This was especially the case with the second calf which received sequential sublethal doses.

In anesthetized rats, intravenous injection of extract blocked responses of the anterior tibialis muscle to stimulation of the sciatic nerve (Fig. 2). The effect was not reversed by neostigmine. In vitro, on the rat phrenic nerve hemidiaphragm preparation, the extract blocked responses of the muscle to nerve stimulation. In anesthetized ducks, responses of the gastrocnemius muscle to stimulation of the sciatic nerve were blocked, and the muscle contracted (Fig. 3) in a manner similar to that reported for other avian species given depolarizing neuromuscular blocking agents. Blockade was not reversed by neostigmine but could be prevented by prior injection of *d*-tubocurarine. In chicks, injection of extract produced a typical depolarizing muscle relaxant syndrome (Fig. 4). On the frog rectus muscle, an extract of the algae gave a contractile response which was qualitatively similar to acetylcholine. *d*-Tubocurarine shifted dose-response lines of acetylcholine or the extract to the right in a parallel manner which indicated competitive inhibition. On the guinea pig ileum, large doses of extract caused a contraction that could be abolished by hexamethonium without affecting responses to acetylcholine.

It is concluded that lyophilized cells and extracts of the toxic strain *A. flos-aquae* NRC-44-1 contain a material which has many of the characteristics of a depolarizing neuromuscular blocking agent (*9*) that is very rapidly absorbed by the oral route. This toxin is considered to be the very fast death factor (VFDF) reported by Gorham *et al.* (*10*) as being produced by the parent strain NRC-44. The structure of the toxin, first described by Stavric and Gorham (*3*) as a tertiary amine, has since been deduced on chemical and spectroscopic grounds (*11*) and confirmed (*12*). An active fraction in crude synthetic material (*13*) shows similar toxicology and pharmacology to that of the lyophilized cells and extract.

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Glucagon: Role in the Hyperglycemia of Diabetes Mellitus

Abstract. *Glucagon suppression by somatostatin reduces or abolishes hyperglycemia in dogs made insulin-deficient by somatostatin, alloxan, or total pancreatectomy. This suggests that the development of severe diabetic hyperglycemia requires the presence of glucagon, whether secreted by pancreatic or newly identified gastrointestinal A cells, as well as a lack of insulin. Glucagon suppression could improve therapeutic glucoregulation in diabetes.*

The metabolic derangements of diabetes mellitus have traditionally been ascribed entirely to insulin deficiency. We propose that, in addition to lack of insulin, the presence of the insulin-opposing hormone glucagon is involved in the development of severe diabetic hyperglycemia. Two lines of evidence favor this. (i) Relative hyperglucagonemia has been found in every form of spontaneous (*1*) and experimental (*2*) diabetes examined thus far, including that produced by total pancreatectomy (*3*), a procedure which had been erroneously assumed to cause a deficiency of glucagon as well as of insulin. (ii) A simultaneous deficiency of both insulin and glucagon produced by the administration of somatostatin (*4*) does not give rise to hyperglycemia (*5*). The obvious physiologic and clinical importance of this question prompted us to study the role of glucagon in the pathogenesis of diabetic hyperglycemia.

Our results suggest that the development of diabetic hyperglycemia does, indeed, require the presence of glucagon. When both insulin and glucagon are suppressed to unmeasurable concentrations by somatostatin, hyperglycemia occurs only when glucagon concentrations are restored exogenously. As for the presence of hyperglycemia after pancreatectomy, we have confirmed the remarkable finding of Vranic *et al.*, of Matsuyama and Foa, and of Mashiter *et al.* (*3*) that glucagon is present in the plasma of totally depancreatized dogs. Moreover, when release of glucagon after pancreatectomy is completely blocked by somatostatin, hyperglycemia is also blocked despite the absence of insulin. Glucagon of depancreatized dogs, which mimics the secretory behavior of pancreatic glucagon with respect to its responses to arginine, insulin, and somatostatin infusion, probably originates in the gastrointestinal tract, where we find cells with granules indistinguishable from those in pancreatic A cells and a glucagon-like polypeptide immunometrically, biologically, and physicochemically similar to pancreatoglucagon. Finally, suppression of glucagon in alloxan-diabetic dogs results in rapid reduction of hyperglycemia to hypoglycemic concentrations, which suggests a potentially valuable new approach to the treatment of diabetes.

