Virchows Arch. A Path. Anat. and Histol. 382, 301–311 (1979)

Virchows Archiv A

Ultrastructural Studies of the Rat Submandibular Gland in Streptozotocin Induced Diabetes Mellitus

Leslie S. Cutler, Harriet E. Pinney, Constance Christian, and Samuel B. Russotto

University of Connecticut School of Dental Medicine, Farmington, Connecticut 06032, USA

Summary. Increased fluid intake (polydipsia) is one of the classic symptoms of diabetes mellitus. Xerostomia (dry mouth) and resultant thirst are other symptoms of the disease and bear a close relationship to polydipsia. The xerostomia in individuals with diabetes is primarily due to decreased saliva flow which appears to be associated with degenerative changes in the salivary glands. This study examines the response of the rat submandibular gland to streptozotocin induced diabetes mellitus. Adult male rats were given a single I.V. dose of streptozotocin (65 mg/kg body weight) in citrate buffer (pH 4.5). Salivary glands were examined by light and electron microscopy at 4, 8 and 24 h and 3, 7, 14 and 21 days posttreatment. The changes in the acinar cells were characterized by an accumulation of secretory material within the cytoplasm. This secretory protein accumulation was followed by degenerative changes in the acinar cells which frequently resulted in cell death and replacement of secretory cells by connective tissue elements. The loss of secretory volume and potential changes in secretory kinetics are discussed with regard to the xerostomia, thirst and polydipsia exhibited by individuals with diabetes mellitus.

Key words: Salivary glands – Diabetes mellitus.

Introduction

The passage of large volumes of urine (polyuria), an increased thirst and fluid intake (polydipsia), loss of weight accompanied by an increased appetite and food intake (polyphagia) and a generalized physical weakness represent the classic symptoms of diabetes mellitus. Xerostomia (dry mouth) is another common symptom of the disease and bears a close relationship to polydipsia. Conner

Send offprint requests to: Dr. L.S. Cutler

0340-1227/79/0382/0301/$02.20

and his coworkers (1970) demonstrated that the xerostomia observed in diabetic patients was a true xerostomia due primarily to a decrease in saliva flow. They found that parotid saliva flow in diabetic patients was about 1/3 that observed in normal control subjects. Additionally, Davidson and associates (1969) reviewed the literature on and presented 14 new cases of diabetic patients with asymptomatic salivary gland enlargement. Histologic study of glands from some of these patients revealed that the glandular enlargement was a non-inflammatory, non-neoplastic fatty infiltration of the parenchyma with a decrease in the number of acinar structures. This non-inflammatory, non-neoplastic type of salivary gland enlargement actually appears to be due to simultaneous fatty infiltration and acinar cell enlargement and has been termed sialadenosis (Seifert, 1967; Thackray and Lucas, 1974). This condition can be found in association with a variety of systemic diseases including diabetes, cirrhosis, malnutrition, alcoholism, thyroid insufficiency and several other states (Rauch, 1970; Thackray and Lucas, 1974; Donath and Seifert, 1975). Donath and Seifert (1975) have attributed the salivary gland changes to degenerative changes in the autonomic nerves innervating the gland. This assertion is supported by their observation of degenerative changes in the nerves within the salivary glands in human sialadenosis and comparable changes in the salivary glands of animals in which nervous innervation of the glands is interrupted experimentally (Peronace et al., 1964; Donath et al., 1974; Garrett and Thulin, 1975; Garrett and Harrop, 1976). The electron microscopic appearance of these salivary gland changes have been examined (Donath and Seifert, 1975) but the pathogenesis and progression of these changes have not been well defined.

Several animal models of diabetes mellitus are available for study. We chose to use streptozotocin induced diabetes in the rat because it appeared to be the most suitable system for the elucidation of the salivary gland changes since streptozotocin appears to be a better drug for inducing diabetes in adult rodents than alloxan (Hoftiezer and Carpenter, 1973; Junod et al., 1969; Mansford and Opie, 1968). Furthermore, the drug induced disease is easier to monitor and control with regards to timing of onset and duration than the hereditary diabetes mellitus in hamsters or mice.

The purpose of the present report is to describe the ultrastructural changes seen in the rat submandibular gland (SMG) during the development of streptozotocin induced diabetes mellitus (a preliminary report of this data has previously been given, Cutler et al., J. Dent. Res. 56(B):B151, 1977).

