Immunoreactive Insulin in Rat Salivary Glands and Its Dependence on Age and Serum Insulin Levels (43899)

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Abstract. **The major salivary glands of adult rodents contain immunoreactive insulin (IRI). To determine if the concentration of IRI in salivary glands is modulated by the level of serum insulin, insulin immunoreactivity in the parotid and submandibular glands of male rats at different ages (sucklings, pubescent, mature, and elderly) was assayed and compared with corresponding serum insulin concentrations. Salivary glands from suckling rats contained 94 ng/g (wet weight) insulin, which is 1.6 times higher than the level in pubescent rats, and about 10 times higher than levels in mature and elderly rats. No direct relationship between salivary gland content and serum IRI levels was indicated by the data. In an attempt to increase insulin levels in serum, insulin-secreting pancreatic islet adenomas were induced in young male rats by injecting streptozotocin (an islet tumor-inducing drug) with nicotinamide (which reduces the drug's β-cell cytotoxicity). The mean insulin content of salivary glands from drug-treated rats that had not yet expressed tumors was no higher than controls. After the development of visible tumors of pancreatic islet tissue, however, salivary gland IRI was markerdly elevated, reaching 40 times control levels, whereas serum insulin, and the immunoreactive insulin content of two insulin-sensitive tissues (*vis.* hepatic, adipose), were elevated only 2-fold. Examination of histologic sections of the parotid and submandibular glands from drug-treated animals revealed no evidence for the formation of salivary tumors. The data indicate that salivary gland insulin content (i) is age-related, being highest in neonates and declining thereafter, (ii) is generally identical in parotid and submandibular glands at a given age, and (iii) is not modulated solely by the animal's serum insulin concentration. These results are discussed in regard to the possible sources of insulin detected in the major salivary glands.**
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The major salivary glands in mammals secrete a saliva that functions in the digestion and lubrication of food, and serves as a vehicle for antibacterial agents, such as lysozyme and lactoferrin, and for secretion of IgA. In addition, a variety of hormones, growth factors, and other biologically active substances are present in salivary glands and saliva (1). Insulin, for example, has been identified in rodent salivary glands by immunoassay and immunohistochemistry (2–7), and in human saliva by immunoassay (8–12). Its apparent molecular structure is identical to pancreatic insulin (2, 6, 10). These reports have given rise to speculations as to the source of the hormone (e.g., from the pancreatic islets by transport from the blood, or by *de novo* synthesis within salivary glands?) and its possible role (e.g., active clearance of insulin, or its passive leakage into saliva?). These questions have not been resolved, although some data support the concept that salivary glands synthesize insulin. In slices of parotid gland Murakami *et al.* (2) demonstrated *in vitro* incorporation of radiolabeled amino acids into insulin that was isolated by immunoprecipitation and gel filtration; Shubnikova *et al.* (4) published a similar study using slices of mouse submandibular

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gland. In a series of reports, Smith presented evidence for specific immunostaining of insulin in rodent parotid and submandibular glands, the hormone being localized to the intercalated ducts in both organs (3, 5, 6, 7). This last group also investigated the immunoassayable insulin content of the salivary glands under two conditions of decreased blood insulin levels: genetic diabetes or treatment with streptozotocin (a selective pancreatic B cell toxin). They observed that plasma and pancreatic insulin levels in diabetics were markedly inhibited, but that salivary immunoreactive insulin (IRI) contents were depressed less dramatically. Furthermore, the incidence of insulin-positive ductal cells were not substantially decreased in the diabetic state. It was suggested that insulin is synthesized by ductal cells of the parotid glands, and that this may account for a significant part of salivary gland insulin (3).

The present study was undertaken to determine how closely the immunoassayable insulin content of rat salivary glands correlates with the concentration of insulin in the blood. This information is important to understanding the role of the major salivary glands in insulin metabolism. Insulin content was determined in salivary glands from normal rats of different ages, and in rats with elevated serum insulin levels resulting from the growth of drug-induced pancreatic insulinomas. The insulin contents of hepatic and adipose tissues also were compared with serum insulin levels, since adipocytes and hepatocytes are known insulin target cells.

