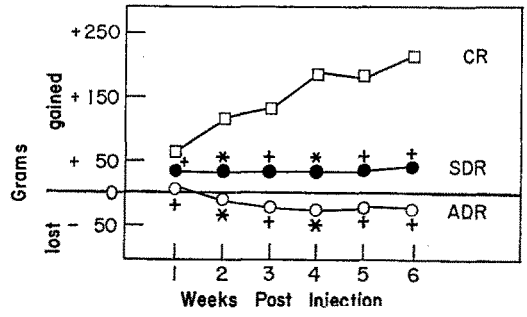

Fig. 2. Mean changes in body weight from injection. The symbols used indicate the same values as those in Fig. 1, with the addition of the * symbol to represent a significant difference of the means at the $p < 0.05$ level

[1] ± one standard deviation of the mean.

The average changes in body weight were inversely related to the blood sugar levels of the animals (Fig. 2). The SDR maintained their injection weight level, while the ADR lost weight. The CR gained an average of 215 g over their initial weight. Fig. 2 does not show actual weight lost or gained since all animals were experimental groups displayed hyperphagia. However, the SDR consumed significantly more food per 24 h period at three, five and six weeks post injection than did the ADR. Polyuria was greater in the ADR than in the SDR during the first and second week after injection, but during the last four weeks, the SDR ex-

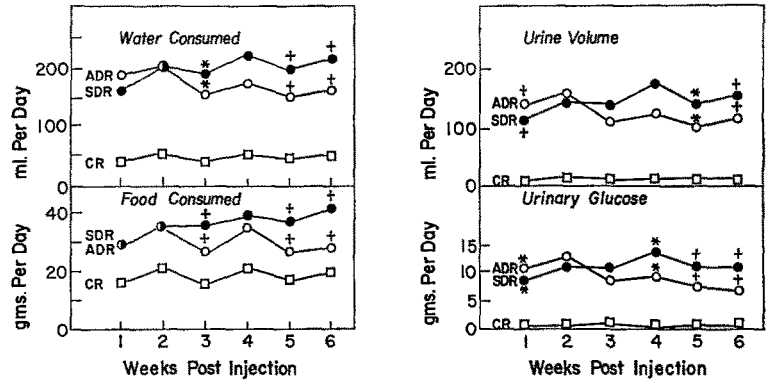

Fig. 3. Water and food consumption, urine volume and amounts of sugar excreted in the urine. The symbols used are the same as those used for Figs. 1 and 2

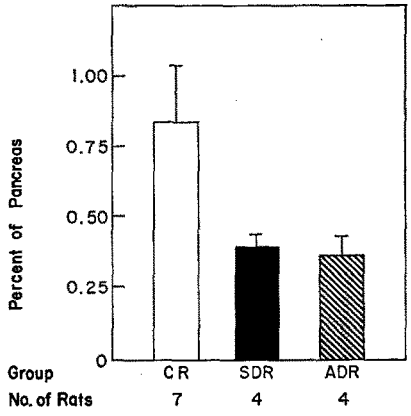

Fig. 4. Mean islet volumes of the pancreas. One standard deviation of the mean is shown by the vertical line (T). The groups are the same as those designated in Table 1

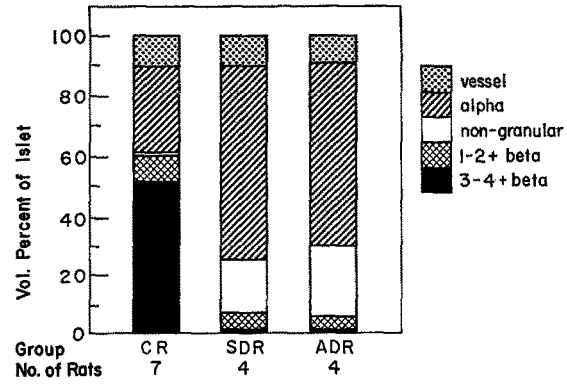

Fig. 5. Mean relative volumes of the components of the islet (as per cent of the total islet). The groups are the same as those designated in Table 1

Table 2. *Mean diabetic index. The groups are the same as those designated in Table 1. The mean diabetic index ± one standard deviation is given for each time period post injection. The number in parenthesis represents the number of rats used for each determination. The asterisk (*) indicates a significant difference of the means at the $p < 0.05$ level*

Group	Weeks post injection 1	2	3	4
CR	0.5±0.4 (4)	0.5±0.1 (5)	0.5±0.2 (4)	0.5±0.2 (5)
SDR	5.5±2.6* (6)	9.5±2.7* (13)	12.7±2.1 (6)	11.4±2.9 (15)
ADR	10.7±1.8* (2)	12.9±1.5* (4)	11.8±9.5 (2)	13.0±5.9 (7)

fasted on alternate weeks for GTT and had to compensate for that weight loss.

