and polarimetrically, was complete within 4 minutes at 5°C, with consumption of 0.6 mole of periodate per galactosamine unit. No further consumption of periodate or change in optical rotation was observed after 3 days. After 1 week in excess periodate the oxidized polysaccharide was recovered by dialysis and lyophilization. The oxidized product did not react with ninhydrin. A significant quantity of galactosamine was identified in the hydrolyzate of the periodate-oxidized polysaccharide.

If the polysaccharide is a simple linear polymer, only an α-(1→4)-glycosidic link between galactosamine units (Fig. 1) is consistent with the observation that a half mole of periodate is consumed per galactosamine residue and a significant amount (half) of the galactosamine is intact after oxidation. The periodate consumed destroys that half of the galactosamine units that are unacetylated by cleavage between carbons No. 2 and No. 3, while *N*-acetylation protects the remainder of the galactosamine moieties from oxidation. An alternating (1→3)-(1→4)-linked galactosaminoglycan in which the (1→3)-linked units are *N*-acetylated would also consume half a mole of periodate in destruction of the *N*-acetylgalactosamine moieties; the unacetylated galactosamine would survive. Such an alternating (1→3), (1→4) pattern occurs in the glucan nigerose (*10*). However, this more complicated structure is ruled out by the observation that periodate-oxidized polymer does not react with ninhydrin, which indicates that the amino groups of surviving galactosamine units are not free but masked by acetyl groups.

Whether the acetylated and unacetylated galactosamine units are ordered in some repeating pattern or whether the polysaccharide results from random acetylation of α-(1→4)-galactosaminoglycan is not yet known. A galactosaminoglycan consisting solely of galactosamine residues which are approximately one-third *N*-acetylated has been isolated from *Aspergillus parasiticus* (*11*). The occurrence of a galactosamine polymer which is 38-percent acetylated in *A. parasiticus* and 50-percent acetylated in *Chondrococcus columnaris* might suggest incomplete and random acetylation of the same simple galactosaminoglycan. However, the galactosaminoglycan from *A. parasiticus* has a much lower reported specific rotation, $[\alpha]^{25}_{D}$ + 51.5°, than that of the *Chondrococcus* glycan, suggesting the presence of comparable amounts of α- and β-glycosidic links in the *Aspergillus* glycan. Since these two *N*-acetylated galactosaminoglycans seem to differ in backbone linkage, it is unlikely that they are examples of a single randomly acetylated aminopolysaccharide.

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Inhibition of Insulin Release by Norepinephrine in Man

Abstract. *Normal subjects were given glucose (300 mg/min) or tolbutamide (1 g, intravenously), alone and during intravenous infusions of norepinephrine (6 μg/min). Immunoreactive insulin concentration was less than expected during the infusions of norepinephrine, but returned to higher values after the norepinephrine infusions. From these data it is concluded that norepinephrine inhibits the release of insulin from pancreatic beta cells.*

Epinephrine infusions in man inhibit the expected rise in peripheral immunoreactive insulin (IRI) when given alone or simultaneously with glucose, glucagon, or tolbutamide (*1, 2*). These observations, together with data obtained with slices of pancreas from rabbits (*3*) and intact dogs (*4*), suggest that epinephrine acts directly on the pancreatic cell to block the release of insulin.

The physiological role of epinephrine may be restricted to its relatively infrequent discharge from the adrenal medulla; but norepinephrine, as the neurochemical transmitter (*5*), would appear to be secreted continuously as the final effector of the sympathetic nervous system. Therefore, it was of interest to test whether norepinephrine inhibited the release of IRI, as has been demonstrated for epinephrine.

The subjects were healthy young men and women 21 to 31 years of age, who were within 15 percent of their ideal body weight and had no known blood relatives with diabetes mellitus. They were maintained at rest in a supine position during the test interval. They were fasted overnight (14 hours), and then in each one an indwelling plastic cannula was inserted into an antecubital vein in one arm for blood sampling, and a scalp vein needle was inserted in a hand vein of the other arm for drug infusions. A slow infusion of 0.85 percent NaCl was given, to prevent clotting, through each catheter throughout the 3-hour period of observation. Blood samples were taken for analysis of glucose, free fatty acids, and insulin at 15-minute intervals. A mean of four values taken during the first 1-hour control period was set to 100 percent, and all these and subsequent values are expressed as a percentage of the mean control value.

