ic control, when it occurred, usually did so at 6 weeks after the secondary transplantation almost exclusively in animals which had exhibited only partial reversal of the diabetic state. The immunoreactive insulin content of transplants removed from six animals displaying failure after a partial (five rats) or complete (one rat) reversal of diabetes was only 9 ± 3.3 milliunits per pancreas compared with 108 ± 16 milliunits per pancreas in rats with complete and permanent reversal of the diabetic state. The low insulin content of the transplants removed from rats following failure of control of diabetes may be due, in part, to intense stimulation of insulin secretion leading to depletion of stored insulin or, alternatively, failure of the pancreatic tissue to grow. To differentiate between depleted storage and limited insulin synthesis and release we would have to measure the secretory capacity of the transplants.

These results suggest the overstimulation of insulin secretion by hyperglycemia at an early stage after fetal pancreas transplantation may limit optimal growth and maturation of fetal beta cells and thus limit insulin secretion. To reduce the amount of pancreatic tissue needed to control the diabetic state, careful control of the blood sugar may be necessary. Since a single donor is desirable in allografts for proper histocompatibility matching to minimize problems of rejection, our observations may be of importance when this method is applied to human beings with diabetes mellitus.

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Salivary Gland Hyperglycemic Factor: An Extrapancreatic Source of Glucagon-Like Material

Abstract. *Extracts of homogenates of rat, mouse, rabbit, and human submaxillary salivary glands contain a significant quantity of a material with glucagon-like immunoreactivity. Fractionation of this material on columns of Sephadex G-100 reveals a single peak immediately following a gamma globulin marker but in advance of a rat growth hormone marker, crystalline amylase, and isotopically labeled porcine insulin and glucagon. This material, which is urea stable, shows identical immunoassay dilution curves when measured with the highly specific K-30 glucagon antiserum. Study of paired glands in vitro shows that low concentrations of glucose stimulate and high concentrations of glucose suppress release of this material. Arginine promotes brisk release in vitro. Somatostatin does not influence arginine-stimulated secretion and insignificantly suppresses basal release in vitro. These findings lend support to previous speculations that the salivary glands may possess endocrine as well as exocrine functions. Salivary gland glucagon may also be the source of circulating glucagon recently reported in pancreatectomized and eviscerated rats.*

By means of the glucagon radioimmunoassay, it was demonstrated that, as with other peptide hormones (*1*), there is considerable molecular heterogeneity among, as well as several sources of, circulating immunoreactive glucagons. Further, it was shown that "pancreatic-specific" antiserums raised to pancreatic glucagon could cross-react with some of these non-islet glucagons. A plasma glucagon of serum globulin dimensions has been observed (*2*), and several laboratories have investigated immunoreactive glucagon-like materials of enteric origin (*3*). There are significant differences in the elution chromatography profiles of serum glucagons in different populations of normal and diseased humans (*4*). In pancreatectomized dogs, glucagon concentrations, measured with a widely used pancreatic-specific glucagon antiserum (*5*), persist and even rise (*6, 7*). Furthermore, arginine, which is known to stimulate the secretion of pancreatic glucagon, elicits a brisk increase in the plasma glucagon concentration in dogs several days after they have been pancreatectomized (*7*).

It was also reported that true pancreatic alpha cells were present in the gastric fundus of dogs and that extracted glucagon from this source possessed essentially all of the physicochemical characteristics of pancreatic glucagon (*8*). A plausible gastrointestinal source of pancreatic glucagon was thus offered to explain the increasing concentrations of glucagon in pancreatectomized animals. However, soon thereafter, it was reported that glucagon levels almost triple over a 48-hour period in eviscerated and pancreatectomized rats maintained alive with a functional liver (*9*). It was these discoveries that prompted us to look for additional extrapancreatic sources of glucagon.

