Effect of Submaxillary Gland Extirpation on Glucose and Insulin Tolerance in Dogs

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SUMMARY

Serum glucose and immunoreactive insulin (IRI) responses to oral glucose and intravenous insulin tolerance tests were determined in dogs before and after total bilateral extirpation of their submaxillary glands. A comparison was made with a control group of dogs before and after sham operations. Postoperative studies were performed at one, three and six months after surgery. No significant differences in glucose and IRI responses were observed between the two groups of dogs, nor upon comparison of the responses within each group at the various periods studied.

A review of the literature which relates the salivary glands to diabetes mellitus in both experimental and clinical studies is presented. Our own experience of diabetes mellitus with apparent salivary gland involvement is briefly discussed.

It is concluded that despite the relatively frequent association of salivary gland involvement and diabetes mellitus, the role of these glands in the pathogenesis and management of diabetes mellitus is obscure. At this time there is no apparent rationale to the performance of total submaxillary gland extirpation for the treatment of diabetes mellitus. DIABETES *21*:722-32, June, 1972.

Several experimental[1-6] and clinical[7-11] studies have resulted in conflicting reports regarding the relationship of the salivary glands to glucose metabolism. Davidson et al.[8] have reviewed the association of parotid gland enlargement and diabetes mellitus and have reported sixteen additional cases. The spontaneous disappearance of insulin-resistant diabetes mellitus coincident with regression in size of enlarged submaxillary glands was reported by Bruce et al.[9] Recently, Godlowski has reported the complete remission of the clinical manifestations of diabetes mellitus in three cases of the maturity-onset type[10] and the return to normal glucose tolerance tests in two[11] of them following the surgical ablation of the submaxillary glands. He postulates that the submaxillary glands produce an insulin antagonist which if removed, would result in an increased hypoglycemic effect from circulating insulin. This hypothesis is based upon his demonstration of increased insulin sensitivity during insulin tolerance tests in dogs following the bilateral extirpation of the submaxillary glands.[5]

The purpose of this study was to determine the effect of bilateral submaxillary gland extirpation in dogs on serum glucose and insulin responses during oral glucose tolerance and intravenous insulin tolerance tests.

MATERIALS AND METHODS

Nine mongrel dogs whose weights ranged from 14.5 to 21.4 kg. were placed on a standard kennel diet. They were weighed three times a week during an observation period of two weeks in order to establish a steady state regarding food intake and maintenance of body weight. On the day of testing, all dogs were in the postabsorptive state. All studies were performed while the dogs were under sodium pentobarbital anesthesia, using 30 mg./kg. of body weight. After anesthesia was attained and the oral-gastric tube and indwelling venous catheter was in place, a thirty-minute period was allowed to elapse before baseline blood specimens were obtained.

Oral Glucose Tolerance Tests

After the dogs were prepared as described above, the head of the table was elevated to an angle of thirty degrees in order to prevent regurgitation of the orally administered glucose solution. Baseline blood specimens were obtained and then each dog received a solution

From the University of California at Irvine.

of glucose containing 1.5 gm. of glucose per kilogram of body weight which was administered via an oral-gastric tube. The glucose solution was diluted so that the concentration was no greater than 30 per cent w/v. The entire solution was delivered within a five-minute period and it was followed by a washing of the intragastric tubing with 50 cc. of tap water. Timing was begun after half of the volume of the glucose solution was delivered. Subsequent blood specimens were obtained at 10, 20, 30, 60, 90 and 120 minutes by means of an indwelling intravenous catheter. All blood specimens were placed in an ice bath immediately after collection and then centrifuged within one hour. The serum was separated and immediately frozen for storage and subsequent analysis.

Insulin Tolerance Tests

After a recovery interval of no less than one week, all dogs were prepared as described above, except that these studies were performed while the dogs were in a horizontal position. After baseline blood specimens were obtained, crystalline insulin in a dosage of 0.05 U./kg. of body weight was injected intravenously in a vein different from that used for obtaining blood specimens. Subsequent blood specimens were obtained via an indwelling intravenous catheter at 3, 5, 7, 10, 15, 20, 30, 45 and 60 minutes following the injection of in-

