Glucagon Secretion from the Perfused Pancreas of Streptozotocin-treated Rats

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SUMMARY

One hour following intravenous streptozotocin, rat pancreases were perfused *in situ*, and, in contrast to saline-injected controls a marked decrease of insulin secretion was observed. In these streptozotocin-treated animals, baseline glucagon secretion was enhanced when the perfusate glucose concentration was either 80 mg./100 ml. or 300 mg./100 ml. In addition there was hypersecretion of glucagon in response to arginine.

Exogenous insulin (20,000 μU./ml.) could suppress glucagon secretion when endogenous insulin secretion was low but had no effect when endogenous secretion was plentiful. Baseline and arginine-stimulated glucagon secretion of the streptozotocin-treated animals was not suppressed by large amounts of glucose and insulin to the degree seen in control animals. The glucagon rise in response to an abrupt fall of glucose from 80 mg./100 ml. to 25 mg./100 ml. was not significantly higher in the control group than in the streptozotocin group.

The results seen with epinephrine were in sharp contrast to those found with arginine. Epinephrine-stimulated glucagon secretion was not enhanced in the streptozotocin group. In addition, epinephrine-induced secretion could be suppressed by exogenous insulin in both the control and streptozotocin groups. The differences may be secondary to differences of endogenous insulin secretion.

The present results are compatible with the hypothesis that local insulin secretion can exert a significant suppressive effect upon the alpha cell and that the inhibition of glucagon secretion by glucose is partially mediated by this mechanism. Furthermore, anomalous local insulin secretion may contribute to the abnormal glucagon secretion of diabetes mellitus. DIABETES *25*:275-82, April, 1976.

It has recently been appreciated that glucagon is secreted in excessive amounts in diabetes mellitus.[1] Furthermore, there is growing evidence that this hyperglucagonemia contributes very significantly to the hyperglycemia of diabetes.[2] The cause of this increased secretion is unknown, but there are several prominent hypotheses. It has been postulated that the hypersecretion may result from beta-cell failure and consequent insulin deficiency.[3] Another suggestion has been that there is a primary alpha-cell defect in diabetes.[4-6] In addition, others have speculated that the increased amounts of glucagon may arise from extrapancreatic sources, possibly the stomach.[7]

The present study utilizes perfused pancreases from rats treated acutely with streptozotocin to examine the hypothesis that local insulin secretion within the islets of Langerhans can exert a suppressive effect upon the alpha cell[8] and that with beta-cell dysfunction, this suppressive effect is reduced resulting in hypersecretion of glucagon.

METHODS

Materials. Glucagon antisera 02K and 30K were kindly supplied by Dr. Roger Unger, and insulin antiserum (Lot K4273) was obtained from Burroughs Wellcome Company (Research Triangle Park, N.C.).

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(^{125}I) Glucagon was purchased from Nuclear Medical Laboratories, Inc. (Dallas, Tex.) and (^{125}I) insulin was obtained from New England Nuclear (Boston, Mass.). Pork glucagon standard (Lot GLF 599A) was supplied by Eli Lilly Company (Indianapolis, Ind.), and rat insulin standard (Lot R355) was from the Novo Research Institute (Copenhagen, Denmark). Trasylol was obtained from FBA Pharmaceuticals (New York, N.Y.) and dextran T70 from Pharmacia Fine Chemicals, Inc. (Piscataway, N.J.). L-arginine-monohydrochloride was purchased from Eastman Kodak Company (Rochester, N.Y.), and pork insulin (Actrapid, monocomponent) was donated by Novo (Copenhagen, Denmark). Streptozotocin was a gift from the Upjohn Company (Kalamazoo, Mich.). Epinephrine was obtained from Parke, Davis and Company (Detroit, Mich.).

Radioimmunoassays. The radioimmunoassay methods for both glucagon and insulin have been previously described.[9] The sensitivity of the glucagon assay was usually about 15 pg./ml., and the insulin assay sensitivity was about 3 μU./ml. Glucagon antiserum 30K was used for the epinephrine experiments and antiserum 02K for all the others. Glucagon concentrations in samples taken during these perfusions were the same when measured with either 30K or 02K.