Heparinized plasma was kept at 0°C, centrifuged in the cold, and frozen at −19°C until analyzed for free fatty acids (*6*), and glucose (*7, 8*). Whole blood was kept at 0°C until the sampling was finished, allowed to clot for 1 hour at room temperature, and centrifuged at 0°C. This serum was then frozen at −19°C until analyzed for IRI by the double-antibody immunoprecipitation technique (*9*). Each subject received intravenously either norepinephrine (6 μg/min) (Levophed bitartrate), in 0.85 percent NaCl for 1 hour, or tolbutamide (1 g) (Orinase) injected within 1 minute as its sodium salt in 20 ml of water.

An infusion (6 μg/min.) of norepinephrine raised plasma FFA markedly and glucose slightly (Fig. 1), but had no effect on serum IRI until after the infusion was stopped, when a small rise in IRI was noted. With this small hyperglycemic response it was impossible to judge whether an increase in IRI during the norepinephrine infu-

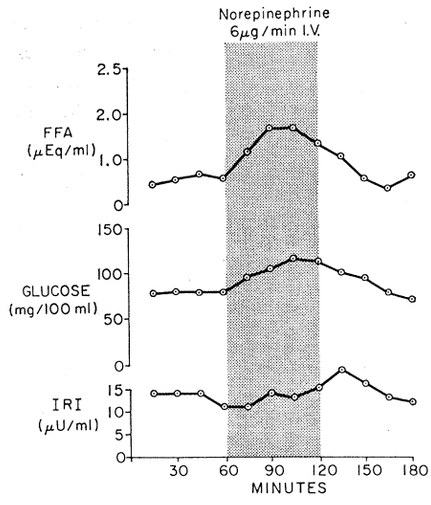

Fig. 1. Effect of norepinephrine infusion (6 μg/min) for 1 hour.

sion could be expected. Therefore, ten subjects were given glucose (300 mg/min) and norepinephrine (6 μg/min) simultaneously. For comparison, ten subjects were infused only with glucose (300 mg/min). Five of the subjects were tested twice, and ten others were given either norepinephrine or glucose (Fig. 2). Mean plasma glucose was significantly higher during the combined infusions, yet serum IRI was lower. This difference in IRI response was significant when the five subjects who were tested twice were compared directly ($P < .02$, $< .02$, $< .05$, and $< .1$ for the four points during infusion), or when all subjects were compared as two groups of ten ($P < .02$, $< .05$, $< .1$, $< .1$ for the four points during infusion). Perhaps of equal importance is the prompt secondary rise in IRI after the combined norepinephrine and glucose infusion, rather than the fall observed after glucose infusion alone. This suggests that the inhibition of an insulin response is promptly reversed as catechol concentration in the plasma falls.

Similar studies with tolbutamide as the stimulus for insulin release were performed on another group of five subjects. Each subject was tested twice, at intervals of at least 1 week; (selected at random) some subjects received 1 g of tolbutamide given alone as the first dose, the second dose being 1 g of tolbutamide given 15 minutes after the start of an infusion of norepinephrine (6 μg/min). There was a significantly smaller insulin response when tolbutamide was given during a norepinephrine infusion ($P < .05$, $< .05$, $< .05$, $< .02$ by paired comparisons for the samples taken 10, 20, 30, and 45 minutes after administration of tolbutamide). After the norepinephrine infusion was stopped, serum IRI concentration again rose, although at this time plasma glucose was already at or below control values.

The values for plasma insulin in the normal fasting subjects given glucose alone (mean ± S.D., 7.9 ± 4.1 microunits per milliliter) were not significantly different from those in fasting subjects given glucose and norepinephrine (12.7 ± 3.0). The plasma insulin varied little in those subjects given tolbutamide, measurements on two different days being 10.0 ± 5.6 and 10.1 ± 3.9 microunits.