Materials and Methods

Animals and Tumor Induction. Male Sprague-Dawley strain rats were obtained from Holtzman, Co. (Madison, WI), housed in a temperature- and light-controlled room, and maintained on Purina Rat Chow and water *ad libitum*. For induction of pancreatic islet tumors, several 6-week-old male Holtzman rats (160–180 g body wt) were fasted overnight and injected ip with nicotinamide (350 mg/kg body wt in saline). Ten minutes later, a single iv injection of streptozotocin (65 mg/kg body wt in citrate buffer, pH 4.5) was given, followed by another ip injection of nicotinamide 180 min after the first dose, as detailed previously (13). Animals maintained for 36 weeks thereafter exhibit slightly lower fasting blood glucose levels than controls during the first 20 weeks; pancreatic tumors first appear in 20 weeks and affect about 85% of drug-treated group by 36 weeks (14). Serum insulin levels in insulin-secreting tumor-bearing rats at this time generally are 2-fold higher than controls (15).

Tissue Collection and Analyses. The animals were anesthesized by ip injection of chloral hydrate at 35 mg/g body wt. After onset of anesthesia, samples were taken via cardiac puncture for blood chemistry determinations. Serum was removed after centrifugation and stored at −20°C until assay. Parotid and submandibular glands were dissected carefully to remove adherent tissues, weighed, and frozen at −20°C until their extraction for hormone assay. Each pair of glands was quickly minced during thawing and homogenized in 20 volumes of ice-cold acid ethanol using motor-driven glass-teflon grinders. The extracts were cleared by centrifugation. The resulting supernatants were brought to pH 8–8.5 and maintained in the cold for 2 hr, and again cleared by centrifugation. Due to their small size, the parotid and submandibular glands from each suckling rat were isolated, trimmed of fat and lymphoid tissue, pooled, and processed *en masse*. Aliquots of the final alcoholic extracts were dried under vacuum, and the immunoreactive insulin contents were estimated by radioimmunoassay as described (13). The minimal detectable insulin concentration of the assay was approximately 40 pg/ml; the ED_{50} was 480 pg. Blood glucose was determined by glucose oxidase method (Glucose Analyser 2; Beckman Instruments, Inc., Brea, CA).

Levels of immunoreactive insulin (IRI) in the glands and tissues were expressed as nanogram IRI per gram wet weight tissue (ng/g), and in serum as nanogram IRI per milliliter. Data were compared by one-way analysis of variance with Student's Newman-Keuls test to evaluate significant F ratios.

Histology of salivary glands was prepared on Bouin's fixed, paraffin-embedded sections stained with aldehyde fuchsin as described previously (14).

Results

Salivary Gland Insulin (IRI) Content and Age. Parotid and submandibular glands were isolated from mixed gender suckling rats with a mean weight of 30.5 ± 0.46 g, and from groups of male rats weighing 85.0 ± 3.4 g (pubescent), 202 ± 9.3 g (mature), and 681 ± 32.1 g (elderly). The glands were processed for insulin assay and the data expressed as nanograms of immunoreactive insulin per gram tissue (wet weight). As illustrated in Figure 1, the insulin content of salivary glands varied with age, being highest in sucklings (94.0 ± 17.2 ng/g wet wt, n = 7). Parotid and submandibular glands from six pubescent rats (85 g body wt) contained IRI of 62.8 ± 22.5 and 58.0 ± 28.9 ng/g, respectively. Glands from mature (202 g) rats contained 10.5 ± 2.52, and 10.6 ± 2.03 ng/g, respectively, and showed no further changes after achieving a body weight of 680 g, where the parotid contained 9.61 ± 1.19 ng/g and the submandibular gland contained 7.34 ± 1.44 ng/g immunoreactive insulin.