The degree of polydipsia was closely related to both polyuria and glycosuria (Fig. 3). The SDR consumed significantly more water per 24 h at the fifth and sixth week post injection than did the ADR. Both creted a larger average volume of urine per 24 h than did the ADR; the differences were not significant until the sixth week when the SDR excreted an average volume of 155±36 milliliters (ml) of urine per 24 h compared to 115±36 ml excreted by the ADR. The urine excreted by the CR never exceeded an average of

10 ml per 24 h. The average amount of glucose excreted in the urine followed the same pattern as the urine volume for each of the three groups (Fig. 3). The levels of glycosuria of the two diabetic groups were significantly different at five and six weeks post injection; the SDR excreted 10.8 ± 2.7 g and 11.7 ± 2.8 g of glucose per 24 h compared to 7.2 ± 2.9 g and 8.8 ± 2.4 g excreted by the ADR. The level of glucose in the urine of the CR averaged less than 0.03 g per 24 h at all intervals tested.

The average diabetic indices (I_D) of the two experimental groups did not differ at the $p < 0.01$ level (Table 2), although the I_D of the SDR were lower than those of the ADR at the first and second weeks post injection ($p < 0.05$). If the two I_D obtained from the same SDR are compared (week one with week three) the difference is significant at the $p < 0.01$ level.

Quantitative Scanning

There was no significant difference in the average islet volume of the pancreas between the SDR ($0.39 \pm 0.06\%$) and the ADR ($0.36 \pm 0.07\%$), although both were significantly reduced from that of the CR ($0.83 \pm 0.20\%$) (Fig. 4). The islet volumes were reduced from the control values by 53.0% in the SDR and by 56.6% in the ADR.

The average relative volumes of the components of the islets of the two diabetic groups did not vary significantly (Fig. 5). In both groups the average total beta cell volume was reduced from that of the CR (59,7% with 50.8% being 3—4+ granulated). Streptozotocin reduced the average total beta cell volume of the animals to 5.9% with only 0.5% being 3—4+ granulated, while alloxanization resulted in an average total beta cell volume of 5.1% with 0.6% having 3—4+ granulation. The non-granular cell volume of the islets averaged 18.5% in the SDR and 23.8% in the ADR compared with 0.4% in the controls. The average alpha cell volumes of the islet of both the SDR (62.2%) of the ADR (61.8%) were greater than that of the CR (29.6%). The average vessel volume of the islet remained unchanged among the three groups — 10.3% in the CR, 10.4% in the SDR and 9.3% in the ADR.

The differences between the volumes of the components of the islet of the diabetic animals and those of the CR are more obvious when the values are expressed as volume per cent of the total pancreas (Fig. 6). The average total beta cell volume of the pancreas in both groups of diabetic animals is reduced by about 96% from that of the controls. Although the average non-granular cell volume of the pancreas increased in the experimental groups, it is not enough to compensate for the loss in the beta cell volume of the pancreas. The combined volumes of the beta cells and the non-granular cells within the two groups represent an 80% reduction from the combined values in the CR. In contrast to the component volume of the islet, the average alpha cell volume of the pancreas did not differ significantly among the three groups. The islet vessel volume as per cent of the total pancreas was reduced from that of the CR by 52.9% for the SDR and by 61.2% for the ADR. The total cell mass of the islet (beta + non-granular + alpha cells) as a per cent of the total pancreas was reduced from that of the CR by 53.3% for the SDR and by 56.4% for the ADR.

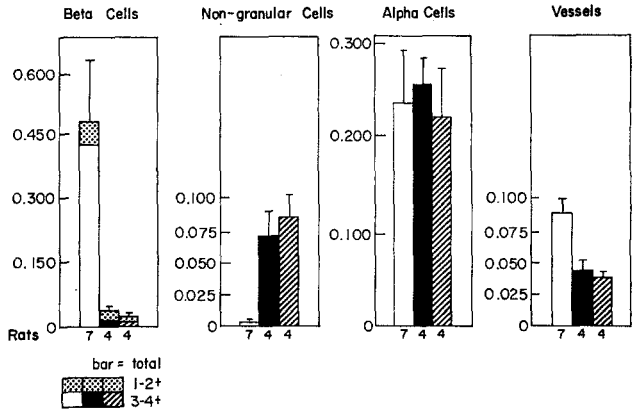

Fig. 6. Mean relative volumes of the components of the islet (as per cent of the total pancreas). The open bars (□) represent the mean values of the control rats, the filled bars (▮) represent mean values of the streptozotocin-diabetic rats and the stripped bars (▨) represent the mean values of the alloxan-diabetic rats. One standard deviation of the mean is shown by the vertical line (T) and the numbers below the bars indicate the number of rats used for each determination

Discussion

The present study confirms the observation of others [1, 4, 8, 9, 15, 23, 24] that streptozotocin is as effective a diabetogen as is alloxan when either agent is given intravenously. Mortality among the rats, excluding GTT deaths, was 10 times greater with alloxan (40.0%) than it was with streptozotocin (3.7%). The clinical courses of the diabetes induced by either streptozotocin or alloxan were similar, but not identical. The levels of hyperglycemia (Fig. 1) and the changes in body weight (Fig. 2) did not correlate with the degree of polydipsia, hyperphagia, polyuria and glycosuria (Fig. 3). The explanation for these differences may be due to extrapancreatic effects, such as a difference in the renal toxicity of the two beta cytotoxins. The nephrotoxic effects of alloxan are well established. It has been suggested that streptozotocin is more selective for beta cells with less general toxicity than is alloxan [1, 8, 9]. Also streptozotocin depresses the hepatic NAD levels, while alloxan does not [51]. There was no significant difference between the diabetic indices of two experimental groups at three and four weeks after injection. The diabetic indices of SDR may reflect a gradual decrease in the tolerance to glucose with time, supporting the suggestion of Dulin, *et al.* [4] that the severity of streptozotocin diabetes increases gradually.