We studied insulin deficiency produced in dogs by somatostatin, by alloxan, and by total pancreatectomy. Pancreatoglucagon was measured with the highly specific antiserum 30K (*6*). Gut glucagon-like immunoreactivity (GLI), a polypeptide which differs immunochemically from pancreatic glu-

cagon, was assayed with cross-reacting antiserum 78J (*6*). Insulin was measured by the Yalow and Berson technique (*7*) and glucose by the glucose oxidase method with the Technicon Autoanalyzer.

We reduced insulin to unmeasurable concentrations in normal dogs by infusing 3.3 μg of somatostatin per minute; glucagon was also virtually unmeasurable (Fig. 1A). Despite the absence of insulin, glucose instead of rising declined slightly. When plasma glucagon was maintained at 350 pg/ml by infusing crystalline glucagon together with 6.7 μg of somatostatin per minute, glucose rose to a peak of 155 mg/100 ml. When the glucagon infusion was discontinued, glucose declined promptly at a rate of about 1 mg/100 ml per minute.

In order to determine whether glucagon suppression would favorably influence glucoregulation in diabetes, we infused somatostatin continuously for 24 hours in four insulin-treated, alloxan-diabetic dogs. Glucose and glucagon concentrations obtained every 2 hours were compared with values obtained at identical times during the preceding and following days, with a saline infusion given as a control. Without exception administration of somatostatin caused profound suppression of plasma glucagon to below 35 pg/ml and as low as 20 pg/ml. In remarkably parallel fashion, hyperglycemia was dramatically reduced from control values ($P < .001$), and in every dog the previously labile hyperglycemia was entirely abolished; glucagon remained below 50 mg/100 ml in every dog throughout the period of glucagon suppression, and below 35 mg/100 ml in three dogs. In insulin-requiring, alloxan-diabetic dogs, somatostatin has a powerful antihyperglycemic effect which parallels its glucagon-suppressing activity. Even after we discontinued insulin therapy in these dogs, glucagon suppression by somatostatin for 3 hours was accompanied by a progressive reduction by 167 mg/100 ml in the hyperglycemia (Fig. 1B), suggesting diminished hepatic glucose production in the face of a continuing glucose clearance by insulin-independent tissues such as the brain.

In depancreatized dogs, during normoglycemic periods resulting from optimal glucoregulation with NPH (isophane) insulin, fasting glucagon concentrations ranged from 20 to 35 pg/ml and averaged 28—not far above the sensitivity limit of the 30K assay. In order to determine whether glucagon-secreting cells were present, in two of these insulin-treated dogs we infused arginine, which in the presence of pancreatic A cells is a potent stimulus of glucagon secretion. In both dogs plasma glucagon more than doubled, indicating the presence of glucagon-secreting cells, as reported previously by Vranic *et al.* and Mashiter and co-workers (*3*). Insulin did not rise, which is evidence for the absence of insulin-secreting cells, nor did GLI increase. Similar experiments by Müller *et al.* in two well-controlled depancreatized humans failed to reveal a rise in glucagon, although pancreatoglucagon-like immunoreactivity was present (*3*).

We also studied two depancreatized dogs 3 days after discontinuation of insulin treatment. Both were hyperglycemic and had 2 μunit/ml or less of plasma insulin. Fasting glucagon was