In order to determine if glandular insulin content

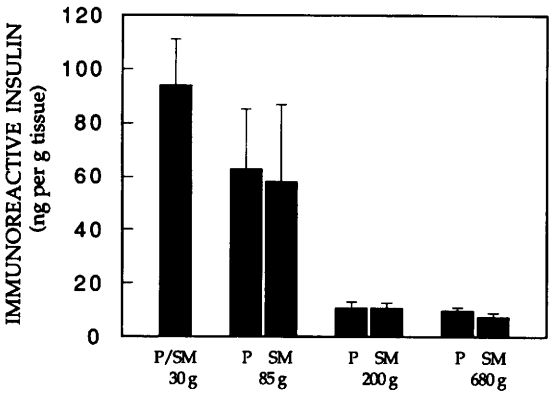

Figure 1. Immunoreactive insulin (IRI) contents of salivary glands from rats of different body weights, expressed as ng IRI/g glandular tissue (wet weight). The parotid (P) and submandibular (SM) glands were combined and extracted in suckling rats. Bar lines represent ±1 SEM. Means of 30.5 versus 202 g, and 30.5 versus 680 g are significantly different ($P < 0.05$ and $P < 0.01$, respectively). Means of 85 versus 202 g are significantly different ($P < 0.05$). Means of 30.5 versus 85 g, and 202 versus 680 g are not significantly different.

depends upon the animal's circulating insulin level, serum immunoreactive insulin concentrations in the suckling and older rats in this experimental series were compared. As illustrated in Table I, the serum insulin in seven sucklings (30.5 g) was estimated as 2.10 ± 0.50 ng/ml, whereas that from six aged (681 g body wt) rats was 7.90 ± 2.50 ng/ml. The mean serum IRI in eight pubescent rats (101 g) and six mature (202 g) male rats were 3.0 ± 0.50 and 2.64 ± 0.40 ng/ml, respectively. Only in aged rats was the serum IRI level significantly different from the level in the sucklings. The data suggest that, with the exception of the aged group, serum insulin levels were constant throughout life. Comparing the data in Figure 1 and Table I indicates that there was no correlation between salivary gland and serum immunoreactive insulin levels in these rats.

Salivary Gland Insulin in Islet Tumor-Bearing Rats. A study was made of the IRI content of salivary glands in rats bearing pancreatic islet cell adenomas induced by an injection of streptozotocin and nictinamide. In the weeks following drug injection, treated rats maintained normal fasting plasma glucose and serum insulin levels, but exhibited subtle changes in the capacity to metabolize a high dose of injected glucose and are thus classified as subdiabetic (14). In the 20- to 36-week period following drug treatment, the appearance of grossly visible tumors of the pancreas has been correlated with changes in blood chemistry: rats with insulin-secreting tumors exhibit a dramatic fall in plasma glucose and a doubling in serum immunoassayable insulin levels. These tumors also secrete increased levels of immunoreactive somatostatin, but serum glucagon levels are lower than normal (13). As illustrated in Figure 2, the mean salivary IRI contents of the tumor-bearing rats in the present study (determined 14 months after drug injection) were several-fold higher than in age-matched controls: parotid gland—588 ± 164 ($n = 5$) versus 9.61 ± 1.19 ng/g ($n = 6$) in controls; submandibular gland—179 ± 83.2 ($n = 5$) versus 7.34 ± 1.44 ng/g ($n = 5$) in controls.

The presence of pancreatic tumors also coincided with increases in the immunoreactive insulin content of two insulin target tissues (i.e., hepatic and adipose). The mean IRI contents of liver tissue from tumor-bearing and age-matched controls were 27.4 ± 6.40 ($n = 8$) and 16.7 ± 2.74 ng/g ($n = 7$), respectively (Fig. 3). The mean IRI content of adipose tissue from tumor-bearing rats was 42.8 ± 9.96 ($n = 8$) versus 22.5 ± 3.92 ng/g ($n = 6$) in controls (Fig. 3).

The mean serum insulin levels of the tumor-bearing rats in the experimental group were estimated at 14.2 ± 8.60 ng/ml ($n = 5$) compared with the controls at 7.91 ± 2.47 ng/ml ($n = 6$) (Table I). These means were not statistically different because of large variations in individual serum concentrations among the tumor-bearing animals. Comparing these data on serum IRI with tissue levels (Fig. 2 and 3) indicates that, while the immunoreactive insulin content of liver and adipose tissue appeared to roughly correlate with serum IRI levels, no linear correlation was seen with salivary gland IRI levels, which were inappropriately increased. Routine histologic examination of the salivary glands from tumor-bearing rats (that had not been processed for insulin assay) appeared normal and revealed no evidence for the occurrence of salivary tumors. Salivary glands from rats that had not yet developed pancreatic tumors (animals sacrificed only 4