Perfused rat pancreas preparation. The *in situ* isolated perfused rat pancreas preparation was a modification of the method of Penhos, Wu, Basabe, Lopez, and Wolff[10] and has been described in detail in an earlier publication.[9] Fasted male Sprague-Dawley rats (Charles River Breeding Laboratories) weighing 350-550 gm. were used. The perfusate was a modified Krebs-Ringer bicarbonate buffer with 4 per cent dextran T70 and 0.2 per cent bovine serum albumin (fraction V) and was bubbled with 95 per cent O_2 and 5 per cent CO_2. Flow rate was frequently monitored and remained constant at 2.4-3.2 ml./min. for the duration of the perfusions, each perfusion lasting 45 minutes. Within any given perfusion, flow rate remained constant, varying not more than about 0.2 ml./min. The first 20 minutes of each perfusion was always an equilibration period and will not be shown on any of the graphs. Perfusate was never recycled through the pancreas.

Once the rats were anesthetized with intraperitoneal Amytal Sodium (Eli Lilly and Company, 160 mg./kg.), 0.4 ml. of blood was drawn from the femoral vein for glucose determination. Immediately following this, a freshly dissolved solution of streptozotocin in saline was infused intravenously via the femoral vein (either 75 or 200 mg./kg.). If the large dose of streptozotocin (given in the same volume as the lower dose) was given more rapidly than 45 seconds, the rats often died suddenly, their death preceded by a few seconds of hyperventilation. Control rats received an equal amount of saline via the femoral vein. Thirty minutes following the injections, surgery was begun; and 45 minutes after the injections, 0.4 ml. of blood was drawn from the inferior vena cava. The blood samples were immediately centrifuged with a Microfuge (Model 152, Beckman Instruments, Inc., Fullerton, Calif.), and serum glucose levels were determined with a Beckman glucose analyzer (Model ERA 2001). Surgery was finished approximately 60 minutes following the injections, and the 20-minute equilibration perfusion period was immediately begun. During the experimental perfusion period, samples from a portal vein catheter were collected over 30-second intervals into glass tubes containing 4 mg. of EDTA to protect against proteolytic degradation by pancreatic enzymes.

The perfusate reservoir used for the 20-minute equilibration period was always the same as that used for the subsequent five-minute baseline period. At the end of the baseline period (25 minutes after the end of surgery), a rapid switch was made to a second perfusate reservoir containing any of three experimental modifications: 10 mM arginine, 2.7×10^{-7} M epinephrine, or 25 mg./100 ml. glucose. (With the exception of the latter case, utilized in a series of specifically identified experiments involving a change in glucose concentration, the insulin and glucose levels in the first and second reservoirs were identical within any given perfusion.) The duration of the second perfusion period was 25 minutes, terminating 50 minutes after the end of surgery. Arginine and insulin were added to the perfusate one to three hours prior to a given perfusion; epinephrine was added to the perfusate eight minutes prior to its infusion.

Data presentation. Glucagon and insulin secretion over a 30-second period is depicted in subsequent graphs as a single point at the beginning of the time interval. For instance, a point at four minutes represents the glucagon concentration of perfusate collected during the 4-4.5-minute period. Baseline secretion for each perfusion is the mean of the glucagon or insulin levels of the 0- and four-minute samples. Total glucagon and insulin output, during arginine or epinephrine stimulation, was estimated by planimetry. The two-tail Student's t test was used to determine significance for all comparisons. All data are ex-

pressed as mean ± standard error of the mean.

It will be noted that baseline secretion is reported as series I and II. Series I refers to baseline secretion from the experiments of figures 2 and 5 and the arginine experiments of figure 4. Series II refers to baseline secretion from the epinephrine experiments of figure 4. The series II (epinephrine) experiments were done five months after all the other studies had been completed. Antiserum 30K was used for series II because our supply of sensitive 02K was exhausted. There is no good explanation for the differences in baseline values between series I and II, but we assume that minor, unapparent technical variation accounts for the discrepancy.