Methods

Male Sprague-Dawley rats (225–250 g) were maintained under environmentally controlled conditions and given standard rat chow and water ad libitum. Streptozotocin (ICN Pharmaceuticals, Inc., Cleveland, Ohio) was dissolved in citrate buffer (pH 4.5) just prior to use. Streptozotocin (65 mg/kg body weight) was administered in a single I.V. (femoral vein) dose at 8:00 A.M. on the day the experiments were initiated do 21 rats. Control animals (21) where similarly injected with an equal volume of citrate buffer (pH 4.5). Animals were examined (3 per time period) at 4, 8 and 24 h and 3, 7, 14 and 21 days after treatment. In all cases blood glucose was determined on experimental and paired control animals using a Worthington glucostat reagent set (Worthington

Biochemical Corp., Freehold, New Jersey) to insure the success of the injection procedure and the development of hyperglycemia.

Electron Microscopy

Animals were anesthetized with ether, and the right SMG was quickly excised, minced into small (1 mm^3) cubes and immersed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). Fixation proceded for 3 h at room temperature. The tissue was then washed in cold (4°C) 0.1 M cacodylate buffer (pH 7.4) (4X – 30 min each) and then post-fixed in 1% cacodylate buffered oxmium tetroxide. Following post-fixation the tissue was washed twice in distilled water and then stained en bloc in 0.25% aqueous uranyl acetate. The tissue was then routinely dehydrated through a graded series of acetones and embedded in Spurr low viscosity media (Spurr, 1969).

Thick (0.5 μ) sections were taken for orientation purposes and then thin (60–90 mμ) sections were cut on an LKB Ultratome III, counterstained with lead citrate (Venable and Coggeshall, 1965) and examined on a Zeiss EM-10 electron microscope.

Results

The blood glucose levels of the experimental animals relative to their paired controls is shown in Fig. 1. Blood glucose levels rose sharply four hours after streptozotocin administration. Glucose levels had dropped below control values eight hours after treatment and were elevated about three fold 24 h after treatment and at all subsequent time points investigated. This blood glucose pattern is consistent with that previously reported by Junod and his associates (1969) in streptozotocin induced diabetes.

Morphological Changes

No fine structural alterations were seen in either the acini or the ducts at four hours or eight hours after streptozotocin administration (Fig. 2). The acinar

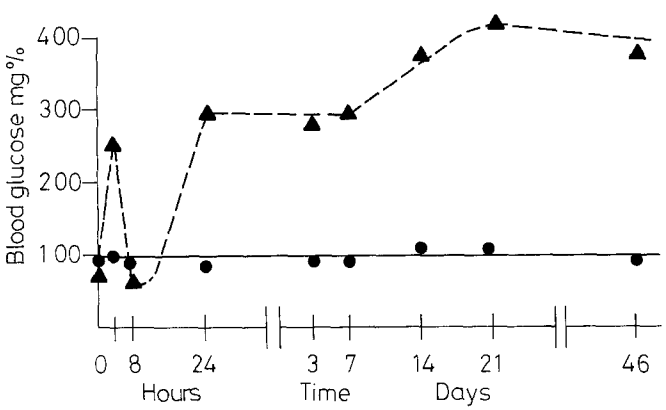


Fig. 1. Graphic representation of the average blood glucose levels (mg%) in streptozotocin treated (▲---▲) and paired control (●–●) rats during the course of the study. Blood was obtained by cardiac puncture and the blood glucose assayed using a Worthington glucostat reagent set (Worthington Biochemical Corp., Freehold, N.J.)

cells showed their typical apical-basal polarization of secretory granules and intracellular organelles. The secretory granules retained their floculant matrix with granules occasionally contiguous with one another (Fig. 2) as described in several reports on the fine structure of the rat SMG (Jacoby and Leeson, 1959; Tamarin and Sreebny, 1964). The first structural alterations observed in the SMG occurred 24 h post-treatment. Several granules seemed to fuse with one another forming relatively large pools of secretory material within the cytoplasm of the acinar cells (Figs. 3 and 4).

By 7 days after streptozotocin administration this phenomenon of granule fusion had become more prominent and most cells in the gland showed several mucin pools in their cytoplasm. Frequently these pools fused with each other thus occupying a large poriton of the cytoplasm and compressing the cellular organelles in the remaining cytoplasmic volume (Fig. 5). Secretory granules were seen fused with the apical plasma membrane and releasing their contents into the acinar lumen in an apparently normal fashion (Fig. 5).