Table I. Serum Immunoreactive Insulin (IRI) of Male Rats by Age and Body Weight

	Age				
	1.4 Weeks	4 Weeks	7 Weeks	56 Weeks	
				Control	Tumorous
Body wt (g)	30.5 ± 0.46	101 ± 0.55	202 ± 9.3	681 ± 32.1	722 ± 30.2
Serum IRI (ng/ml)	2.1 ± 0.50 (7)	3.0 ± 0.50 (8)	2.6 ± 0.40 (6)	7.9 ± 2.5 (6)	14 ± 8.6 (5)

Note. Serum IRI values for Week 56 means are significantly different from 1.4, 4, and 7 week means ($P < 0.05$). There are no significant differences among 1.4, 4, and 7 week group means. n is indicated by number in parentheses.

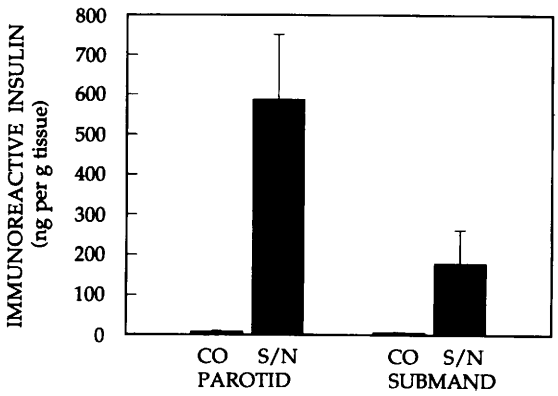

Figure 2. Immunoreactive insulin contents of salivary glands from 56-week-old pancreatic adenoma-bearing rats (S/N) and age-matched control (CO) rats. Body weights were: 681 ± 32.1 g in six S/N-treated rats, and 722 ± 30.2 g in five controls. Means of S/N versus control groups are significantly different ($P < 0.05$).

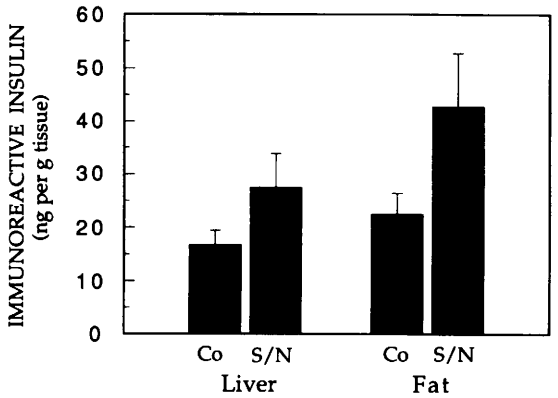

Figure 3. Immunoreactive insulin contents of liver and adipose tissues from 56-week-old rats bearing pancreatic adenomas (S/N), and from age-matched controls (Co). The means are not significantly different.

weeks after drug treatment) showed no differences in IRI content: parotid gland—9.00 ± 1.71 ($n = 5$) versus 14.4 ± 2.40 ng/g ($n = 4$) in controls; submandibular gland—6.14 ± 1.03 ($n = 5$) versus 7.26 ± 0.78 ng/g ($n = 4$) in controls (data not illustrated).

Discussion

The detection of insulin in the major salivary glands and saliva has been a topic of interest to both endocrinologists and clinicians. Reports on the nature of insulin in saliva (6) and salivary glands (2, 10) emphasize that the immunoreactive material is indistinguishable in size and charge from pancreatic insulin and exhibits an identical dilution curve to insulin.

Several reports suggest that concentrations of IRI in human saliva are related to serum levels in normal, nondiabetic obese, and in type 1 and type 2 diabetic patients (8–12). Moreover, in euglycemic clamp experiments on normal human subjects, where blood glucose levels are stabilized as insulin is being infused, insulin in the saliva rises with a lag time of about 30 min to a level only slightly lower than the serum insulin level (10). These observations support the view that in humans the major salivary glands transport insulin from blood to saliva, and that blood insulin levels directly regulate saliva insulin concentrations.