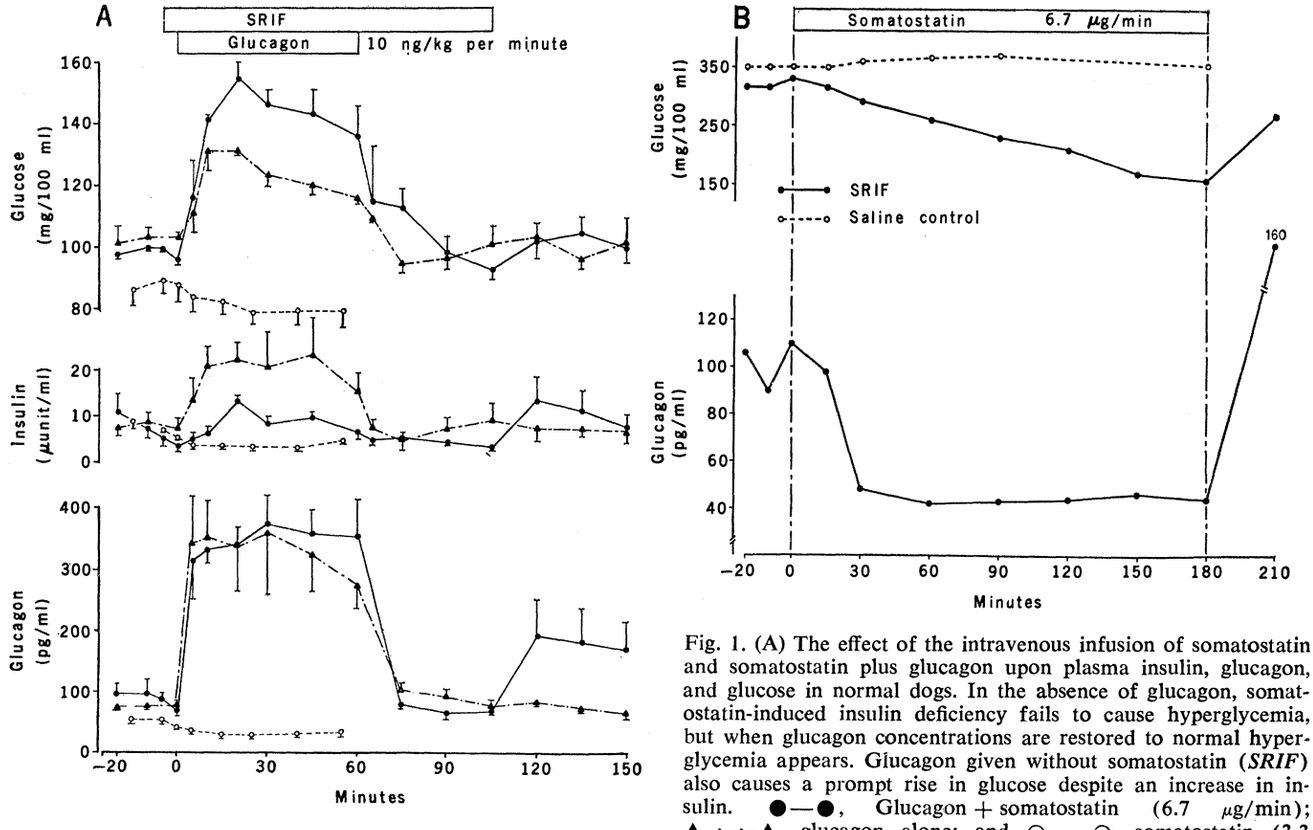

Fig. 1. (A) The effect of the intravenous infusion of somatostatin and somatostatin plus glucagon upon plasma insulin, glucagon, and glucose in normal dogs. In the absence of glucagon, somatostatin-induced insulin deficiency fails to cause hyperglycemia, but when glucagon concentrations are restored to normal hyperglycemia appears. Glucagon given without somatostatin (*SRIF*) also causes a prompt rise in glucose despite an increase in insulin. ●—●, Glucagon + somatostatin (6.7 μg/min); ▲-·-·-▲, glucagon alone; and ○- - -○, somatostatin (3.3 μg/min) alone. (B) A representative experiment in a dog with long-standing insulin-requiring alloxan diabetes. The last dose of regular insulin was given 18 hours previously. Glucagon suppression by intravenous infusion of somatostatin is accompanied by a progressive decline in glucose at a rate averaging 56 mg/100 ml per hour, indicating that glucose production is now substantially less than glucose utilization. When glucagon rises, hyperglycemia increases.

at least 200 pg/ml, about ten times that of well-regulated dogs, while GLI concentrations were normal. In both dogs arginine stimulated a rise in glucagon to at least 300 pg/ml (Fig. 2A), but neither insulin nor GLI changed. When we infused insulin in five such dogs, without exception plasma glucagon declined rapidly to below 35 pg/ml (Fig. 2). Somatostatin, which suppresses the hyperglucagonemia of untreated alloxan-diabetic dogs (*5*), also suppressed hyperglucagonemia after pancreatectomy to 35 pg/ml or less in each of three insulin-deprived dogs (Fig. 2B).