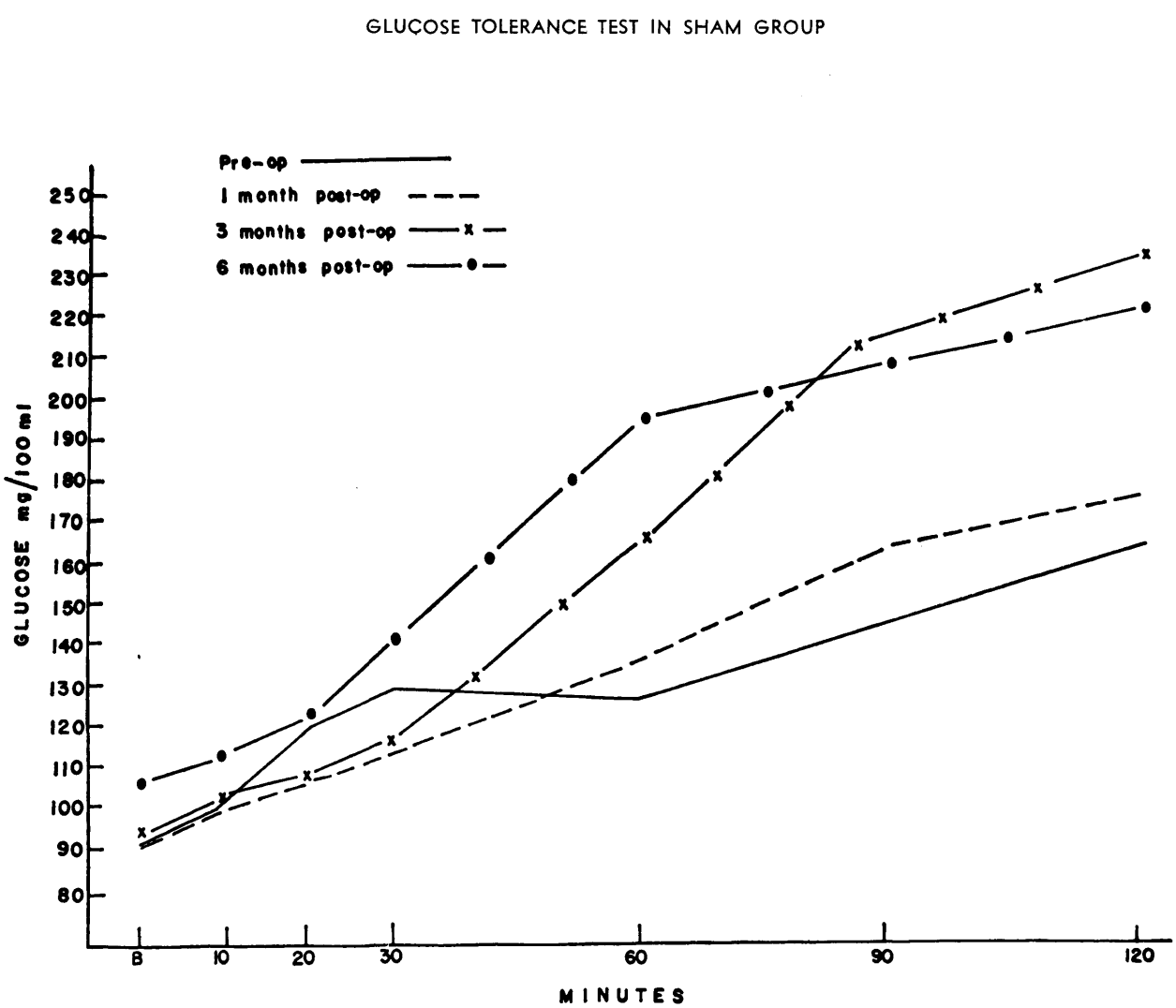

FIG. 1. Mean serum glucose responses of four dogs to 1.5 gm./kg. glucose administered orally before and at one, three and six months after sham operation. B = baseline

sulin. All blood specimens were immediately placed in an ice bath and after the last specimen was obtained, they were centrifuged and the serum was separated. The serum was immediately frozen for storage and subsequent analysis.

Submaxillary Gland Extirpation

When all baseline studies above were completed, five dogs had total bilateral extirpation of the submaxillary glands, while four dogs had sham surgeries in which similar incisions were made exposing the superficial portion of the submaxillary gland without disturbing the gland itself, followed by routine skin closure. Intratracheal anesthesia was used for better exposure and dissection. All hair was shaved around the operative site. The dogs were thoroughly prepared and draped. The skin incision extended linearly downward from the inferior portion of the dog's pina well into the nape of the neck, approximately 6 cm. Careful semisharp and blunt dissection exposed the submaxillary gland which was found to be straddled by the superficial facial veins. The gland itself was encapsulated with thick fascia and was easily enucleated from the fossa surrounding the gland. The dissection was carried anteriorly to include approximately 3 cm. of the main duct. All the surgical procedures were done with relative ease without complications. It was necessary to ligate the anterior superficial facial veins for removal of the gland. The remaining fossa was obliterated with 4-0 chromic suture. Fascia and skin were approximated with 4-0 chromic suture. Intensive medical therapy was given all dogs postoperatively, until final healing resulted in about ten days.