Although norepinephrine appears to inhibit insulin secretion in a manner similar to that of epinephrine, at an equivalent dose its effects are quantitatively less (*1*, *2*). The concentration of catecholamine in blood was not determined, but it is likely that the amounts were higher during these infusions than in any physiological variation measured in normal man, since each infusion was accompanied by

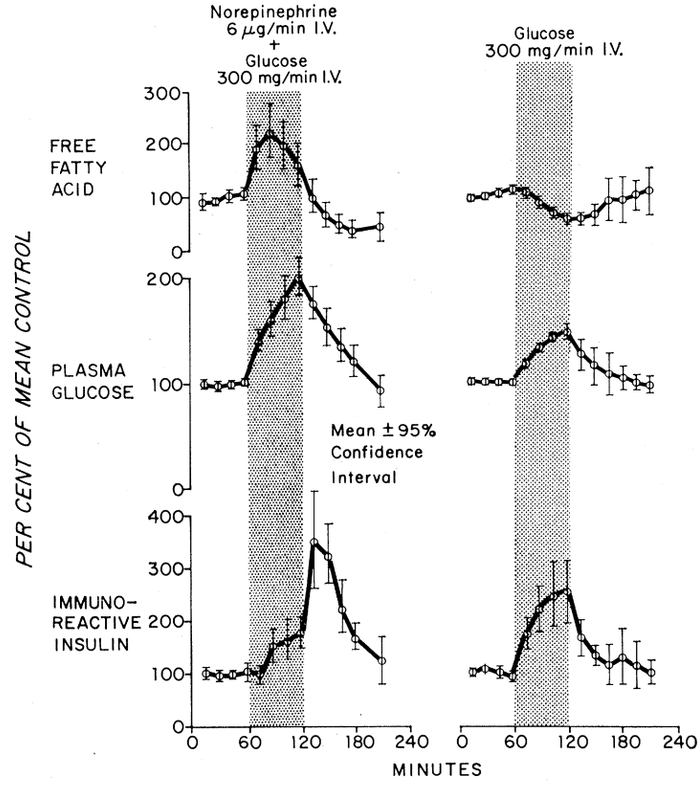

Fig. 2. Comparison of glucose with norepinephrine-plus-glucose infusion. Ten subjects for each group. Values whose brackets do not overlap are significant at $P < .05$ or better.

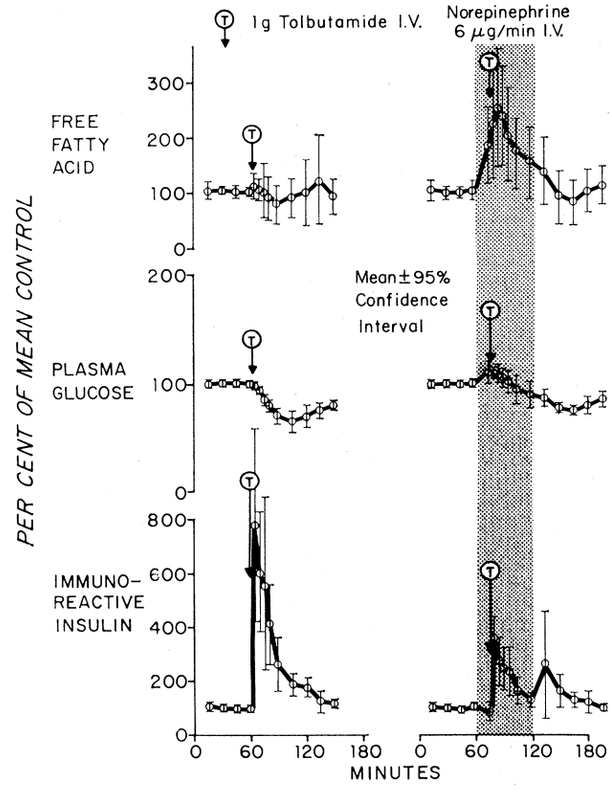

Fig. 3. Effect of norepinephrine infusion on the response to acute injection of tolbutamide. Five subjects were studied twice.