Fourteen days after treatment many of the acinar cells showed large vacuoles in the cytoplasm (Fig. 6). These vacuoles appeared to be of a lipid nature since in some sections the material was osmophilic. However, most of the lipid seems to have been removed during fixation and processing of the tissue. This is a common finding in glutaraldehyde fixed tissue. Additionally the cells frequently contained dense bodies and other secondary lysosomal variants (Figs. 6 and 7). The overall appearance of many of the cells was indicative of the advanced stages of degeneration often leading to cell death.

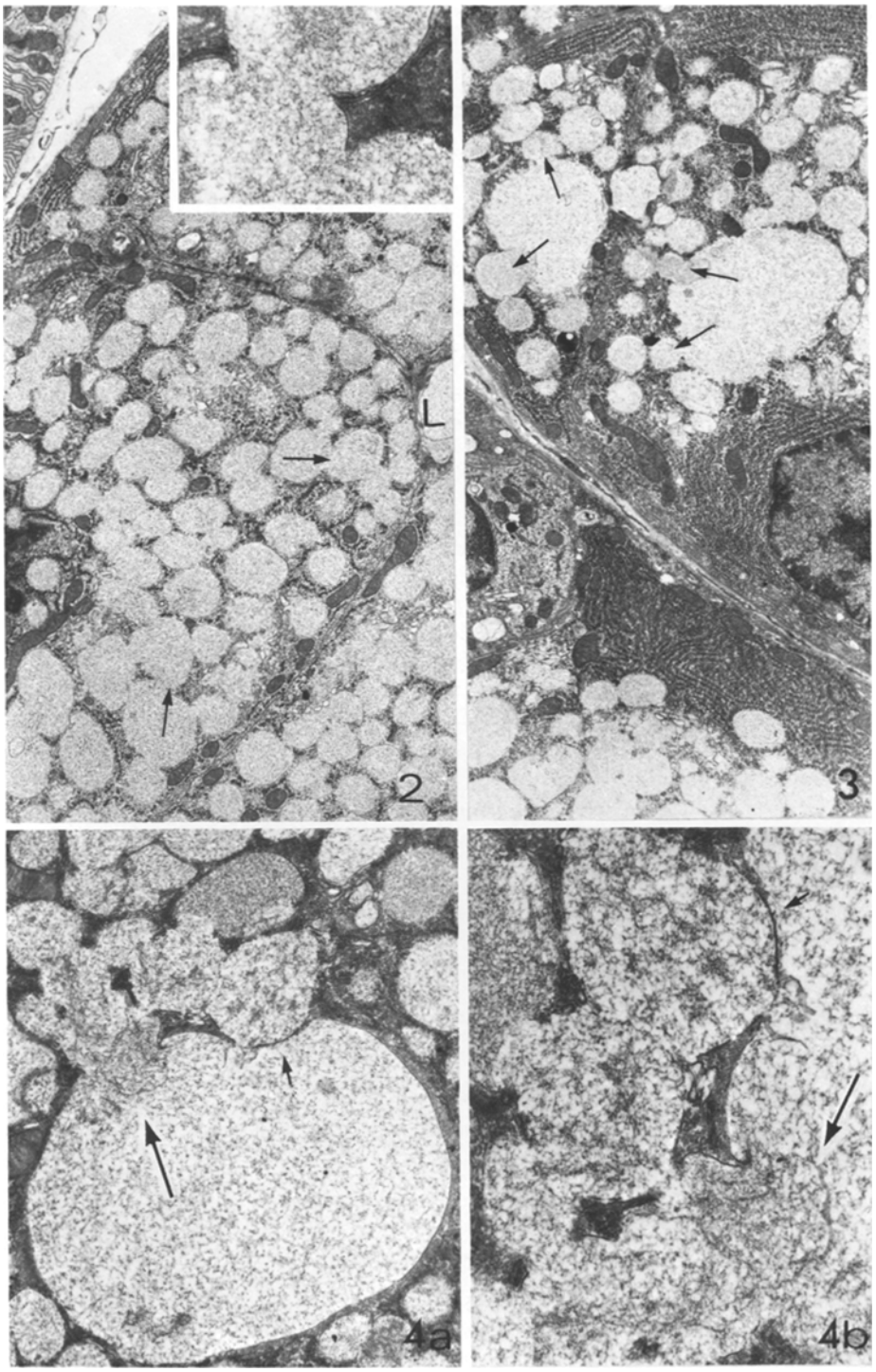
By three weeks after treatment many cells were showing frank signs of necrosis. Other cells had completely lost their normal architecture and their cytoplasm was filled with degenerating organelles with various forms of secondary lysosomes (Fig. 8).