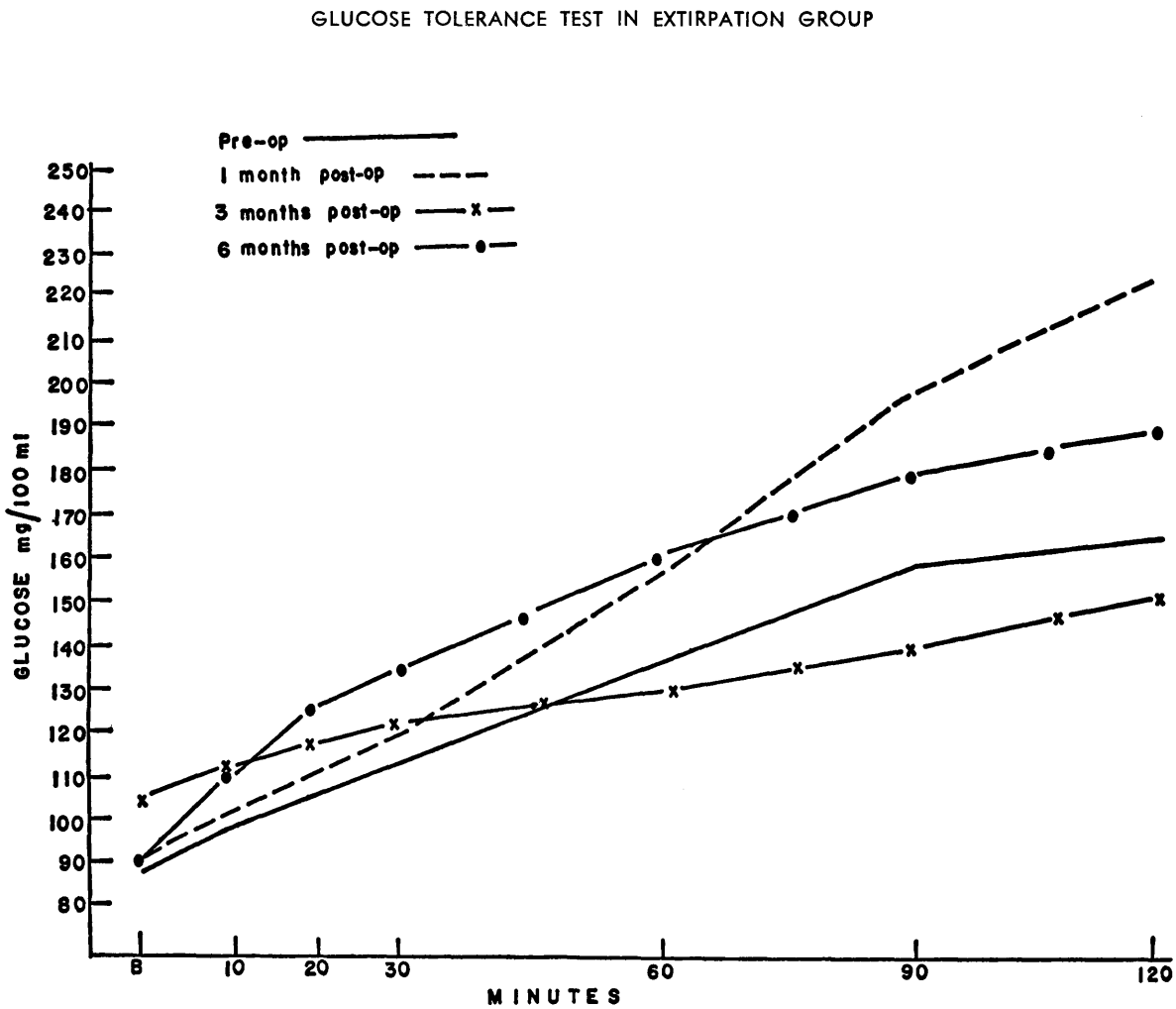

FIG. 2. Mean serum glucose responses of five dogs to 1.5 gm./kg. glucose administered orally before and at one, three and six months after total bilateral extirpation of submaxillary glands. B = baseline

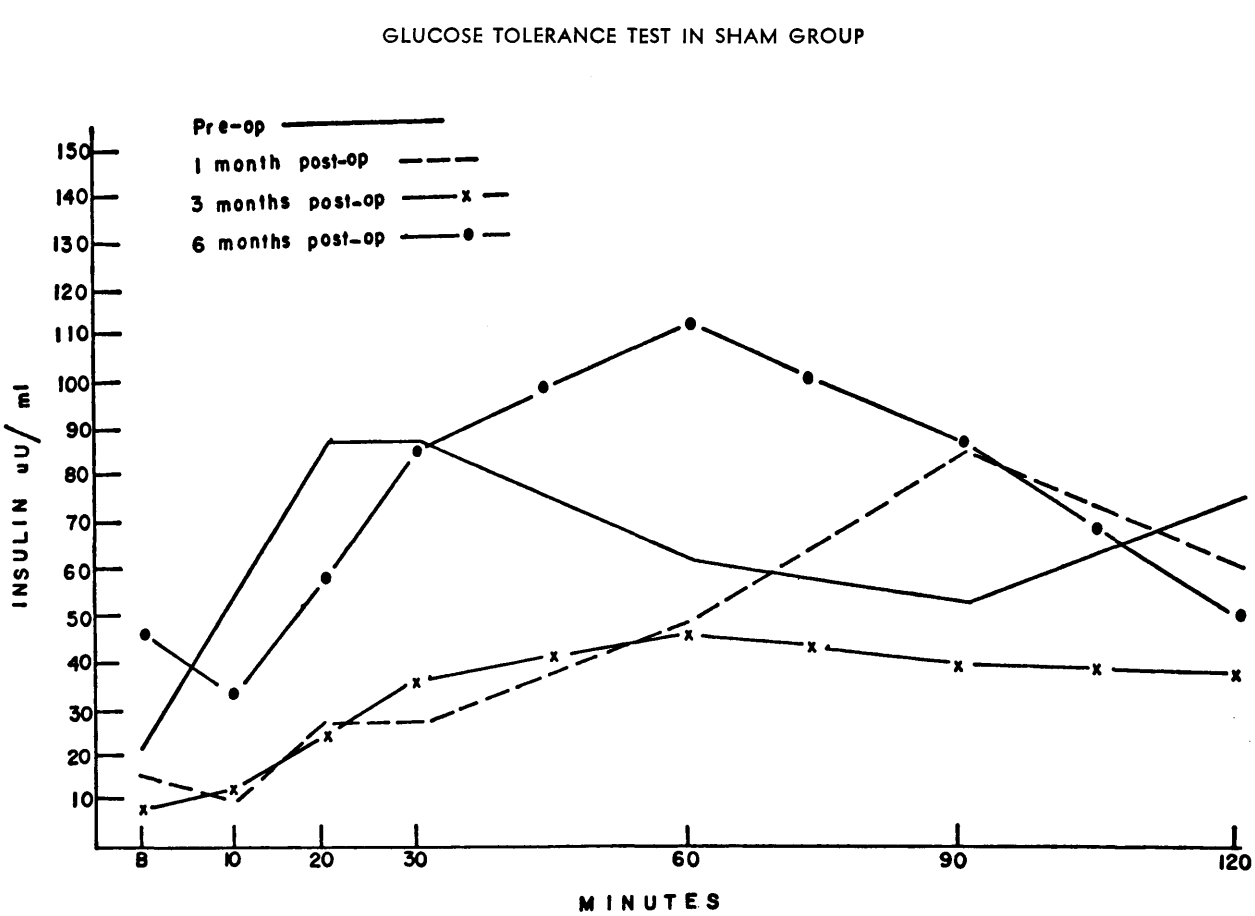

FIG. 3. Mean serum IRI responses of four dogs to 1.5 gm./kg. glucose administered orally before and at one, three and six months after sham operation. B = baseline

Postoperative Studies

Oral glucose and intravenous insulin tolerance tests as described above were repeated on all dogs at one, three and six months, postoperatively. The glucose tolerance and insulin tolerance studies on each dog were performed with an interval of one week between tests.

All specimens were analyzed for glucose by the Technicon-AutoAnalyzer procedure[12] and immunoreactive insulin concentrations were determined by the procedure described in the Phadebas Insulin Test Radioimmunoassay kit.[13]* All specimens were assayed as a single batch at the completion of the study.

RESULTS

At the conclusion of the study, the maximum weight changes in the dogs ranged from an increase of 16 per cent to a loss of 23 per cent. However, there was no significant difference between the initial mean weight for each group of dogs when compared with the final mean weight for each group. Each dog was weighed on the day of study and the dosage of glucose and insulin was adjusted accordingly as described above.