RESULTS

Effect of acute streptozotocin treatment on serum glucose and secretion of insulin. Forty-five minutes following an intravenous injection of streptozotocin (75 mg./kg.), serum glucose levels rose significantly (table 1). A statistically indistinguishable rise, however, also occurred following a control injection of saline. Surgery was begun 30 minutes following the injections and continued through the drawing of the second glucose sample, so perhaps the stress of surgery accounted for the glucose rise following both types of injections.

Despite the lack of a significant effect of streptozotocin per se on glucose levels, there was pronounced impairment of insulin secretion from the pancreas perfusions following administration of the toxin (figures 1-3). With perfusate glucose at 80 mg./100 ml., baseline insulin secretion was indistinguishable between streptozotocin-treated and normal pancreases, with levels being close to the detection limit of the insulin radioimmunoassay (<5μU./ml.). A marked reduction of arginine-stimulated insulin secretion was observed, however, with the cumulative output of the streptozotocin group being only 16 per cent of the control response (figures 1 and 3). A similar effect of streptozotocin was observed when perfusate glucose was at 300 mg./100 ml., with both baseline and arginine-stimulated insulin secretion being markedly reduced (figures 2 and 3).

Experiments were performed with a larger intravenous dose of streptozotocin (200 mg./kg.) to determine whether a toxic effect on the alpha cell might be uncovered. Baseline insulin secretion stayed at or below the detection limit of the immunoassay. Arginine-stimulated insulin output was significantly less than that found with the lower dose of 75 mg./kg. (0.27 ± 0.12 vs. 0.78 ± 0.02 mU., P<0.01). Baseline and arginine-stimulated glucagon secretion

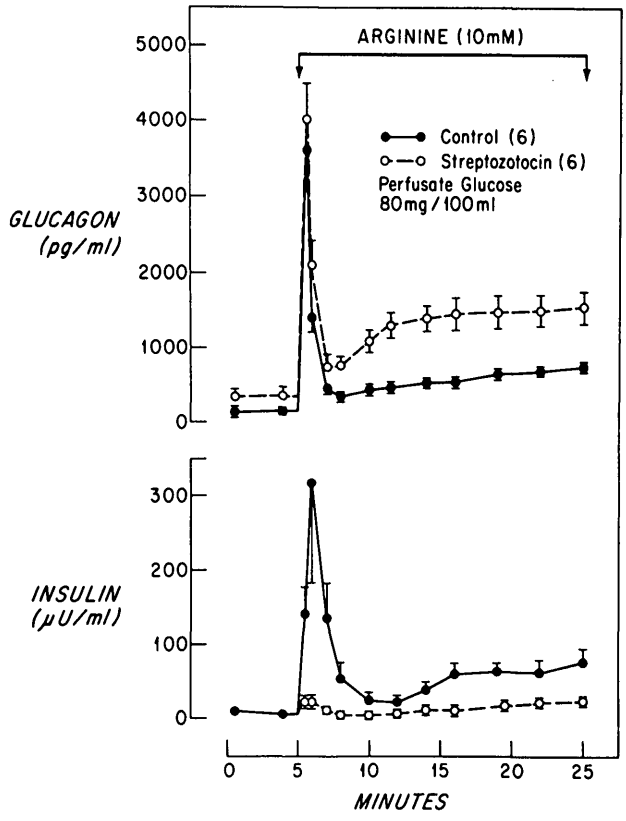

FIG. 1. Baseline and arginine-stimulated glucagon and insulin from perfused pancreases of control and streptozotocin-treated rats.

were not significantly different between the two doses (311 ± 73 vs. 358 ± 56 pg./ml., and 66.1 ± 15.8 vs. 92.7 ± 10.4 ng.).

Baseline glucagon secretion from control and streptozotocin-treated pancreases: Effects of exogenous glucose and insulin. Baseline glucagon secretion under several different perfusate conditions is depicted in figures 1 and 2 and table 2. Baseline secretion in control perfusions was considerably higher at a perfusate glucose level of 80 mg./100 ml. than at 300 mg./100 ml. (190 ± 33 vs. 10 ± 4 pg./ml., P<0.001).