The intercalated and striated ducts showed essentially no changes throughout the experimental period while the secretory duct cells (CGT) showed changes generally analogous to those seen in the acinar cells. This process was not synchronous throughout the gland and some areas appeared relatively normal while others were in transitional states and still other areas showed dramatic

Fig. 2. Electron micrograph showing portions of 3 SMG acinar cells surrounding a lumen (*L*) 4 h after streptozotocin administration. The floculant secretory granules are distinct with occasional granules showing local fusion (*arrows*). No signs of secretion or resynthesis (×5,250). Higher magnifification electron micrograph showing normal granule fusion (×22,000)

Fig. 3. Electron micrograph showing a portion of a SMG acinar cell 24 h after streptozotocin administration. Several secretory granules can be seen fused together (*arrows*) to form rather large accumulations of secretory material within the cytoplasm (×5,000)

Fig. 4. **a** Somewhat higher magnification electron micrograph of an area of granules fusing to form a large pool of secretory material analogous to that shown in Fig. 3. The fusion of a granule with the pool of material can be seen (*small arrow*) and the secretory product from another granule can be seen streaming into the pool (*larger arrow*) (×7,000). **b** Higher magnification electron micrograph of areas indicated by arrows in 4a. Note the fusion (*small arrow*) of granule membrane with limiting membrane of pool material and apparent membrane fragment in area where material seems to be streaming into the pool (*large arrow*) (×22,000)