Oral Glucose Tolerance Tests

Serum glucose responses to the oral administration of glucose are illustrated in figures 1 and 2. Although the mean responses during each study period were quite variable, there was no significant difference ($p > 0.5$) in the glucose response during any time interval when comparing the preoperative values with each of the postoperative periods in both the sham operated and extirpated groups. The corresponding insulin responses during the oral glucose tolerance tests are depicted in figures 3 and 4. Although it appears that the extirpated

*Generously donated by Pharmacia Laboratory, Inc., Piscataway, New Jersey.

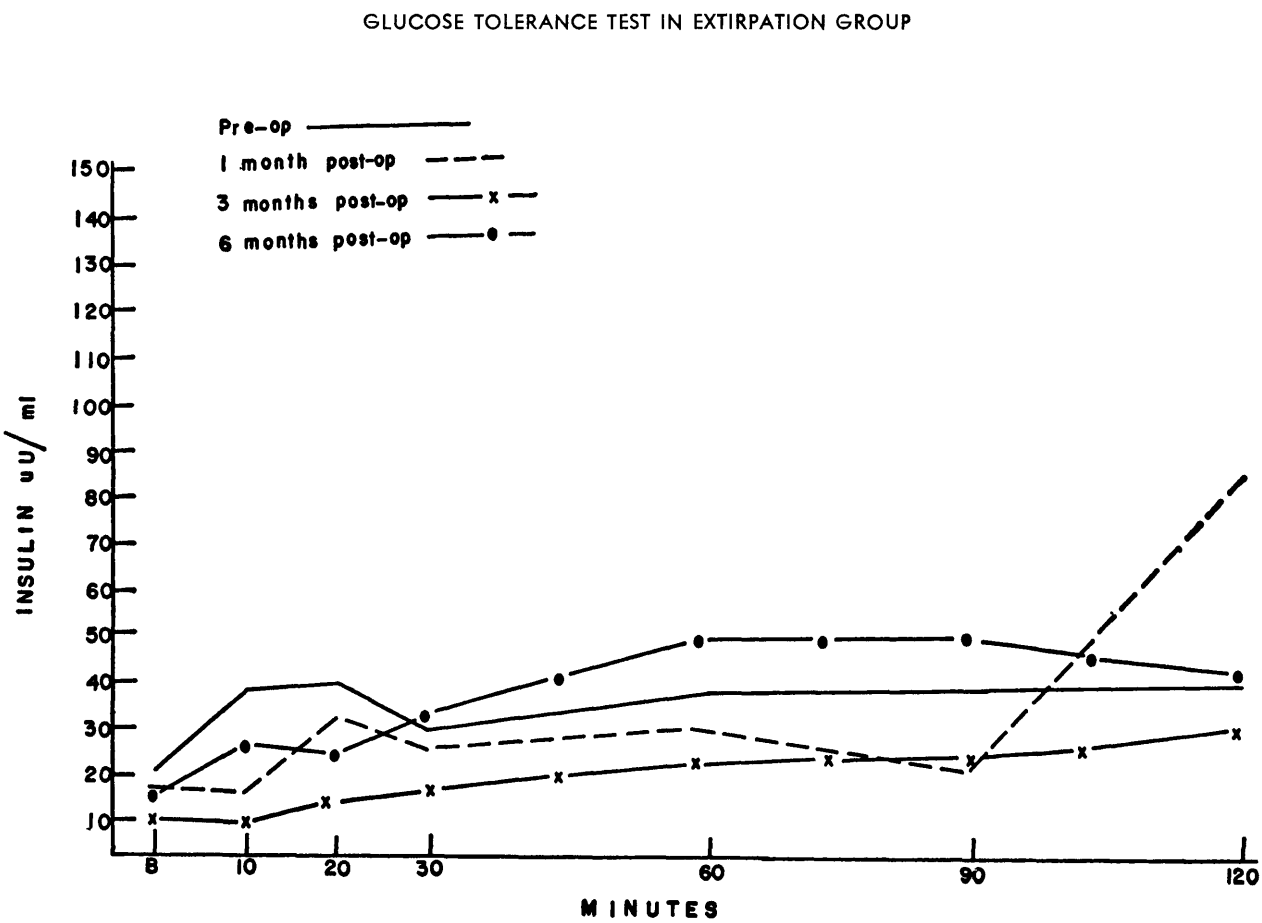

FIG. 4. Mean serum IRI responses of five dogs to 1.5 gm./kg. glucose administered orally before and at one, three and six months after total bilateral extirpation of submaxillary glands.

group had a diminished insulin response to the oral administrations of glucose, there was actually no significant difference between the two groups, nor was there any significant difference during any individual time period when comparing the preoperative values with each of the postoperative periods.

Insulin Tolerance Tests

The glucose responses to the intravenous administration of insulin are illustrated in figures 5 and 6. There was no significant difference in the responses of the sham operated group as compared with the extirpated group. Although the greatest degree of hypoglycemia was attained in the sixth month of the postoperative period for both groups of dogs, these values were not significantly different from the preoperative values at any point during the one hour observation period. The insulin responses to the intravenous administration of insulin are shown in figures 7 and 8. The results were nearly identical for both groups of dogs. Although the highest insulin levels were attained after the sixth postoperative month in both groups of dogs, these values were not significantly different from the preoperative insulin levels ($p > 0.5$).

Neither standard deviations nor standard errors of the means are shown in any of the figures as they were very great and were virtually superimposable at all points in these studies.

DISCUSSION

The concept that the salivary glands are related to endocrine physiology first appeared in the English literature in 1886.[14] At that time, it was proposed that the parotid glands are associated with the activity of the reproductive organs.[15] Morgan and Raven[7] unify the

above concept with diabetes mellitus in their review of an endocrine theory based upon estrogen deficiency as being etiologic of Sjogren's syndrome. A rather frequent association of Sjogren's syndrome with hyperglycemia and hypoglycemia has been reported.[16,17]

A number of reports which relate the salivary glands to glucose metabolism was well reviewed by Zimmerman[1] in 1932. In his own studies he showed that ligation of the parotid ducts in normal dogs resulted in increased glucose tolerance, but extirpation of the parotid glands gave inconsistent results. In the next decade, the work of Birnkrant[2,3] on rat parotid glands supported the studies of Zimmerman. In the decade that followed, Gault[4] showed no effect on blood sugar levels after parotidectomy in rats and mice.