TABLE 1

Serum glucose levels (mg./100 ml.) in rats following injection of either streptozotocin (75 mg./kg.) or saline

	n	0 min. (injection)	45 min. after injection	p*
Control (saline)	29	102.7 ± 3.6	125.9 ± 5.9	<0.01
Streptozotocin	48	103.9 ± 4.0	136.2 ± 5.9	<0.001
p†		N.S.	N.S.	

*Significance between 0 and 45 minutes.
†Significance between control and streptozotocin groups.

TABLE 2

Effect of exogenous insulin on baseline glucagon secretion of control and streptozotocin-treated rats*

	Control	p	Exogenous insulin†	Streptozotocin	p	Exogenous insulin†
Series I	190 ± 33 (16)	<0.01	74 ± 21 (6)	358 ± 56 (12)	N.S.	272 ± 71 (6)
Series II	126 ± 17 (11)	<0.01	71 ± 6 (7)	276 ± 38 (8)	<0.001	61 ± 7 (3)

*Perfusate glucose 80 mg./100 ml. in all experiments; glucagon secretion, expressed as pg./ml., represents mean of one- and four-minute samples.

†Exogenous insulin in perfusate at concentration of 20,000 μU./ml.

Streptozotocin treatment led to increased baseline glucagon secretion. As shown in table 2, glucagon secretion was higher in series I ($P<0.01$) and series II ($P<0.005$) when perfusate glucose was 80 mg./100 ml. It was also higher when perfusate glucose was 300 mg./100 ml. (90 ± 33 vs. 10 ± 4 pg./ml., $P<0.05$), as depicted in figure 2.

Exogenous insulin at a concentration of 20,000 μU./ml. exerted a significant suppressive effect on baseline secretion in the control group with perfusate glucose at 80 mg./100 ml. (series I and II of table 2), and in the streptozotocin series II group with perfusate glucose at 80 mg./100 ml. The suppression seen in the streptozotocin series I group lacked statistical significance. In all of the additional experiments, exogenous insulin at a concentration of either 2,000 or 20,000 μU./ml. did not significantly suppress baseline glucagon secretion (figure 2).

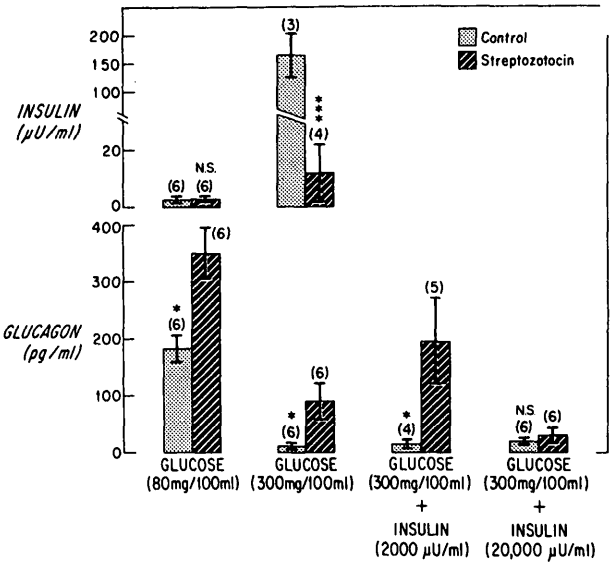

FIG. 2. Baseline glucagon and insulin secretion at varying glucose insulin concentrations. Baseline secretion in each perfusion was the mean glucagon level of the 0- and four-minute samples. Significance figures represent comparison between control and streptozotocin-treated groups as follows: *P<0.05, ***P<0.001, N.S. not significant.