L
2
3
4a
4b

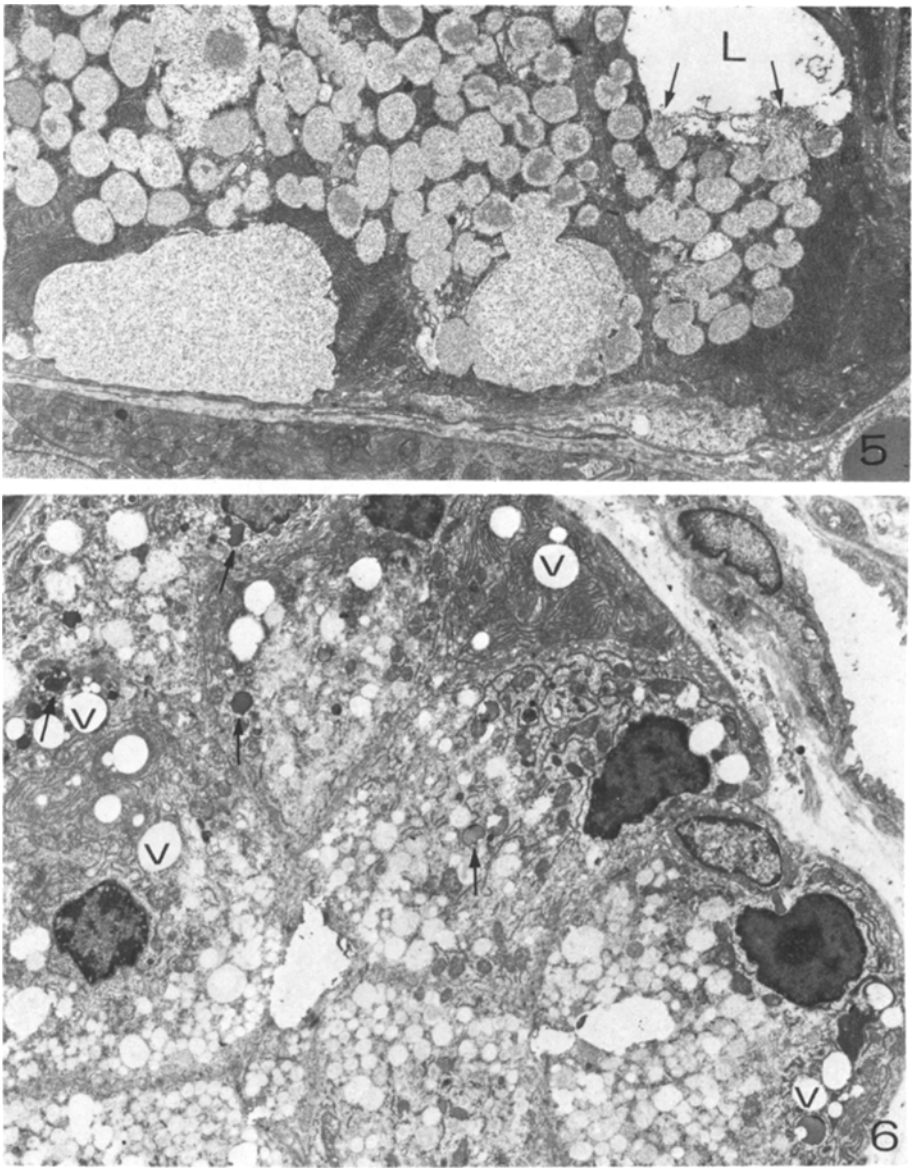


Fig. 5. Electron micrograph showing portions of SMG acinar cells 7 days after streptozotocin administration. Many pools of secretory material can be seen. The pools are large and compress the cellular organelles. A lumen (*L*) is present and secretory granules can be seen in the process of releasing their products into the lumen (*arrows*) (×4,400)

Fig. 6. Electron micrograph showing SMG acinar cells 2 weeks after streptozotocin treatment. The cell contains numerous vacuoles (*V*) which probably represent lipid vacuoles with the lipid having been extracted during the processing of the tissue. Several lysosomal variants or cytosegresomes (*arrows*) can also be seen in these cells. The general architecture of the cells is disrupted and the cells appear to be in the early phases of degeneration (×2,800)