We have found a significant amount of a large immunoreactive glucagon-like material in the submaxillary salivary glands of rabbits, rats, and mice, and a measurable amount in the submaxillary glands of humans (Table 1). Insignificant amounts are present in the parotid and sublingual glands of rodents and man. Small amounts of a glucagon-like immunoreactive substance were reported earlier in the submaxillary glands (*10*), but using the cited method of extracting these tissues, we were unable to confirm such findings. The apparent discrepancy stemmed from the fact that immunoreactive glucagon of salivary gland origin is a large peptide that is not extractable by the traditional acid-alcohol method of Kenny (*11*) used in that study. The peptide can be obtained, however, by simple, acid-saline extraction in the presence of Trasylol, an effective protease inhibitor. After thorough homogenization in 0.9 percent saline acidified with 0.1*N* HCl to *p*H 2.8, samples from the submaxillary glands of the rat are spun at 2000 rev/min for 15 minutes, and the supernatant is removed and frozen. It is then thawed and recentrifuged, and the supernatant is applied to a Sephadex column (G-100; 50 by 1.5 cm) and eluted with either 0.2*M* glycine buffer, *p*H 7.5, or with 0.09 percent NaCl.

Using this approach we find significant amounts of a large immunoreactive glucagon-like material which appears in a discrete peak of fractions immediately following an isotopically labeled gamma globulin marker, suggesting a molecular weight of 70,000. This peak retains its chromatographic profile after repeated chromatography and after incubation with 8*M* urea and 1*M* acetic acid for 16 hours. Serial dilutions of this glucagon-like material show identical immunoassay curves when measured with the Unger K-30 glucagon antiserum, even though the column elution pattern for

submaxillary gland glucagon appears well in advance of isotopically labeled growth hormone of the rat and before labeled pancreatic glucagon and insulin markers. Acrylamide gel electrophoresis [tris, EDTA (disodium salt), boric acid buffer, 0.1*M*, *p*H 8.9; or sodium dodecyl sulfate and 8*M* urea] revealed a major immunoreactive band immediately following marker albumin, as well as two much fainter and more diffuse bands which migrated with marker chymotrypsinogen and pancreatic glucagon, respectively. These studies also suggested a molecular weight for this material of approximately 70,000.

Intravenous injections of immunoequivalent amounts of this large immunoreactive glucagon from the submaxillary gland of the rat elicits a hyperglycemic effect comparable to that obtained with porcine pancreatic glucagon (Fig. 1); insulin release in response to the induced hyperglycemia was similar in both groups of animals. No significant amounts of immunoreactive glucagon were found in either acid or acid-alcohol extractions of brain, pituitary, muscle, tongue, kidney, or liver. No immunoassayed glucagon was found in saliva of man or rats.

In ten separate experiments in vitro, glands in glucose (0.3 mg/ml) released 200 to 300 percent more glucagon than glands incubated in buffer alone ($P < .001$). Furthermore, high glucose concentration significantly suppressed glucagon release from submaxillary glands incubated in vitro. When paired glands were incubated in glucose concentrations of 0.3 or 3.0 mg/ml, release of glucagon in the former medium was 150 to 200 percent greater than in the higher glucose concentration (ten experiments, $P < .001$). Arginine, 16 m*M*, was a potent stimulus to the release of this salivary gland glucagon-like material in vitro (Fig. 2). Preliminary studies with somatostatin, the hypophysiotropic tetradecapeptide, which has been shown to suppress pancreatic glucagon secretion, failed to show any significant suppression of the release of submaxillary gland glucagon in vitro (*12*).

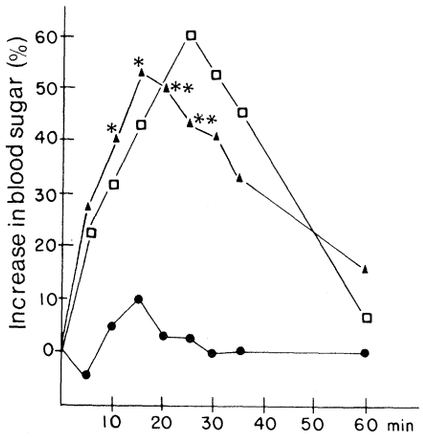

Fig. 1. The effect of tail vein injection into overnight fasted, lightly anesthetized (Nembutal) male rats of 0.1 ml of: □, porcine glucagon (0.65 μg) ($N = 3$); ▲, an immunoequivalent amount of rat submaxillary gland immunoreactive glucagon (after tissue extraction, column separation of the immunoreactive glucagon, dialysis to remove the glycine buffer, lyophilization, and reconstitution in normal saline) ($N = 5$); and ●, normal saline ($N = 3$). After the injections, 0.25 to 0.5 ml of blood was drawn from the external jugular vein at the times indicated. Plasma glucose was determined by the Beckman glucose-oxidase autoanalyzer. There were five rats in each group. A single asterisk denotes a significant difference from the saline controls at $P < .05$; a double asterisk, $P < .001$.