It has been assumed tacitly that blood insulin levels also regulate the concentration of IRI in extracts of salivary glands, but this hypothesis has not been directly examined. In short-term experiments, the development of hypoinsulinemia in rodents as the result of chemical destruction of islet β cells (3, 7), or pancreatectomy (4), or during the onset of overt diabetes in genetically susceptible rats (5), has been observed to markedly reduce the content of insulin in the major salivary glands, which implies that salivary gland insulin levels are responsive to ambient blood insulin levels. In these studies, however, the changes in blood and salivary insulin levels, albeit in the expected direction, were poorly correlated. In the present study, immunoreactive insulin (IRI) was estimated in salivary glands from groups of rats of several ages *viz.*, in sucklings, and in pubescent, mature, and elderly adult males. The results indicate that IRI levels vary widely with age, being highest in the neonatal animals, and tending to plateau in mature and elderly rats. The levels of IRI in serum did not appear to be the sole determinant of glandular IRI contents, since serum IRI concentrations were fairly constant throughout life, only diverging from this level in the group of elderly animals studied. Additional evidence for the poor correlation of salivary IRI levels with blood levels was obtained when we compared salivary and serum IRI levels in rats bearing chemically induced islet tumors. Certainly, factors other than serum IRI may have influenced salivary IRI levels in this study, including an elevated somatostatin level, which is known to accompany tumor development (13, 14). In the same tumor-bearing animals, by contrast, IRI levels of hepatic and adipose tissues both exhibited appropriate (i.e., 2-fold) increases over the control levels (Fig. 3). We conclude that the insulin content of these salivary glands is regulated in rats only in part by ambient blood insulin levels.

The glandular IRI levels in adult rats observed in this study (11 ng/g parotid in 202 g Sprague-Dawley rats) can be compared to the levels reported previously. In 250–280 g Wistar males and 315 g mixed sex Wistar rats, parotid IRI levels of 5.6 (2) and 26 ng/g (5), respectively, have been reported. In this study, the level of submandibular gland IRI (11 ng/g in 202 g rats) is approximately the same as reported in 350–370 g adult Wistar rats (16), but lower than previously reported 32 ng/g in adult Sprague-Dawley rats (4).

Several mechanisms may be considered to explain the observed age differences in salivary IRI levels. An

age dependence of salivary insulin levels suggests the possibility that the hormone may be differentially internalized or bound to salivary gland membranes during life, or that the efficiency of hormone binding in the younger animals is higher than in adults, due to higher receptor concentration or affinity. These may be aspects of salivary gland function in younger animals that become less pronounced in pubescent and adult rats. Insulin-sensitive tissues might trap insulin by a receptor-mediated mechanism. At the onset of diabetes, for example, acute decreases in blood IRI levels are followed by slower changes in the insulin content of target tissues. The sequestering of receptor-bound insulin in liver and intestine has been hypothesized to account for this observation (17). The ubiquitous presence of insulin in tissues (18) may also be explained by receptor-bound insulin (see Ref. 19). Although the salivary glands are insulin-sensitive organs (20), and insulin receptor-ligand kinetics have been reported (21), little is known about the over-all transport and metabolism of insulin by these glands. The acini are known to be insulin sensitive based on the observed modulation of submandibular peroxidase by insulin (20), so it is assumed that receptor-bound insulin is internalized and the ligand is degraded in endocytic vesicles, as in hepatocytes (22). However, since substantial quantities of insulin also appear in saliva without undergoing appreciable degradation (10), then a separate transcytotic pathway may operate within these glands. This hypothetical transport system may be situated in acinar cells or, more likely in intralobular duct cells.

Increased salivary insulin contents in immature rats also might relate to the prolonged postnatal development of the acinar and intralobular duct systems in rodent salivary glands (23, 24). Acini are not yet formed at birth, but arise during the first 6 weeks of life by proliferation from terminal tubules, which are highly granulated cells of the proximal intralobular duct system. Definitive intercalated ducts differentiate only at 5 weeks of life; intralobular striated ducts arise by growth and differentiation at 6 weeks. If the development of the epithelial structures were dependent upon insulin, it would be reasonable to expect expression of high densities of insulin receptors and increased levels of receptor-bound insulin during the first 4 weeks of life, where in the present study we observed elevated insulin levels. The underlying explanation for the observed increases in insulin binding during postnatal development of rodent salivary glands must await further study.