The results of quantitation of the pancreas show no significant difference between SDR and ADR (Fig. 4). The average islet volume of the seven CR was $0.83 \pm 0.20\%$ of the total pancreas. Other investigators using the linear scan method on rats have reported normal average islet volumes of 0.69, 1.16 and 1.29 % [33] and 1.22% [3, 34]. The lower islet volume in the present study is attributed to the younger age of the rats used. Both the number [35, 36, 37] and the volume [38, 39, 40, 41] of islets are reported to increase with age. The average age of the rats used here was 49 days. Presumably, the other workers used rats considerably older than 50 days. The size of the litter from which an animal comes also is reported to influence islet volume [42].

The component volumes of the islet found in this study (Fig. 5) compare favorably with those previously reported using the same method, with the exception of the alpha cell volume of the islet [32, 33]. The beta cell volume in our study, 59.7%, is within the range, 56.6 % to 71.3 %, reported by Carpenter and Lazarow [32] and Wright [33]. These same authors [32, 33] found alpha cell volumes ranging from 13.9 to 21 % and vessel volumes of 11.8 to 16 %. The vessel volume from this study (10.3 %) falls into the lower range of the previous studies. The alpha cell volume is higher than that found in comparable studies of normal rats. However, Hellman [43], using another method of quantitation, found that age influences the alpha:beta cell ratio in rats. He found more alpha cells in newborn animals than in adult rats 100 days of age. Petersson, *et al.* [42] noted that rats from large litters had increased beta:alpha cell ratios, due to lower numbers of alpha cells, than animals of the same age from small litters. Thus, it is felt that the difference in alpha cell volume in this study is due to factors other than variations in cell identification or application of the linear scan method. Non-granular cells have not been reported in the islets of normal animals by investigators using similar staining and identification methods [32, 33, 34, 44]. Carpenter and Lazarow [32] reported that non-granular cells appeared in normal animals four hours after glucose injection. The non-granular cells seen in CR in this study occurred in only three rats which had received two injections of glucose for the GTT during the six week observation period. The true identity of these cells is unknown [32, 34].

The decrease in islet volume of the pancreas (Fig. 4) of the diabetic animals could be due to a decrease in either the size or the number of islets in the pancreas. If entire islets were destroyed a decrease in the alpha cell volume of the pancreas would be expected. However, the fact that the alpha cell volume of the total pancreas remains within normal limits (Fig. 6), suggests that the decreased islet volume of the pancreas is a result of a decrease in the size of the islets. A normal alpha cell volume of the pancreas also indicates that the increase in the alpha cell volume of the islet (Fig. 5) is a relative increase due to loss of beta cells rather than an actual increase due to proliferation of alpha cells. Wright and Carpenter [45] did not, although Wright [33] did, report a decrease in alpha cell volume of the pancreas of alloxan diabetic and subdiabetic animals concurrent with a decrease in beta cell volume.

A significant decrease in islet vessel volume of the total pancreas was observed in both groups of diabetic animals from that of the controls (Fig. 6). A diminished vessel volume of the pancreas has been observed in alloxanized animals [33], which was attributed to total destruction of some islets. If this were the case in the present study, an actual increase in alpha cells in the remaining islets would have been required to compensate for loss of cells in destroyed islets. Another explanation may be the loss or disappearance of vessels no longer needed to supply the destroyed beta cells. The reduction of the total islet cell mass (the sum of the beta, alpha and non-granular cell volumes of the pancreas) of the pancreas from that of the controls for the SDR (53.3 %) and ADR (56.4 %) was approximately the same as the reduction of the islet vessel volumes (52.9 % and 61.2 % respectively) of the pancreas. This explanation adds further empirical support for the accuracy of the linear scan method of quantitation.

Both streptozotocin and alloxan induce diabetes by acting to produce relative or absolute deficiencies of insulin [9, 15, 23, 46, 47, 48, 49]. Presuming that aldehyde fuchsin is insulin [50], the scanning data indicate no morphological differences in the amount of insulin (beta cell granulation) present in the pancreas of the two groups six weeks after the administration of streptozotocin or alloxan. However, similar morphometric measurements would not reflect a differential rate of insulin turnover (ie. synthesis, storage and/or release) between the two groups. The diabetic index was the clinical parameter which best reflected the data obtained from quantitation of the pancreatic beta cell volume.

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