Table 1. Immunoreactive glucagon in salivary glands of mouse, rat, dog, rabbit, and man. Results are expressed as nanograms of immunoreactive glucagon per gram of tissue (wet weight).

Gland	Amount (ng/g)				
	Mouse	Rat	Dog	Rabbit	Man
Submaxillary	7000	5000	Trace	8.7	20*
Sublingual	Trace	Trace	5	Trace	7†
Parotid	Trace	20 to 80	0.8	Trace	None*

*Surgical specimens. †Four hours postmortem.

These findings add additional weight to the cautionary note that the source of blood glucagon measured in normal or diabetic man and animals not be overstated. We suggest that the pancreatic specificity of any of the available antiserums to glucagon should not be stressed until these antiserums have been subjected to rigorous examination in pancreatectomized, eviscerated, and now sialectomized animals. Although we have not examined the glucagon concentrations in such operated animals, it seems likely that the submaxillary glands are the source of circulating glucagon in pancreatectomized and eviscerated rats (*9*). Also, it may be that salivary gland glucagon contributes significantly to the arginine-induced glucagon increase in pancreatectomized dogs because the elution pattern of plasma glucagon from such animals appears in advance of the isotopically labeled pancreatic glucagon marker (*7*). Furthermore, our experiments in vitro have shown arginine to be a potent stimulus to the release of glucagon immunoreactive material from the submaxillary glands of rats.

Although the exact role of pancreatic glucagon in the metabolic events of the diabetic state is currently disputed (*13*), it seems likely that the very high levels that occur in the poorly controlled diabetic may contribute to early acceleration of ketogenesis and hepatic glycogenolysis and gluconeogenesis. It is also possible that in the severely insulinopenic diabetic, glucagons of extrapancreatic sources may contribute to measurements of total circulating glucagon. Although little is known of the biologic activities of the extrapancreatic glucagons, it may be that they also act to hasten or worsen the metabolic events of the uncontrolled diabetic state. Finally, it is of interest to note there are reports in the literature of enlarged salivary glands in diabetics, including a case of insulin-resistant diabetes in which the patient had enlarged submaxillary glands and an extensive eczematoid skin eruption (*14*); in this patient, the diabetes and skin disease spontaneously remitted at the same time that the submaxillary glands returned to normal size. These observations seem similar to what has been described in the glucagonoma syndrome where diabetes and an eczematoid dermopathy disappear with successful resection or cytotoxic therapy of an alpha cell, glucagon-secreting neoplasm (*15*).

For many years there have been sporadic reports that the salivary glands serve both an exocrine and an endocrine function. Nerve growth factor (*16*) and

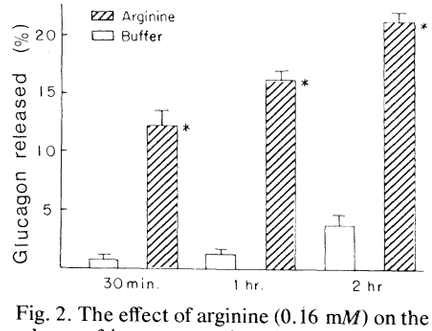

Fig. 2. The effect of arginine (0.16 m*M*) on the release of immunoreactive glucagon from rat submaxillary glands quartered and incubated in 10 ml of Ringer-lactate solution alone or in buffer with added arginine. A single asterisk denotes a significant difference between glucagon released in buffer alone compared to the presence of arginine at each time period noted; $P < .001$. The amount of glucagon released is expressed as a percentage of the total gland glucagon content after 2 hours of incubation. Five experiments were performed. The vertical lines represent the standard errors of the mean for each group of experiments.

epidermoid growth factor (*17*) have both been described in submaxillary glands of male mice. Epidermoid growth factor, the polypeptide that promotes precocious eye opening and toothbed eruption in mice, has also been described in human urine (*18*), and gastrin-like immunoreactivity has been found in bovine parotid glands and in saliva (*19*). Our studies, reported here, dealing with the presence and release of a large glucagon-like material from the submaxillary gland, even possibly a proglucagon, lay the groundwork for additional investigations to determine the biologic significance of these findings.