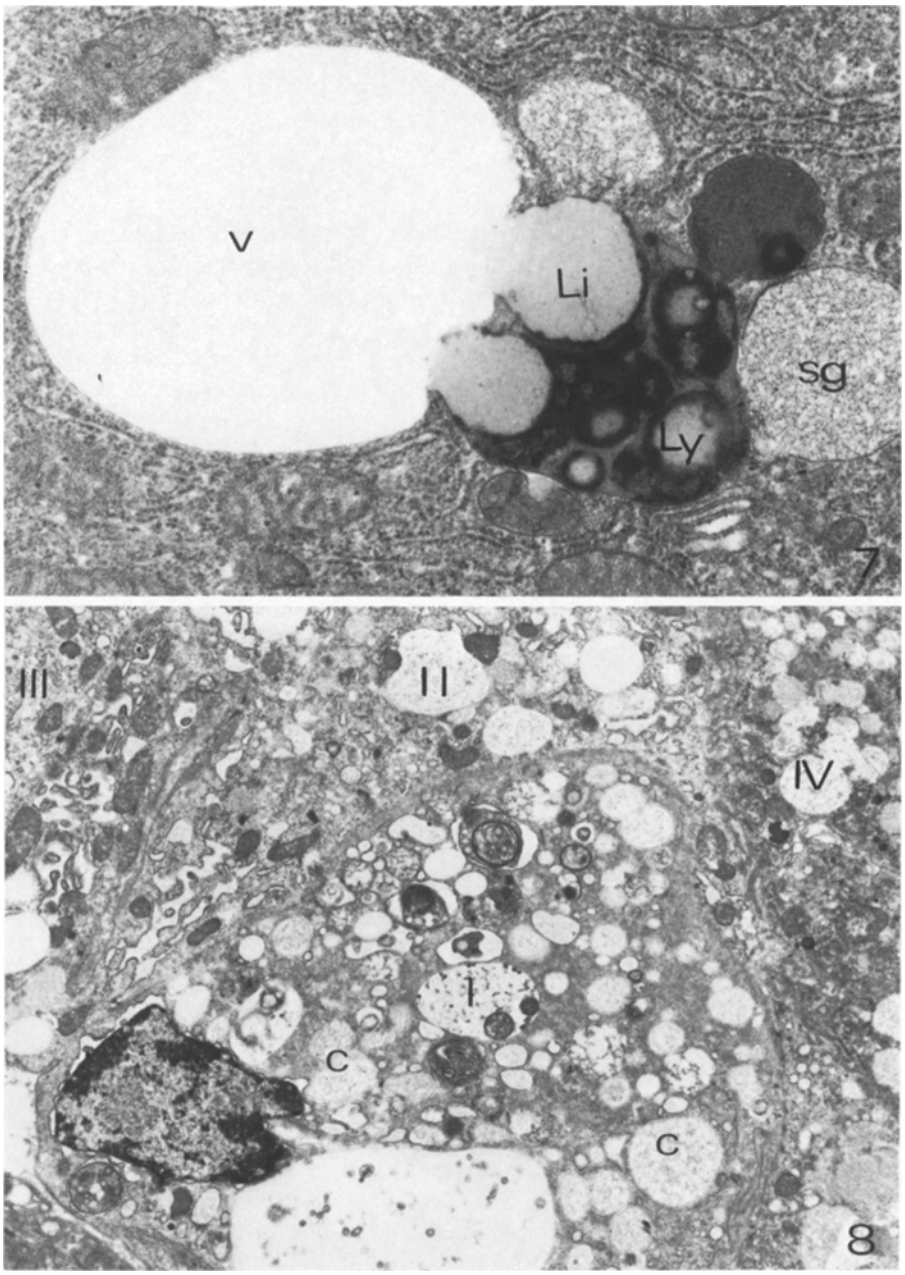


Fig. 7. Electron micrograph showing a lysosomal varient (*Ly*) associated with a lipid vacuole like those seen in Fig. 6. The lipidlike material (*Li*) can be seen incorporated within the lysosome and the continuity between the lipid and the vacuole is evident at one pole of the lysosome. A secretory granule (*sg*) appears to be fusing with the lysosome at the opposite pole (×28,000)

Fig. 8. Electron micrograph showing SMG acinar cells 3 weeks after streptozotocin treatment. One of the cells (*I*) is filled with cytosegresomes and other degenerative bodies which appear to involve the secretory granules (*C*). Other cells (*II, III*) appear to be less affected than cell I while yet another cell (*IV*) appears relatively normal in architecture (×4,400)

degenerative changes. Occasional inflammatory cells were seen in the connective tissue, probably in response to products released upon the death of the secretory cells.

Discussion

The results of the present study indicate that streptozotocin does not cause secretion or have any other immediate direct effects on the morphology of the adult rat submandibular gland. No morphologic evidence of secretion or resynthesis of exocrine protein was seen at four or eight hours after injection of the drug. This observation is in agreement with the reported specificity of action of streptozotocin on pancreatic islets (Karunanayake et al., 1976; Rakieten et al., 1963).

The morphologic changes seen in the acinar cells of the SMG of the streptozotocin induced diabetic rats are consistent with the appearance of the parotid gland acinar cells in human sialadenosis (Donath and Seifert, 1975). Direct correlation between the changes in the rat SMG model and in human diabetes associated sialadenosis cannot be made since changes in the human SMG have not been reported. Further, the exact types of sialadenosis studied by Donath and Seifert (1975) were not listed in their report. In human parotid gland sialadenosis the acinar cell changes were associated with changes in the autonomic nerves innervating the gland. However, the structural changes in nerves seen in human sialadenosis (Donath and Seifert, 1975) were not seen in the present study on the rat (Fig. 9).

In contrast to the absence of neural changes, the morphologic changes in the acinar cells of the SMG of diabetic animals seem in many ways to parallel those alterations seen in salivary gland secretory cells when they are deprived of neural stimulation (Garrett and Harrop, 1976; Peronance et al., 1964) or after treatment with drugs which block sympathetic or parasympathetic stimulation of the gland (Donath et al., 1974). Enlargement and fusion of secretory granules was described as an early event which followed preganglionic sympathectomy of the rat SMG (Garrett and Harrop, 1976) while acinar enlargement followed by degeneration was seen subsequent to parasympathetic denervation (Peronace et al., 1964). Somewhat analogous findings have been made in the rat parotid gland following post-ganglionic sympathectomy (Garrett and Thulin, 1975) or after treatment with sympathetic or parasympathetic blocking agents (Donath et al., 1974). Perhaps the changes seen in the SMG of the streptozotocin treated rats are due to alterations in neuroreceptors rather than in the nerve. Such a defect would explain the similarities and differences seen in the diabetic rat and human sialadenosis and would provide a common mechanism for the changes. This mechanism would revolve around an abnormal neural stimulation of the gland. In one case the defect might lie at the level of the neuron and in the other case at the level of the neural receptor on the responding cell surface.