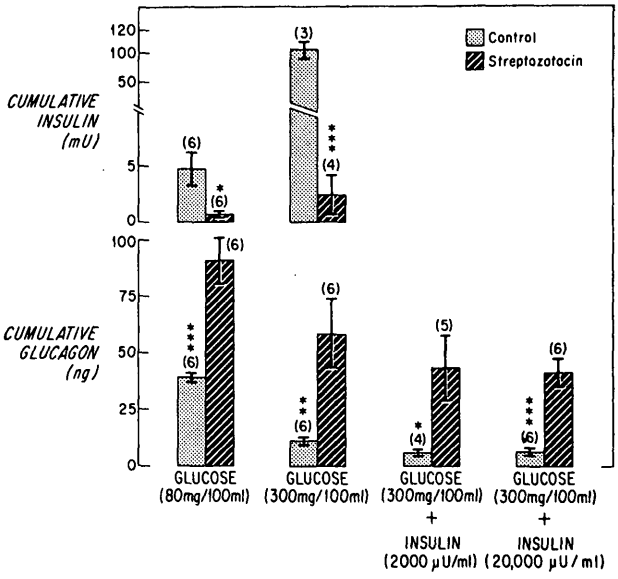

FIG. 3. Arginine-stimulated glucagon and insulin output at varying glucose and insulin concentrations (output determined by planimetry). Significance figures represent comparison between control and streptozotocin-treated groups as follows: *P<0.05, **P<0.01, ***P<0.001, N.S. not significant.

The suppressability of baseline glucagon secretion by combinations of exogenous glucose and insulin is depicted in figure 2. As already mentioned, baseline secretion in the control group was markedly suppressed by a glucose concentration of 300 mg./100 ml. This finding of suppression to assay detection limits was not altered by further addition of either 2,000 or 20,000 μU./ml. of insulin. In the streptozotocin group, glucagon secretion with a perfusate glucose level of 300 mg./100 ml. remained significantly higher than the comparable control, as noted above, and was not changed significantly by the addition of exogenous insulin at a concentration of 2,000 μU./ml. With the addition of 20,000 μU./ml. of

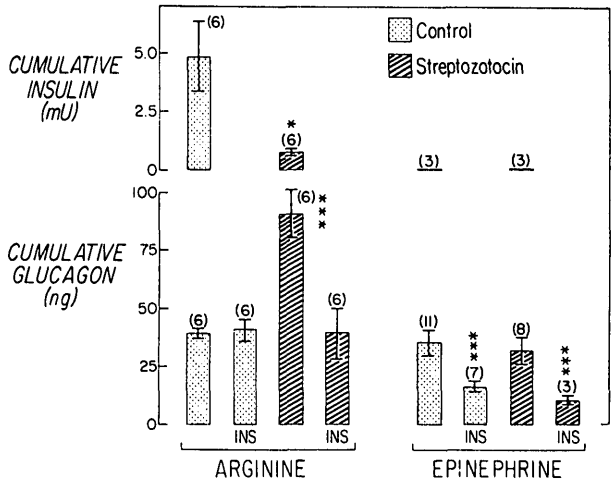

FIG. 4. Comparison of arginine- and epinephrine-stimulated glucagon and insulin secretion with demonstration of the effects of exogenous insulin (INS; 20,000 μU./ml.) and streptozotocin treatment. Perfusate glucose was 80 mg./100 ml. Significance figures represent comparison with the control (without insulin) within either the arginine or epinephrine groups as follows: *P<0.05, ***P<0.001.

insulin, however, glucagon secretion of the streptozotocin group was markedly suppressed to levels indistinguishable from those of the corresponding control group (32 ± 13 vs. 20 ± 6 pg./ml.). In summary, baseline glucagon secretion from pancreases of streptozotocin-treated rats was relatively resistant to suppression by 300 mg./100 ml. glucose and 2,000 μU./ml. exogenous insulin.

Arginine- and epinephrine-stimulated glucagon secretion from control and streptozotocin-treated pancreases: Effects of exogenous glucose and insulin. Arginine- and epinephrine-stimulated glucagon secretion at a perfusate glucose concentration of 80 mg./100 ml. are compared in figure 4. Streptozotocin treatment led to enhancement of arginine-stimulated secretion, yet epinephrine stimulation was unaffected. Exogenous insulin at a concentration of 20,000 μU./ml. had no effect on the control arginine result but lowered hypersecretion of the streptozotocin group to control levels, yet no further. The same exogenous insulin concentration suppressed epinephrine-induced secretion in both the control and streptozotocin groups.