In the 1960's, the focus of attention was suddenly shifted from the parotid glands to the submaxillary and sublingual glands in the studies of Godlowski et al.[5,6,10] It appears that the submaxillary glands were chosen for further study because they are histologically more similar to the pancreas than the parotids.[6] Despite the histologic similarity, Godlowski does not propose a functional similarity. On the contrary, he hypothesizes that the submaxillary glands secrete an insulin antagonist, the removal of which will result in an enhancement of insulin sensitivity.

Our study did not demonstrate any effect of total submaxillary gland extirpation on glucose and insulin re-

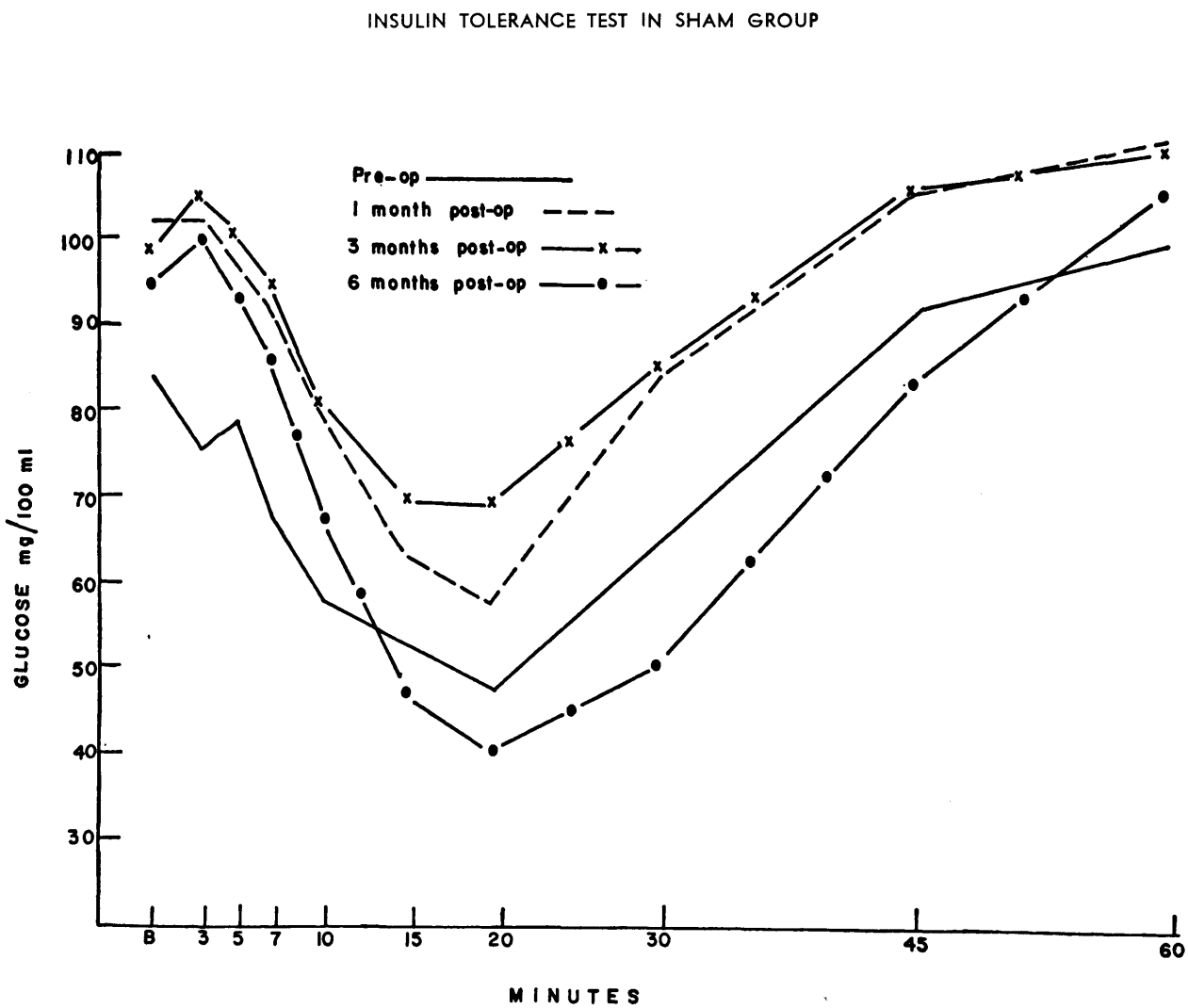

FIG. 5. Mean serum glucose responses of four dogs to 0.05 unit/kg. crystalline insulin administered intravenously before and at one, three and six months after sham operation. B = baseline

sponses during oral glucose and intravenous insulin tolerance tests in dogs. This is in marked contrast to the results reported by Godlowski and Calandra.[5] The glucose responses to intravenously administered insulin in their study were highly significantly different ($p < 0.001$) upon our comparison of their preoperative and sixth month postoperative data at both the thirty- and forty-five-minute intervals. Perhaps this discrepancy is due to the fact that we removed only the submaxillary glands and they extirpated both the submaxillary and sublingual glands. However, in their original[5] and subsequent studies,[6,10,11] Godlowski et al. stressed that the source of the insulin antagonist was in the submaxillary glands and designated it "submaxillary insulin inhibitor."

The present study corroborates the recent work of Garcia et al.[18] Glucose and immunoreactive insulin responses to intravenously administered glucose following submaxillary gland extirpation were essentially identical to their preoperative values. Their postoperative studies were performed in the fourth week after surgery. Godlowski et al.[5] felt that the stress of surgery was still present by the fourth week since they observed *reduced* sensitivity to insulin within that postoperative time. Our study also suggests reduced insulin sensitivity one month postoperatively (figures 1, 2, 5, 6).

However, our repeated studies at the third and sixth postoperative months showed no significantly increased insulin sensitivity.