Wright and Giammara (1976) have reported that in alloxan induced diabetes in the mouse, both amylase and protease activities of the SMG were significantly

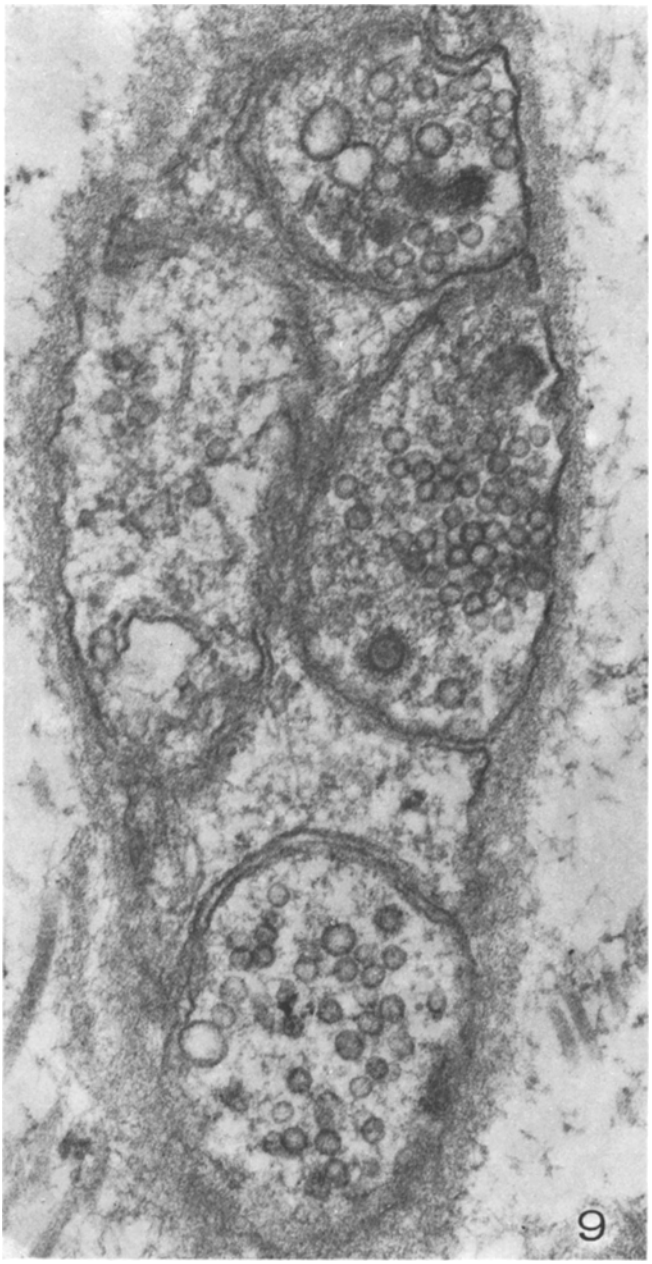


Fig. 9. Electron micrograph of a typical cholinergic nerve ending from the SMG 21 days after streptozotocin treatment. The nerve ending looks normal (×62,500)

elevated over normal animals for the first nine days after treatment. Subsequently both amylase and protease activities began to decrease and were at normal levels by 11 days post-treatment. Morphologic changes were seen that were consistent with those reported in the present study (Wright and Giammara, personal communication). These morphologic observations were mentioned but not detailed in their abstract (Wright and Giammara, 1976).

During the first phase of streptozotocin induced diabetes (7–14 days) there appears to be an accumulation of secretory product within the acinar cells of the SMG. This accumulation may lead to cell injury with subsequent autophagy which can result in cell death. There appears to be a replacement of secretory tissue with connective tissue elements. This degenerative phase seems to begin about two weeks after treatment with streptozotocin and thus coincides with decrease in enzymatic activities described by Wright and Giammara (1976). Thus, it appears that in the early phases of streptozotocin induced diabetes mellitus there is a shift in secretion kinetics such that secretory product accumulates within the acinar cells. Some of the morphologic aspects of this accumulation resemble changes seen when the glands are deprived of neural stimulation or in human sialadenosis. However, the exact reason for this accumulation of secretory granules can not be attributed to neural changes in this study. This secretory product accumulation does not appear to be due to an inability of granules to fuse with the plasma membrane and release their contents since granule fusion and release were seen. The pooling of secretory protein within the acinar cells apparently leads to chronic degenerative changes in the cells characterized by lipid accumulation, autophagy and eventual cell death. The summation of altered secretion and the loss of secretory cell mass may result in decreased salivary flow (loss of secretory reserve). Such a mechanism may account for the decreased salivary flow observed by Conner and coworkers (1970) and may account for the xerostomia and its part in the polydipsia commonly see in individuals with diabetes mellitus.