We determined the molecular size of the postpancreatectomy glucagon immunoreactivity by chromatographing the plasma on P-10 Bio-Gel columns. Approximately half of the immunoreactivity eluted with the [^{125}I]glucagon marker, and, in confirmation of Mashiter *et al.* (*3*), a substantial fraction appeared before the [^{125}I]insulin marker. "Big plasma glucagon," the globulin-sized fraction of Valverde *et al.* (*8*), was present in only minimal amounts. Chromatography of plasma obtained at the peak of arginine stimulation revealed most of the increased plasma immunoreactivity to be in the glucagon-sized fraction. This fraction was undetectable in plasma obtained during glucagon suppression with insulin or somatostatin.

In order to determine whether suppression of nonpancreatic glucagon would prevent hyperglycemia following pancreatectomy, we attempted to block glucagon release by infusing somatostatin in five dogs before, during, and for 3.5 to 4 hours after total pancreatectomy. In two of the dogs glucagon remained 20 pg/ml or more below the preoperative basal concentrations for 4 hours following the removal of the pancreas. In these dogs, instead of the expected progressive hyperglycemia, plasma glucose rose less than 20 mg/100 ml, despite the absence of immunoassayable insulin throughout the 4-hour somatostatin infusion, after which hyperglycemia appeared. In the other three dogs hyperglycemia appeared as expected, but glucagon had not been suppressed to below basal concentrations.

In an effort to determine the source of glucagon after pancreatectomy we searched for biochemical and morphologic evidence of its gastrointestinal origin. We found in extracts of porcine duodenal mucosa, chromatographed on P-10 Bio-Gel columns, a small fraction of GLI, approximately 20 percent of the total, that resembled pancreatic glucagon far more closely than GLI does. Whereas the main GLI peak had a molecular weight of 2900, reacted 30 times more avidly with cross-reacting antiserum 78J than with specific antiserum 30K, and had an isoelectric point of 10, the lesser fraction of immunoreactivity, like glucagon, had a molecular weight of 3500, exhibited equal reactivity with both antiserums, and had an isoelectric point of 6.2. Moreover, we were able to identify electron microscopically, in the canine gastric fundus, a second type of "A-like cells" with secretory granules indistinguishable from pancreatic A cells. These cells differ from previously described "A-like" gastrointestinal cells, the so-called "L cells" or "EG cells" of the lower small bowel, believed to produce GLI (*9*). The "true A cells" of the gut could well be a source of extrapancreatic production of glucagon.

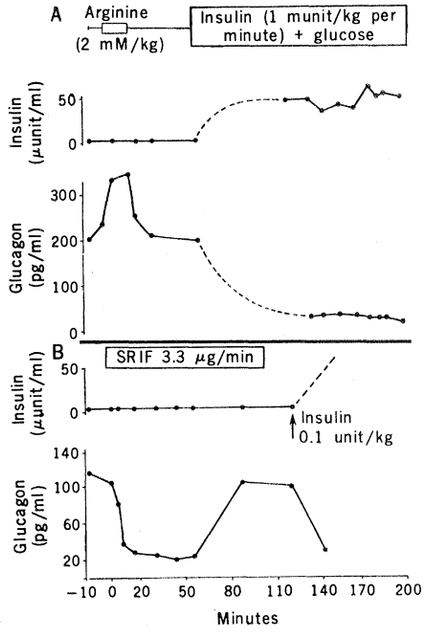

Fig. 2. (A) Typical response of plasma glucagon to intravenous infusion of arginine and of insulin in a totally depancreatized dog deprived of insulin for 3 days. Plasma glucose was maintained at 300 mg/100 ml throughout these experiments by the intravenous infusion of 12 mg of glucose per kilogram per minute. (B) Response of plasma glucagon to intravenous infusions of somatostatin (*SRIF*) and of insulin in a totally depancreatized dog after 3 days without insulin treatment. Plasma glucose remained at approximately 300 mg/100 ml throughout these experiments, varying by no more than 20 mg/100 ml.

The results of this study challenge several long-accepted concepts of diabetes. They suggest that, together with lack of insulin, the presence of glucagon may be important, if not essential, for the development of the full hyperglycemic syndrome. The increase in hepatic glucose production after total pancreatectomy, previously ascribed entirely to lack of insulin, could well be due to hyperglucagonemia after pancreatectomy (*3*), and, conceivably, overproduction of glucose observed in all forms of diabetes could be secondary to relative hyperglucagonemia derived from pancreatic or gastrointestinal "true A cells," or both. The effect of insulin upon hepatic overproduction of glucose, heretofore attributed to direct insulin action upon the liver, could just as well be mediated by the prompt suppression of hyperglucagonemia by insulin. Finally, the antihyperglycemic effect of glucagon blockade suggests a new means of achieving sustained euglycemia in diabetic patients, a goal not attained by present methods of treatment.