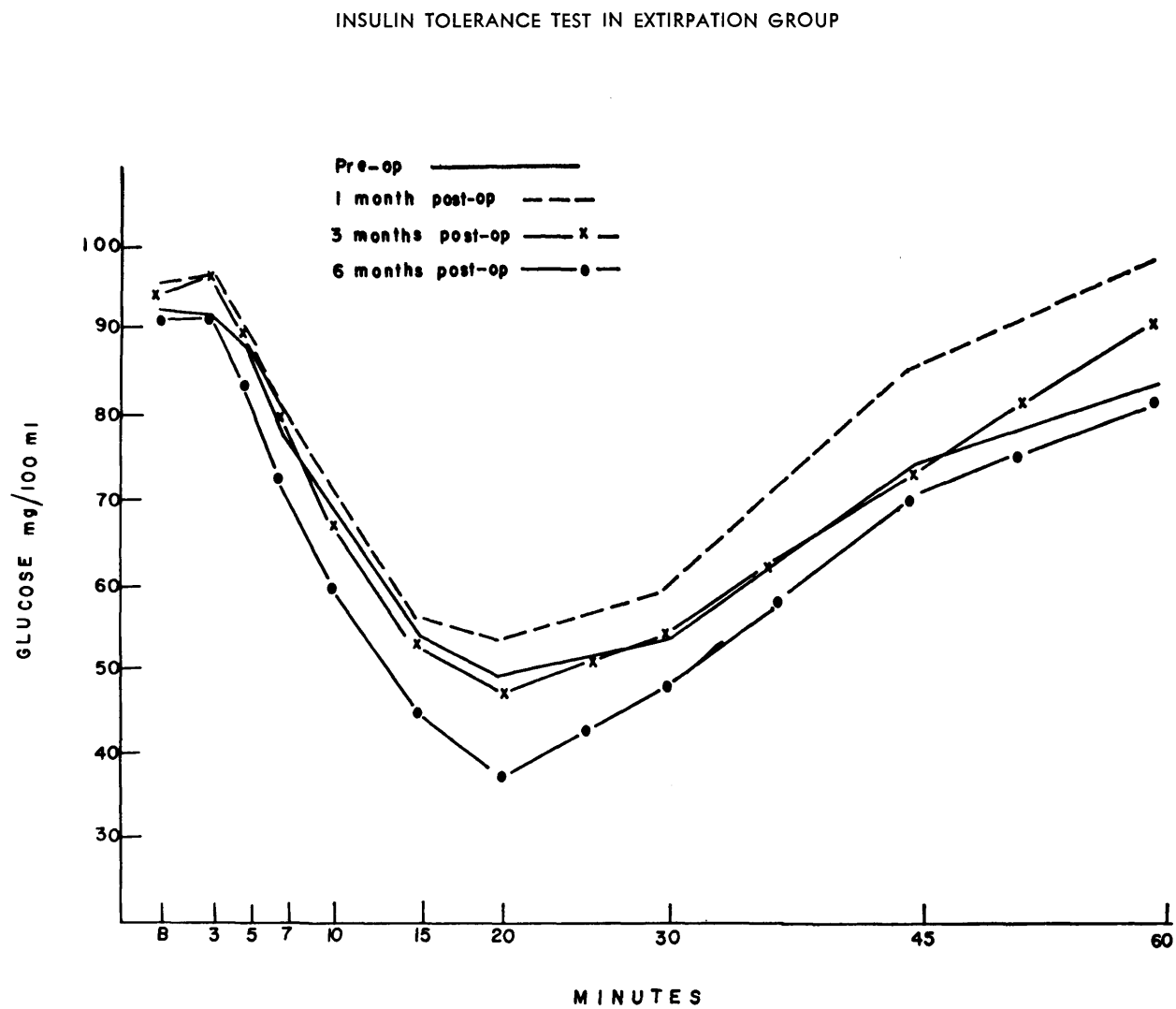

FIG. 6. Mean serum glucose responses of five dogs to 0.05 unit/kg. crystalline insulin administered intravenously before and at one, three and six months after total bilateral extirpation of submaxillary glands. B = baseline