It has been suggested that a significant portion of the insulin content of salivary glands may arise by *de novo* insulin biosynthesis in these organs. In two studies, the production of labeled insulin from radioactive amino acids was observed during *in vitro* culture of salivary gland preparations from adult rodents and humans (2, 4). Unfortunately in neither study were the ordinary controls performed to prove that the observed uptake of label by insulin represented insulin biosynthesis. The demonstrated absence of glandular proinsulin labeling in both reports also weakens the case for insulin biosynthesis. The search for salivary proinsulin, an obligatory precursor for insulin synthesis as currently understood, also has yielded only negative results to date (2, 7, 10). In view of these considerations, it would be difficult to argue that the elevated salivary insulin levels in immature rats observed in the present report arose from insulin-synthetic activity. Also, a dramatic increase in insulin synthesis by salivary glands from insulinoma-bearing rats would be a counter-regulatory response to increased serum insulin levels, and thus an unlikely explanation for the marked increases that were observed.

Localizing insulin within salivary glands is an important prerequisite to understanding variations in insulin content with age and serum insulin levels. The immunohistochemical localization of insulin to striated ducts and granular convoluted tubules in mouse submandibular glands (4), but to intercalated ducts in rat parotid and submandibular glands (3, 7) seems contradictory, and such isolated observations have not been confirmed by reports from other laboratories, nor have we been able to repeat them in our laboratory. Of special concern with these reports is the absence of data on the nature of the observed insulin (i.e., nascent insulin within secretory granules, or receptor-bound, or within pinocytotic vesicles?).

Finally, it should be recognized that the observed age variations could be related to technique: the efficiency of extracting insulin may depend on the animal's age, since connective tissue in older rats may be tougher than in younger rats and might therefore resist solubilization. This possibility is considered remote, because of the demonstrated efficiency of the extraction method that was used. Also, the extractable IRI from parotid and submandibular glands from each age group of animals was identical, which is an unlikely observation if either gland were to differ in hormone extractability with age.

In a recent study (16), insulin immunoreactivity levels of submandibular gland extracts were reported higher in 11-month (44-week) than in 3.5-month (14-week) Wistar rats. Although small (55%), the difference was significant ($P < 0.05$). The plasma IRI of these mature rats also was significantly higher in 11 month than in 3.5-month animals by 80%. The authors concluded that IRI levels in submandibular glands increase during maturation. In the context of the present study, however, we would conclude that salivary IRI was relatively stable in two populations of mature rats, but that each group responded to the ambient blood IRI level.

The detection of high concentrations of IRI in salivary glands of suckling rats and the inappropriately elevated glandular IRI levels during hyperinsulinemia and hypoglycemia raises questions on the role of these glands in nutrient metabolism. A salivary-pancreas endocrine axis has been described to explain changes observed in the parotids of diabetic patients (25). The effects of sialadenectomy on pancreatic insulin output in rats (26) also suggest that the major salivary glands have a role in the modulation of insulin release. The nature and extent of the participation of salivary glands in nutrient homeostasis are topics for future study.