Note added in proof: While this report was in press Gerich *et al.* (*11*) have demonstrated remarkable antihyperglycemic effects in human diabetics requiring insulin, thus providing further support for this thesis.

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Increased Lever Pressing for Amphetamine after Pimozide in Rats: Implications for a Dopamine Theory of Reward

Abstract. *Low and high doses of a dopamine blocking agent had effects on lever pressing for intravenous amphetamine reward which resembled the effects of reward reduction and reward termination, respectively. Noradrenaline blockade had no such effects. A role in central mediation of reward perception is suggested for dopamine but not for noradrenaline.*

Research on the nature of central reward mechanisms generally utilizes the intracranial self-stimulation paradigm (*1, 2*) and currently focuses on the question of which of several catecholamine pathways are primarily involved in reward mediation (*3–5*). Noradrenaline and dopamine blocking agents have both been shown to reduce the rate of lever pressing for stimulation (*4, 5*). Similar response decrements occur when the reward value (current intensity) of stimulation is reduced, and consequently both noradrenaline (*2, 6*) and dopamine (*5*) have been proposed as neurotransmitters in a central reward mechanism. There is controversy, however, over this interpretation since response decrements might alternatively be due to arousal deficits associated with noradrenergic blockade (*5, 7*). Increased distractability, sedation, or other nonspecific drug consequences might also be argued to account for response decrements with noradrenaline blockade, and a motor deficit might account for response decrements with dopamine blockade. The usual self-stimulation data do not resolve this question, since they do not dissociate reward deficits from these other, nonspecific, types of deficit.

Rats will also lever press for intravenous injections of amphetamine (*8*), and amphetamine, like rewarding brain stimulation, seems to activate catecholamine mechanisms (*9*). Many response characteristics of intracranial self-stimulation are also typical of amphetamine self-administration (*8*); these common characteristics suggest that a common reward mechanism mediates the two behaviors. Human subjective report supports this suggestion: A patient with a history of amphetamine abuse who was subsequently given septal brain stimulation likened his responses to stimulation with the "pleasurable states he had sought and experienced through the use of amphetamines" (*10*, p. 26).

The most obvious differences between lever pressing for amphetamine and lever pressing for intracranial stimulation is a difference in rate; this difference and its underlying causes allow amphetamine self-administration to be used to dissociate true reward deficits from secondary deficits. The lever-press rate for intravenous amphetamine depends primarily on the duration of effectiveness of each injection and therefore varies inversely with the injection dose (*11*). Thus, in the case of amphetamine self-administration, when the amount of reward (dose) per injection is reduced, the lever-press rate increases. Since any nonspecific interference with the animal would decrease lever pressing, the self-administration paradigm permits clear dissociation of true reward deficits from nonspecific response disruption. The fact that the catecholamine synthesis–blocking agent α-methyl-*p*-tyrosine causes increased lever pressing for intravenous amphetamine (*12*) indicates that one of the catecholamines does, in fact, play a primary role in the reward function. We now report that it is dopamine, not noradrenaline, that plays this role. Increased rates of amphetamine self-administration (followed by extinction when high doses are used) are seen after treatment with the dopamine blocking agent pimozide, whereas decreased rates are seen with the α- and β-noradrenaline blocking agents phentolamine and *l*-propranolol.

Each of 22 adult male Sprague-Dawley rats was prepared with a permanent jugular catheter that passed subcutaneously to an exit anchored to the skull (*13*). The infusion tubing was interrupted by a feed-through swivel, so that the animal could move freely in a test box containing two levers: one lever activated a syringe pump that delivered 0.25 mg of *d*-amphetamine sulfate per kilogram of body weight with each lever press; the other caused the same relay noise but led to no injection.

The animals were trained to lever press in one or two overnight sessions in the test box. Lever pressing on the control lever was seen at first but was not sustained. Once self-administration was initiated, animals continued pressing at their characteristic rates unless they were treated with a drug, or unless amphetamine injections were ceased.

At the beginning of each test the animals were given 2 to 4 hours to settle into regular response patterns (*8*). The effects of catecholamine blocking agents were assessed over 10 hours after this stabilization period. The animals were taken from the test box, given a preassigned drug injection, and replaced in the box. Each animal was