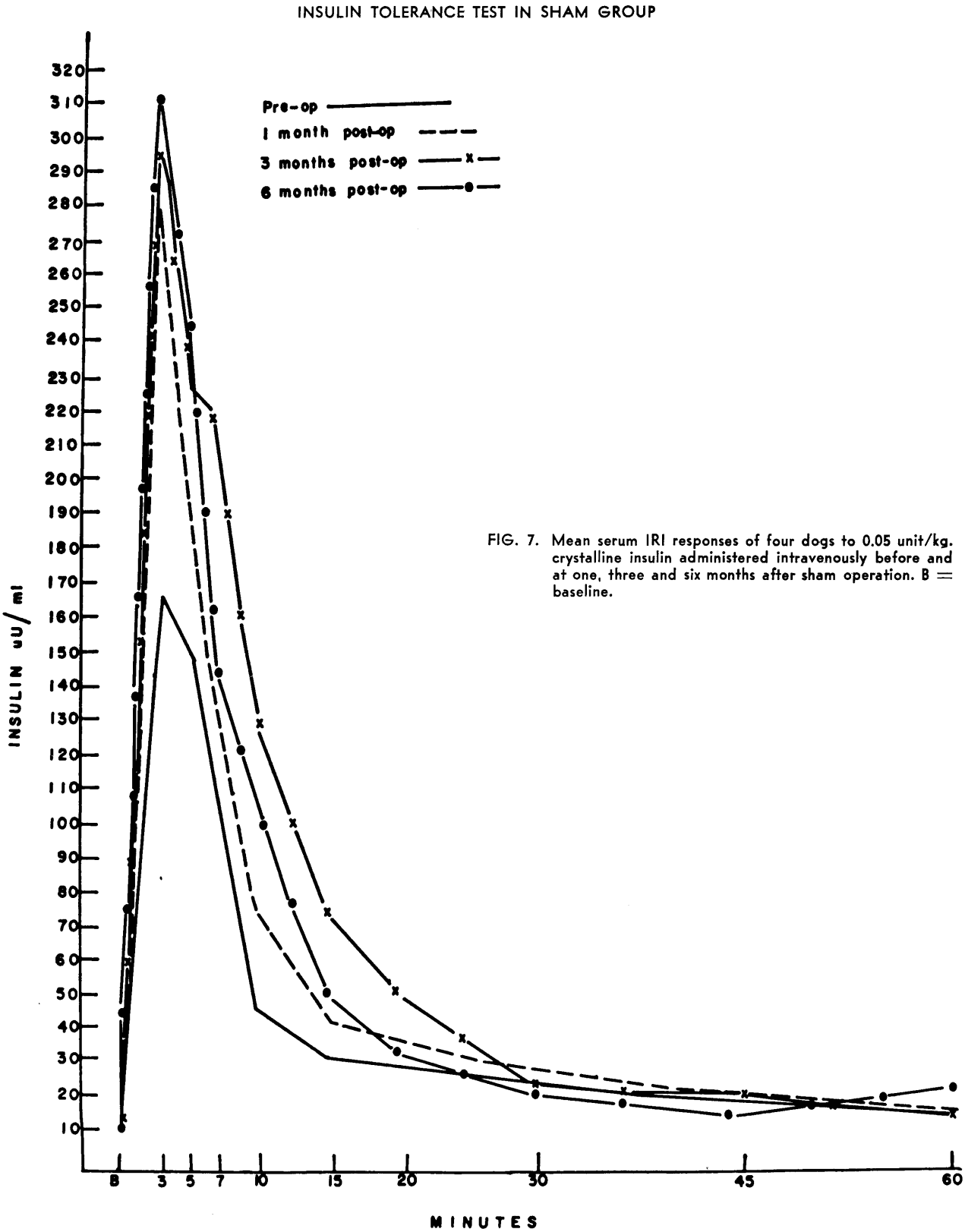

FIG. 7. Mean serum IRI responses of four dogs to 0.05 unit/kg. crystalline insulin administered intravenously before and at one, three and six months after sham operation. B = baseline.

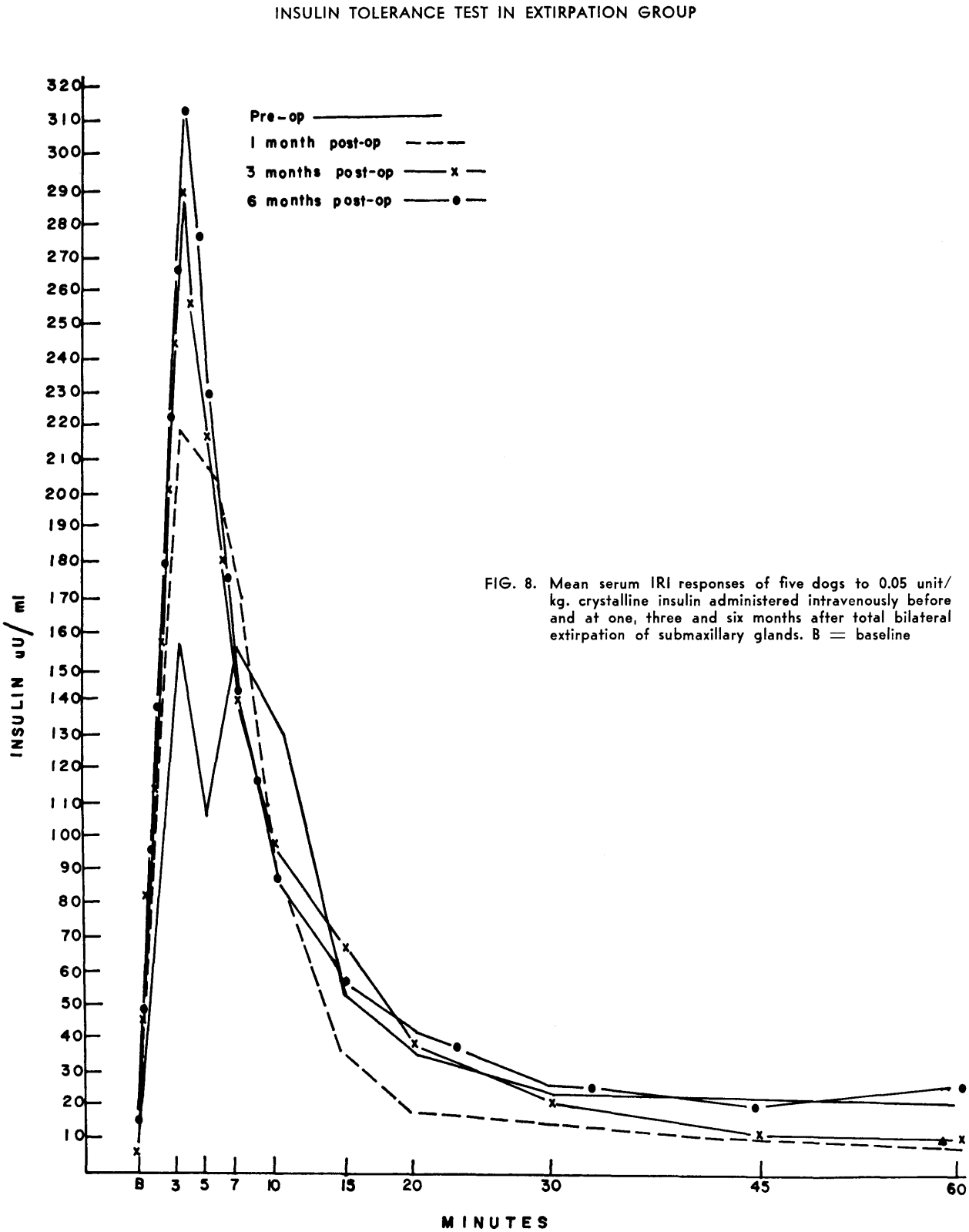

FIG. 8. Mean serum IRI responses of five dogs to 0.05 unit/kg. crystalline insulin administered intravenously before and at one, three and six months after total bilateral extirpation of submaxillary glands. B = baseline

We chose to administer the glucose orally in order to obtain an insulin response that we anticipated would be greater than if given intravenously. However, the glucose absorption was apparently erratic and unusually delayed since mean peak levels were not achieved until or perhaps after the termination of the two-hour study period (figures 1 and 2). This effect in dogs has been noted previously[1] and we were unsuccessful in avoiding it by diluting the glucose solution. The irregular absorption of glucose probably accounts for the erratic insulin responses (figures 3 and 4). The fact that these studies were performed on anesthetized animals may also have contributed to the observed effects on serum glucose and insulin levels. Our failure to demonstrate increased insulin sensitivity to *exogenous* insulin in the postoperative periods is less easily explained.