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1. Barka T. Biologically active polypeptides in submandibular glands. J Histochem Cytochem **28**:836–859, 1980.
2. Murakami K, Taniguchi H, Baba S. Presence of insulin-like immunoreactivity and its biosynthesis in rat and human parotid gland. Diabetologia **22**:358–361, 1982.
3. Smith PH, Patel DG. Immunochemical studies of the insulin-like material in the parotid gland of rats. Diabetes **33**:661–666, 1984.
4. Shubnikova EA, Volkova EF, Printseva OY. Submandibular glands as organs of synthesis and accumulation of insulin-like protein. Acta Histochem **74**:157–171, 1984.
5. Smith PH, Leone JP, Stearns SB. Immunochemical studies of an insulin-like material in the parotid gland of diabetic BB rats. Diabetes **35**:106–109, 1986.
6. Smith PH, Toms BB. Immunocytochemical localization of insulin- and glucagon-like peptides in rat salivary glands. J Histochem Cytochem **34**:627–632, 1986.
7. Patel DG, Begum N, Smith PH. Insulin-like material in parotid and submaxillary salivary glands of normal and diabetic adult male mice. Diabetes **35**:753–758, 1986.
8. Sweeney EA, Antoniades HN. The presence of immunologically reactive insulin in parotid saliva and its relation to changes in serum insulin concentration. Vox Sang **13**:54–56, 1967.
9. Sweeney EA, Juan CS, AvRuskin TW. Turner's syndrome and carbohydrate metabolism. II. Parotid salivary insulin concentration in normal subjects and in patients with gonadal dysgenesis. Am J Med Sci **277**:153–162, 1979.
10. Vallejo G, Mean PM, Gaynor DH, Devlin JT, Robbins DC. Characterization of immunoreactive insulin in human saliva: Evidence against production *in situ*. Diabetologia **27**:438–440, 1984.
11. Marchetti P, Benzi L, Masoni A, Cecchetti P, Giannarelli R, Di Cianni G, Ciccarone AM, Navalesi R. Salivary insulin concentrations in Type 2 (non-insulin-dependent) diabetic patients and obese non-diabetic subjects: Relationship to changes in plasma insulin levels after an oral glucose load. Diabetologia **29**:695–698, 1986.
12. Pasic J, Pickup JC. Salivary insulin in normal and Type I diabetic subjects. Diabetes Care **11**:489–494, 1988.
13. Bauer GE, Dixit PK, Sorenson RL, Wobken JD. Glucose-stimulated hormone release in rats bearing streptozotocin/nicotinamide-induced islet adenomas: Evidence for slow and fast responders. Pancreas **4**:464–471, 1989.
14. Michels JE, Bauer GE, Johnson DE, Dixit PK. Morphometric analysis of the endocrine cell composition of rat pancreas following treatment with streptozotocin and nicotinamide. Exp Mol Pathol **44**:247–258, 1986.
15. Dixit PK, Bauer GE. Studies on rats with islet beta cell tumors induced by nicotinamide and streptozotocin. Proc Soc Exptl Biol Med **152**:232–236, 1976.
16. Deville de Périère D, Puech R, Hillaire-Buys D, Ribes G. Maturation and insulin-like immunoreactivity in rat submandibular salivary glands: Possible implication of G regulatory proteins. Acta Endocrinol **126**:282–284, 1992.
17. Smith SS, Bhathena SJ, Nompleggi D, Penhos JE, Recant L. Studies on persistent circulating immunoreactive glucagon (IRG) and immunoreactive insulin (IRI) found in eviscerated rats with a functional liver. Diabetologia **14**:177–184, 1978.
18. Rosenzweig JL, Havrankova J, Lesniak MA, Brownstein, Roth J. Insulin is ubiquitous in extrapancreatic tissues of rats and humans. Proc Natl Acad Sci USA **77**:572–576, 1980.
19. Eng J, Yalow RS. Insulin recoverable from tissues. Diabetes **29**:105–109, 1980.
20. Anderson LC, Shapiro BL. The effect of alloxan diabetes and insulin *in vivo* on peroxidase activity in the rat submandibular gland. Archs Oral Biol **24**:343–345, 1979.
21. Scacchi GE, Turyn D, Dellacha JM. Specific binding sites for ^{125}-iodo insulin in the rat submandibular gland: *In vivo* and *in vitro* studies. Horm Metab Res **15**:167–171, 1983.
22. Williams FW, Johnson DE, Bauer GE. [^{125}I]-Insulin metabolism by the rat liver *in vivo:* Evidence that a neutral thiol-protease mediates rapid intracellular insulin degradation. Metabolism **39**:231–241, 1990.
23. Jacobi F, Leeson CR. The post-natal development of the rat submandibular gland. J Anat **93**:201–216, 1959.
24. Redman RS, Sreebny LM. Morphologic and biochemical observations on the development of the rat parotid gland. Devel Biol **25**:248–279, 1971.
25. Levine SB, Sampliner RE, Bennett PH, Rushforth NB, Burch TA, Miller M. Asymptomatic parotid enlargement in Pima Indians. Ann Intern Med **73**:571–573, 1970.
26. Leonora J, Tieche J-M, Cook DS. Evidence for insulinotropic effect from rat parotid glands. Diabetes **37**:441–445, 1988.