Glucagon output in response to arginine was significantly greater in control perfusions at a perfusate glucose level of 80 mg./100 ml. than at 300 mg./100 ml. (39.5 ± 1.7 vs. 11.7 ± 2.6 ng., P<0.001). The enhancement of arginine-stimulated glucagon secretion by streptozotocin was seen when perfusate glucose was at 80 mg./100 ml. and also when perfusate glucose was 300 mg./100 ml. (figure 3).

Suppression of arginine-stimulated glucagon secretion by combinations of exogenous glucose and insulin is shown in figure 3. In the control pancreases glucagon secretion was significantly suppressed by a glucose concentration of 300 mg./100 ml. (P<0.001), but further suppression by either dose of exogenous insulin (2,000 or 20,000 μU./ml.) lacked statistical significance. In the streptozotocin-treated group there was insignificant suppression with a glucose level of 300 mg./100 ml., but significant suppression was seen with addition of exogenous insulin at a concentration of either 2,000 μU./ml. (43.8 ± 14.2 vs. 92.7 ± 10.4 ng., P<0.05) or at 20,000 μU./ml. (43.7 ± 6.8 vs. 92.7 ± 10.4 ng., P<0.01). Nonetheless, despite the addition of high glucose and either dose of exogenous insulin, the glucagon secretion of the streptozotocin group remained significantly higher than the secretion of corresponding controls (figure 3). It is of interest that even with the addition of 300 mg./100 ml. glucose and the higher dose of insulin (20,000 μU./ml.), glucagon secretion of the streptozotocin-treated pancreases was essentially equal to the secretion of control pancreases at 80 mg./100 ml. glucose with no exogenous insulin (39.5 ± 1.7 vs. 43.7 ± 6.8 ng.). Therefore, in the present streptozotocin model, arginine-induced glucagon secretion is relatively resistant to suppression by 300

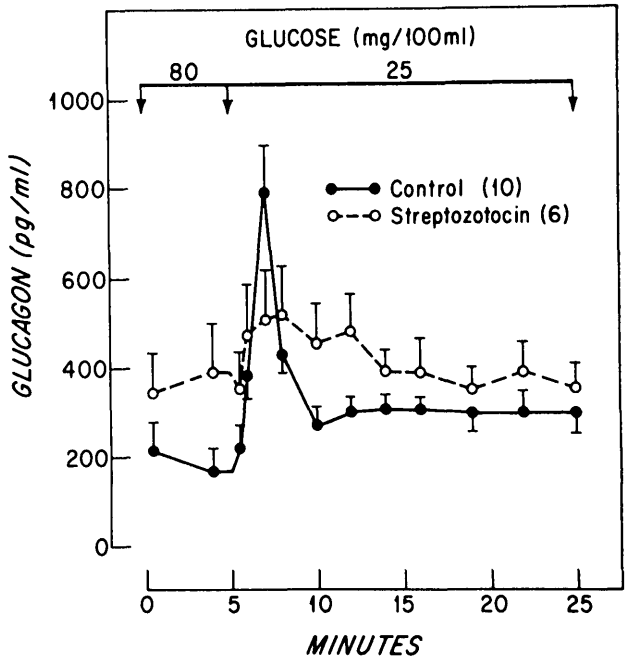

FIG. 5. Glucagon secretion from perfused pancreases of control and streptozotocin-treated rats, in response to a fall of perfusate glucose from 80 to 25 mg./100 ml.

mg./100 ml. glucose and very large amounts of exogenous insulin (20,000 μU./ml.).