Although the relationship between the salivary glands and glucose metabolism is unclear at this time, it is difficult to avoid being impressed by the rather frequent clinical association of salivary gland enlargement and diabetes mellitus.[7-11,16,17,19] Of the sixteen patients reported by Davidson et al.[8] with asymptomatic parotid gland enlargement and glucose intolerance, twelve were discovered during a four-month prospective study. Three of these patients also had submaxillary gland enlargement. In their review of diabetes mellitus and painless enlargement of the parotids, McFadzean and Yeung[19] noted that the diabetes in all cases was regarded as mild or moderate in severity. However, Bruce et al.[9] reported a case in which over 1,000 units of insulin per day was required for control of the diabetes during a ten-month period, and the patient had asymptomatic enlargement of the submaxillary glands. A most striking feature of this case was that there was *complete* remission of the diabetes coincident with regression in size of the submaxillary glands. This case is believed to be the second of only two documented cases of *complete* spontaneous remission of insulin-dependent diabetes mellitus.

During the routine screening of patients for macroamylasemia[20] by the Division of Gastroenterology of our Department of Medicine, one case was found in a patient whose clinical manifestations of diabetes mellitus suddenly appeared during his hospitalization for an unidentified dermatologic disorder. There was no appreciable salivary gland enlargement or discomfort nor any manifestations of pancreatitis other than hyperamylasemia found during the routine screening. Isoenzyme analysis disclosed both pancreatic and salivary amylases with the latter being the predominant type. The hyperglycemia and glycosuria were poorly responsive to several hundred units of insulin per day during a period of two weeks. The patient then became very responsive to insulin and the diabetes was easily controlled with 30 units of NPH insulin daily coincident with the disappearance of the macroamylasemia and hyperamylasemia.

Finally, Godlowski et al.[11] performed total ablation of the submaxillary glands in eighty-six patients with diabetes mellitus of various degrees of severity. At first[10] he reported complete remission of diabetes mellitus in three patients with the adult-onset type but later[11] claimed two remissions. He also stated that a number of insulin-dependent diabetics had a significant reduction in their insulin requirement following surgery. We feel that the above findings are not unlike those seen in most large diabetic clinics with unoperated patients.

From our study and a review of others, it appears that the role of the salivary glands in the pathogenesis and management of diabetes mellitus is not at all clear at this time. It has been suggested that salivary gland ablation should be performed in selected forms of diabetes mellitus. However, we fail to understand the basis for making such selections, other than perhaps in desperate situations unresponsive to conventional therapy.

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REFERENCES

[1] Zimmerman, L. M.: Effect of ligation of the parotid ducts on the carbohydrate tolerance of normal dogs. Arch. Intern. Med. *49*:409-20, 1932.

[2] Birnkrant, W. B.: The influence of the parotid gland on blood sugar. J. Lab. Clin. Med. *26*:1009-11, 1941.

[3] Birnkrant, W. B., and Shapiro, R.: The influence of a parotid extract on the blood sugar and structure of the pancreas of the rat. J. Lab. Clin. Med. *27*:510-18, 1942.

[4] Gault, S. D.: The effect of parotidectomy on blood sugar levels in the rat and mouse. J. Lab. Clin. Med. *43*:119-22, 1954.

[5] Godlowski, Z. Z., and Calandra, J. C.: Salivary glands as endocrine organs. J. App. Physiol. *15*:101-05, 1960.

[6] Godlowski, Z. Z.: Endocrine function of submaxillary glands. Arch. Otolaryngol. *75*:346-63, 1962.

[7] Morgan, A. D., and Raven, R. W.: Sjogren's syndrome: A general disease. Brit. J. Surg. *40*:154-62, 1952.

[8] Davidson, D., Leibel, B. S., and Berris, B.: Asymptomatic parotid gland enlargement in diabetes mellitus. Ann. Intern. Med. 70:31-38, 1969.

[9] Bruce, D. H., Bernard, W., and Blackard, W. G.: Spontaneous disappearance of insulin-resistant diabetes mellitus in a patient with a collagen disease. Am. J. Med. *48*:268-72, 1970.

[10] Godlowski, Z. Z.: The role of submaxillary glands in homeostasis of insulin. Diabetes *17*:325-26, 1968 (Supplement 1).

[11] Godlowski, Z. Z., Gozada, M., and Withers, B. T.: Ablation of salivary glands as initial step in the management of selected forms of diabetes mellitus. Laryngoscope *81*:1337-58, 1971.

[12] Technicon AutoAnalyzer procedure method N-2b, Tarrytown, New York.

[13] Phadebas Insulin Test Radioimmunoassay Kit, Pharmacia Laboratory, Inc. Piscataway, New Jersey.

[14] Paget, S.: The relation of the parotid to the generative organs. Lancet *1*:86-87, 1886.

[15] Harkin, A.: The connection between the parotid glands and the generative organs. Lancet *1*:374-75, 1886.

[16] Critchley, M., and Meadows, S. P.: Xerostomia and xerophthalmia. Proc. R Soc. Med. *26*:308-09, 1932.

[17] Weber, F. P., and Schluter, A.: Non-ocular features of Sjogren's syndrome. Med. Press *213*:253-54, 1945.

[18] Garcia, A. R., Blackard, W. G., and Trail, M. L.: Submaxillary gland removal: Effects on glucose and insulin homeostasis. Arch. Otolaryngol. *93*:597-98, 1971.

[19] McFadzean, A. J. S., and Yeung, R.: Diabetes among the Chinese in Hong Kong. Diabetes *17*:219-28, 1968.

[20] Fridhandler, L., Berk, J. E., and Ueda, M.: Macroamylasemia: Rapid detection method. Clin. Chem. *17*:423-26, 1971.