Further studies into the kinetics of secretion in response to various neurohomonal secretogogues in these diabetic animals are currently in progress.

This work was supported in part by N.I.H. grant DE-03933. We would like to thank Mrs. Valerie Every for her secretarial assistance.

References

Conner, S., Iranpour, B., Mills, J.: Alteration in parotid salivary flow in diabetes mellitus. Oral Surg. **30**, 55 (1970)

Davidson, D., Leibel, B.S., Berris, B.: Asymptomatic parotid gland enlargement in diabetes mellitus. Ann. Intern. Med. **70**, 30 (1969)

Donath, K., Spillner, M., Seifert, G.: The influence of the autonomic nervous system on the ultrastructure of the parotid acinar cells. Virchows Arch. A Path. Anat. Histol. **364**, 15 (1974)

Donath, K., Seifert, G.: Ultrastructural studies of the parotid glands in sialadenosis. Virchows Arch. A Path. Anat. Histol. **365**, 119 (1975)

Garrett, J.R., Thulin, A.: Structural changes associated with parotid "degeneration secretion" after post-ganglionic sympathectomy in rats. Cell Tiss. Res. **162**, 1 (1975)

Garrett, J.R., Harrop, T.F.: Short-term effects of pre-ganglionic sympathectomy on the structure of submandibular salivary acinar cells in rats. Archs. Oral Biol. **21**, 161 (1976)

Hoftiezer, V., Carpenter, A.M.: Comparison of streptozotocin and alloxan-induced diabetes in the rat including volumetric quantitation of the pancreatic islets. Diabetologica **9**, 178 (1973)
Jacoby, F., Leeson, C.R.: An electron microscopic study of the rat submaxillary gland during its postnatal development and in the adult. J. Anat. **93**, 287 (1959)
Junod, A., Lambert, A.E., Stanffacher, W., Renold, A.E.: Diabetogenic action of streptozotocin: Relationship of dose to metabolic response. J. Clin. Invest. **48**, 2129 (1969)
Karunanayake, E.H., Baker, J.R.J., Christian, R.A., Hearse, D.J., Mellows, G.: Autoradiographic study of the distribution and cellular uptake of (C^{14})-streptozotocin in the rat. Diabetologica **12**, 123 (1976)
Mansford, K.R.L., Opie, L.: Comparison of metabolic abnormalities in diabetes mellitus induced by streptozotocin and alloxan. Lancet **I**, 670 (1968)
Peronace, A.A.V., Davison, T.A., Houssay, A.B., Perec, C.J.: Alterations in submandibular and retrolingual glands following parasympathetic denervation in rats. Anat. Rec. **150**, 25 (1964)
Rakieten, N., Rakieten, M.L., Nadkarni, M.V.: Studies on the diabetogenic action of streptozotocin. Cancer Chemotherap. Rep. **29**, 91 (1963)
Rauch, S.: Sialadenosis. In: Thoma's Oral Pathology, R.S. Gorlin and H.M. Goldman (eds.), pp. 986–997. St. Louis: C.V. Mosby, Co. 1970
Seifert, G.: Experimental sialadenosis by isoproterenol and other agents: Histochemistry and electron microscopy. In: Secretory Mechanisms of Salivary Glands, L.H. Schneyer and C. Schneyer (eds.), pp. 191–218. New York: Academic Press 1967
Tamarin, A., Sreebny, L.M.: The rat submaxillary gland: correlative study by light and electron microscopy. J. Morph. **117**, 295 (1965)
Thackray, A.C., Lucas, R.B.: Tumors of the major salivary glands. Atlas of Tumor Pathology, second series, Fascicle 10. Washington: D.C., Armed Forces Institute of Pathology 1974
Spurr, A.R.: A low-viscosity eposy resin embedding medium for electron microscopy. J. Ultrastruct. Res. **26**, 31 (1969)
Venable, J.H., Coggeshall, R.: A simplified lead citrate strain for use in electron microscopy. J. Cell Biol. **25**, 407 (1965)
Wright, B., Giammara, B.: Morphologic and enzymatic alterations in alloxan diabetic mouse submandibular glands. J. Dent. Res. **55B**, B231 (1976)

Received December 11, 1978