Glucagon response to reduction of perfusate glucose. When perfusate glucose was abruptly changed from 80 to 25 mg./100 ml., control pancreases responded with a two-phase pattern of glucagon release (figure 5). Under identical experimental conditions, the streptozotocin group responded with an increment of glucagon release that was not clearly biphasic. With the use of planimetry, estimates were made of the increase of glucagon output over the 20-minute period at 80 mg./100 ml. This increase was then compared with what secretion would have been had basal secretion (0- and four-minute levels) remained unchanged during the same 20 minutes. The mean of per cent increments for the control perfusions was 210 ± 144 per cent, which was not significantly greater than that found with the streptozotocin experiments, 57 ± 41 per cent. When the increment was determined by planimetry on the mean values of figure 5, the control increment was 71 per cent and the streptozotocin group increase was 13 per cent.

DISCUSSION

The present data are consistent with the hypothesis that local insulin secretion has an important influence on glucagon secretion. Acute treatment with streptozotocin was followed by a marked reduction of arginine-stimulated insulin release and hypersecretion of glucagon. This was also seen during baseline conditions with perfusate glucose at 300 mg./100 ml. In the baseline state with glucose at 80 mg./100 ml. glucagon secretion was also increased, but a reduction of insulin secretion was not seen. It would have been difficult to find diminished secretion because the perfusate insulin levels in even the control experiments were so close to the detection limit of the assay. It can be postulated that local islet interstitial insulin concentrations were reduced, leading to the enhanced glucagon output. Also noteworthy is the finding that glucagon secretion can be suppressed by exogenous insulin, but this was observed only when endogenous secretion was low. This suggests that when endogenous secretion was plentiful glucagon secretion was already suppressed and therefore not suppressed further by exogenous insulin. The contrast between arginine- and epinephrine-stimulated glucagon secretion seems particularly revealing. Following streptozotocin treatment, there is hypersecretion in response to arginine that occurs when endogenous insulin secretion is inhibited. As opposed to arginine, epinephrine is a potent inhibitor of insulin secretion.[11,12] Our data show that insulin secretion remains minimal following streptozotocin treatment, and no enhancement of epinephrine-stimulated glucagon secretion occurs. It is also noteworthy that exogenous insulin has no effect on arginine-stimulated glucagon release, a situation with plentiful endogenous insulin, yet suppresses epinephrine-induced glucagon secretion. Suppression of glucogon secretion by exogenous insulin has been observed in several situations with low endogenous insulin, including fasting diabetics and nondiabetics,[13,14] islets from streptozotocin-treated rats,[15] and the perfused pancreases from alloxan-treated rats[16] and normal dogs.[35]

These data also indicate that glucagon secretion from pancreases of streptozotocin-treated rats is relatively resistant to suppression by large amounts of exogenous glucose and insulin. There is some evidence that glucagon secretion of diabetics may be relatively resistant to suppression by large amounts of glucose and insulin,[4] but this may not be true for all types of diabetes.[13,14] Glucagon of gastrointestinal origin in alloxan-diabetic dogs can be easily suppressed by small amounts of exogenous insulin,[17] and the origin of the hyperglucagonemia of human diabetics has not yet been elucidated. Pancreatic alpha cells, being juxtaposed to beta cells, may have a sensitivity to exogenous insulin different from that of gastrointestinal alpha cells. Furthermore, pancreatic alpha cells of diabetics may have variable sensitivity dependent on the number of beta cells within the islets. The results from the present diabetic rat model therefore suggest that alpha cells of acutely insulin-deficient islets are relatively resistant to suppression. These findings, coupled with the observation of increased baseline and arginine-stimulated glucagon secretion following streptozotocin treatment, are consistent with the hypothesis that at least some of the abnormalities of glucagon secretion in diabetes mellitus may be secondary to beta-cell failure and do not represent a primary alpha-cell defect.

The finding of glucagon hypersecretion in the present model is in agreement with in vivo and in vitro studies of rats and dogs made diabetic with alloxan or streptozotocin,[3,18,19] and also with perfused pancreas studies using genetically diabetic mice and Chinese hamsters.[20,21] The present findings are, however, in marked contrast to studies using perfused pancreases from chronically streptozotocin- and alloxan-treated rats by Pagliara et al.[16] These workers found very-low-baseline glucagon secretion from the perfused

pancreases of rats that had hyperglucagonemia and plentiful pancreatic glucagon content. They did not find hypersecretion in response to amino acids or suppression of glucagon secretion by exogenous insulin in the streptozotocin-treated rats. There is no clear explanation for these discrepancies, but the chronicity of the diabetes in their animals may have been a factor.