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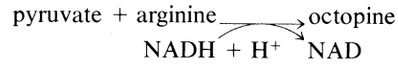
Octopine as an End Product of Anaerobic Glycolysis in the Chambered Nautilus

Abstract. *The terminal step in the anaerobic glycolysis of muscle in the chambered nautilus,* Nautilus pompilius, *is not pyruvate reduction to lactate as in vertebrate muscle. Instead of lactate dehydrogenase, these organisms utilize octopine dehydrogenase (E.C. 1.5.1.11), catalyzing the reductive condensation of pyruvate and arginine, which is dependent on the reduced form of nicotinamide adenine dinucleotide, to form octopine and the oxidized form of the coenzyme. The kinetic properties of octopine dehydrogenase favor the production of octopine, which accumulates under a variety of conditions.*

Across the animal kingdom, there is the problem of producing mechanical work from chemical stores under both aerobic and anaerobic conditions. In the vertebrates, the efficiency of conversion is high during aerobic glycolysis, 38 moles of adenosine triphosphate (ATP) being produced for each mole of glucose 6-phosphate (G6P) oxidized. Under anaerobic conditions, the efficiency drops to 3 moles of ATP per mole of G6P converted to lactate, but the accumulated lactate can be recycled when or where aerobic conditions prevail and so be fully oxidized. Do important invertebrate groups like the cephalopods follow a similar strategy or have they evolved different mechanisms for coping with the differing conditions under which work is demanded by their life-styles?

Until recently not much was known about metabolic organization in the cephalopods, even though the squid is a favorite experimental material for electrophysiologists and the octopus has been used to open new frontiers in comparative animal behavior. This situation was at least partially remedied in the fall of 1973 when a group of us had the opportunity to study a fast-swimming predaceous squid, *Symplectoteuthis* (*1*). We found the mantle muscle packed with mitochondria and displaying an extremely aerobic metabolism primed by a functional α-glycerophosphate cycle, with both cytoplasmic α-glycerophosphate dehydrogenase (E.C. 1.1.1.8) (α-GPDH) and the mitochondrial α-glycerophosphate oxidase occurring in substantial activities (*1*). On the other hand, the anaerobic capabilities of this muscle did not depend on lactate dehydrogenase, which is in fact absent, but upon octopine dehydrogenase (ODH) (E.C. 1.5.1.11), which catalyzes the reductive condensation of pyruvate and arginine (*2*) (NAD is nicotinamide adenine dinucleotide; NADH, reduced form of NAD):



Since simultaneous function of ODH and α-GPDH would lead to a carbon and energy drain off main-line glycolysis (*3*), it was not surprising to find (i) that under most physiological conditions α-GPDH activity could exceed that of ODH by a factor of nearly 100:1 (*4*) and (ii) that therefore octopine typically did not accumulate in fast-swimming cephalopods (*5*). By implication, the reverse of this situation should prevail in more anaerobic cephalopods. Our first opportunity to test these ideas came during the expedition of R.V. *Alpha Helix* to the Philippines, where we studied the chambered nautilus (*Nautilus pompilius*), a phylogenetically ancient animal, shelled and slow swimming. We found α-GPDH at barely detectable levels but ODH at high activities and displaying kinetic properties consistent with function under physiological substrate concentrations. Predictably, octopine accumulation in muscle could be demonstrated under a variety of situations.

Nautilus ODH [see (*6*) for assay method] occurs in highest activities in spadix, funnel, and retractor muscles [about 44, 42, and 38 μmole of product formed per minute per gram wet weight (*p*H 7 and 25°C)]; in the heart, the activities are reduced to about one-fourth of these values. With electron microscopy we observed that mitochondria are least abundant in the spadix and most abundant in the heart, but in none of these muscles are they as abundant as in squid muscle (*1*, *7*). In contrast to the squid, where the ratio of α-GPDH to ODH is high, α-GPDH in the *Nautilus* spadix occurs at less than 1/40 the ODH activity; in retractor muscle it is about 1/13 the ODH activity. This arrangement is metabolically "sensible," since simultaneous function of both enzymes would lead to a carbon drain from glycolysis (to α-glycerophosphate) and to a serious drop in glycolytic energy yield. The activity ratios in *Nautilus* are of course just opposite to those found in the squid (*1*). Thus the absolute activity of ODH as well as the activity ratio of ODH to α-GPDH seem to roughly parallel the anaerobic capabilities of the muscle and indeed of the whole animal,