significant bradycardia (decrease of 10 to 20 beats per minute), and hypertension (increase of 20 to 40 mm systolic pressure; 10 to 30 mm diastolic pressure). However, this degree of hypertension is common in patients with pheochromocytoma, and therefore the hyperglycemia and glycosuria observed in such patients (*10*) may reflect, in part, insulin inhibition from either epinephrine or norepinephrine. Indeed, in one such patient with an abnormal oral glucose tolerance test, there was no rise in IRI until 120 minutes after glucose. This early inhibition was no longer present during a later glucose tolerance test, repeated after surgical removal of the pheochromocytoma (*11*). This inhibitory effect of infused norepinephrine on plasma insulin responses should raise doubts about the validity of any conclusions drawn from experiments in which norepinephrine is given to study the effects of an increased free fatty acid concentration upon glucose turnover. Although Nestel *et al.* have suggested that blockage of FFA mobilization during norepinephrine infusions by nicotinic acid reversed the effects of norepinephrine on glucose disposal (*12*), we have found no reversal of epinephrine inhibition of plasma IRI despite complete nicotinic acid blockade of epinephrine-induced lipolysis (*1*).

Although little is known of the local concentration of norepinephrine at sympathetic effector sites after stimulation of sympathetic nerves, conceivably these concentrations are similar to the concentrations of norepinephrine in the plasma achieved by our infusion, and therefore the sympathetic nervous system may play a tonic role in the regulation of insulin release. Certainly the pancreas has been shown to be innervated by sympathetic nerve fibers (*13*).

There was a prompt return of plasma IRI to normal concentrations 30 minutes after tolbutamide was given alone. When tolbutamide was given during a norepinephrine infusion there was a definite secondary peak in IRI although the tolbutamide had been given 60 minutes before. This "rebound" of serum insulin suggests a prolonged effect of the tolbutamide since plasma glucose was already at or below the values prior to infusion. This prolonged effect is not surprising in view of the 4-hour half-life of tolbutamide (*14*), and suggests that the prompt decline of the insulin concentrations in the course of a tolbutamide tolerance test in normal subjects may reflect counter regulation by epinephrine or norepinephrine secretion rather than lack of effective tolbutamide levels at 30 minutes. Compatible with this suggestion is the rise in heart rate of 10 to 20 heart beats per minute, 20 to 30 minutes after tolbutamide administration, which occurred in each subject. Therefore, lack of such counter regulation, rather than excessive drug response, may be responsible for part of the abnormal insulin response to tolbutamide noted in patients with organic hyperinsulinism.

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Chimeric and Ex-Parabiotic Frogs (Rana pipiens): Specificity of Tolerance

Abstract. *Rejection of orthotopic neural-fold transplants may be prevented by either embryonic parabiosis or reciprocal exchange of presumptive blood. These observations form the basis of the no blood–no tolerance hypothesis, which states that persistent tolerance by the host requires that the somatic transplant be accompanied by presumptive blood from the donor. Regarding parabionts and chimeras as special cases of transplantation of whole or partial animals, I found that ex-parabionts accept subsequent skin exchanges only from the homologous ex-parabiont; reciprocal chimeras are compatible provided each animal portion contains a primary blood source, and will accept transplants as frogs only from the homologous recombinant. Chimeric recombinations made anterior to the heart field prove incompatible and fail to survive to maturity. Successful chimeras as well as ex-parabionts survive to maturity and are apparently normal in every respect.*

When single neural folds are exchanged between embryos of *Rana pipiens* they are incorporated into the embryos, but the pigment cells derived from the donor neural crest are selectively rejected from the host skin during middle life of the larva. Rejection is accompanied by simultaneous repair from host sources, resulting in a host-pigment pattern at metamorphosis. In exceptional instances, repair may be incomplete by metamorphosis, at which time the pattern stabilizes, leaving permanent pigment-free areas in the skin of the frog (*1*). The incompatibility of tissue exchanges between sibling animals supports the findings of Hildemann and Haas (*2*) that skin transplants among some 600 larvae of *R. catesbeiana* were invariably rejected, indicating a large degree of variability in histocompatibility in the wild populations of these animals. Rejection has been avoided by two methods: After the exchange of neural folds, the animals were joined in parabiosis in tail-bud stages; or exchange of neural folds was followed by exchange of portions of the ventral belly region, to include cells destined to give rise to the blood of the animal (blood island). The second method amounts in effect to a surgical exchange of blood, comparable to parabiosis, as indicated by the observa-