There may be several advantages in using rats made acutely insulin-deficient with intravenous streptozotocin. It is convenient and avoids some of the variables inherent in rats used several weeks following streptozotocin or alloxan administration—namely, variations of weight loss, fluid shifts, electrolyte imbalance, and ketone and lipid metabolism, as well as other unknown factors. A concern about the use of streptozotocin, either acutely or chronically, is that it might exert a direct effect on the alpha cell. There is no evidence for such an influence, however. If there had been nonspecific alpha-cell hypersensitivity, one might have expected glucagon hypersecretion in response to epinephrine, but no such enhancement was found. Finally, it must be recognized that beta-cell damage by the toxin might have led to alpha cell dysfunction by a mechanism independent of local insulin secretion.

At this time very little is known about the microcirculation within the islets of Langerhans, and it is not yet possible to determine the insulin concentration of the interstitial fluid surrounding the alpha cell, but certain assumptions are reasonable. To enter the blood, insulin presumably must traverse the islet interstitial space, which probably results in some diffusion of insulin throughout this space. As found in the present study, a perfused rat pancreas can secrete between about 3 to 10,000 μU. per minute. If this amount of insulin all emanates from the islet interstitial space and the volume of this space is about 3 μl.,* one might expect enormous variation of the concentration of insulin within the interstitial fluid. The insulin concentration surrounding the alpha cell may reach levels even higher than 100,000 μU./ml., yet at other times may be very low. Local secretion of other hormones may also have physiologic importance. There is much evidence indicating that the local secretion of norepinephrine and acetylcholine modulates insulin and glucagon release.[25] Glucagon is a potent stimulator of insulin secretion.[26] Somatostatin has recently been identified in islets[27,28] and is a potent inhibitor of both insulin and glucagon.[29,30] The relative numbers of the cells secreting these hormones may be an important determinant of their effectiveness. For instance, every alpha cell is probably surrounded by beta cells, whereas the converse is not true. Therefore, many beta cells may be relatively separated from glucagon-secreting cells and uninfluenced by their secretion. Another possible factor is seen in recent work suggesting that hormone concentration can lead to changes in receptor number and cellular responsiveness.[31] Thus, those beta cells that are exposed to high concentrations of glucagon may be relatively resistant to its action. In addition, alpha cells may be relatively resistant to large amounts of insulin. The possible importance of somatostatin within the islets is unknown.

Following reduction of perfusate glucose from 80 to 25 mg./100 ml., the increment of glucagon secretion in the control group is only a 71 per cent increase. In normal humans, following insulin-induced hypoglycemia, peripheral levels of glucagon increase approximately 400 per cent.[6] The data from this study do not explain why some diabetics fail to secrete glucogon in response to insulin-induced hypoglycemia.[6] If the glucagon-secretory characteristics in rat and man are comparable, one must conclude that much of the glucagon rise in humans following insulin-induced hypoglycemia is due to factors other than the reduction of glucose concentration. Studies of glucagon secretion following insulin injections in calves suggest that much of the glucagon rise is secondary to alpha-cell stimulation by the autonomic nervous system.[32] Therefore, it seems reasonable to postulate that this defect in diabetics is secondary to dysfunction of the autonomic nervous system.[33] Hypersecretion of glucagon by juvenile diabetics in response to epinephrine infusions has recently been reported,[33] which may, among other possibilities, represent denervation hypersensitivity.[34] Increased secretion by epinephrine was not observed in the present model.

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*This calculation is based on the following assumption: pancreatic weight is 1 gm., 1 per cent of the pancreatic volume is islets,[22] and 30 per cent of islet tissue is interstitial fluid.[23,24]

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