1978

Compensatory Changes In The Parotid Gland Of The Immature Rat Induced By Partial Desalivation And Denervation.

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COMPENSATORY CHANGES IN THE PAROTID GLAND OF THE IMMATURE RAT INDUCED BY PARTIAL DESALIVATION AND DENERVATION

by

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A DISSERTATION

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in the
Department of Anatomy and the Department of
Physiology and Biophysics in The Graduate School,
University of Alabama in Birmingham

BIRMINGHAM, ALABAMA

1978

ACKNOWLEDGMENTS

This research was directed by Dr. Charlotte A. Schneyer, an internationally respected salivary physiologist. She has worked tirelessly with me on this project, been readily accessible, has exemplified and encouraged me toward the ideals of a researcher, and has become a dear friend and valued colleague.

Dr. James H. Sheetz, Jr. has patiently instructed and assisted me in the techniques of electron microscopy. His comments, criticisms, and encouragement have been most helpful in the completion of this study.

I wish to thank Mrs. Freddie M. Thomas, Mr. Herman Forrest and Miss Jean Clark for their technical assistance and friendship. I am especially grateful to Mrs. Thomas, who is also my typist, for her extraordinary efforts in helping me to meet various deadlines.

The members of my graduate committee, Drs. Earl G. Hamel, Jr., Jerry W. Brown, Thomas E. Hunt, Leonard H. Robinson, and Samuel B. Barker have provided helpful advice and given freely of their time. Dr. Leon H. Schneyer served as a member of my committee prior to his untimely death and taught me an appreciation of the biophysical aspects of salivary research.

Finally, I wish to acknowledge the unfailing support and patience of my husband, Don, without whose sacrifices this degree would not have been possible.

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INTRODUCTION

Literature Review

Saliva is a fluid of complex composition that is produced primarily by three major gland pairs, the parotid, submandibular, and sublingual glands, located in the oral cavity of most mammalian forms. Some minor contribution is made by scattered buccal, labial, palatal, and lingual salivary glands. The fluid produced by the glands has digestive, cleansing, emulcent, and lubricating functions in most animals, but its most important function is protection of the teeth and buccal mucosa (Schneyer and Emmelin, 1974). In animals such as dogs and cats, it also has a role in regulation of body temperature; panting results in elaboration of copious quantities of saliva, and evaporation of this, in turn, causes cooling (Schneyer and Schneyer, 1967). In the rat, body temperature is regulated by evaporative cooling following spreading of saliva over the body (Stricker and Hainsworth, 1970). Saliva provides large quantities of a well buffered solution for digestive functions of ruminant animals such as sheep (Kay, 1960; van Lennep et al., 1977). In young animals such as rat pups, saliva acts as a mechanical sealant of the pup's lips and mouth to the maternal nipple and allows the pup to suckle (Epstein et al., 1970). Without the presence of a specific chemical substrate coating the maternal

nipples and providing appropriate olfactory stimulation, suckling is eliminated. Pup saliva is a source for this olfactory cue (Teicher and Blass, 1976).

Mature salivary glands are composed of a series of secretory units or salivons (Schneyer and Emmelin, 1974) which consist of secretory endpieces and a network of ducts (intercalated, striated, and excretory) which terminate in the oral cavity. Secretory endpieces or acini form the blind ends of the ductal tree and discharge the main characteristic components of the saliva. They vary greatly in shape and configuration in the various mammalian species (Shackleford and Wilborn, 1968). The lumen of the secretory endpieces is continuous with the lumen of the first portion of the duct system, the intercalated duct or striated duct. Although many ductal cells do add components to the secretion, none of the duct system, including granular ducts, is included in the term secretory endpieces because of their primary function of conduction of fluid. Intercalated ducts are poorly developed in the sublingual gland and are entirely absent in some species. Their length is variable, and the ductal epithelium consists of a simple cuboidal epithelium ranging from columnar to very low cuboidal surrounding a narrow lumen (Tamarin and Sreebny, 1965; Tandler et al., 1970). A few secretory granules of unknown nature are present in the intercalated ducts of most species studied (Shear, 1969; Dorey and Bhoola, 1972; Robinovitch and Iversen, 1977).

Intercalated ducts converge to form striated or intralobular ducts which are lined by a simple low columnar epithelium. Striated

duct cells exhibit radial, basophilic striations or infoldings of the plasma membrane with vertically oriented mitochondria associated with the folds in the basal third of the cell below the nucleus. The prevalence of and extent of development of striated ducts varies markedly among different species and also among the major glands of a single species. They are generally most conspicuous in parotid and submandibular glands and least so in sublingual glands (Leeson, 1967). In the submandibular gland of some animals, particularly of many rodents, the proximal ends of striated ducts are modified to form granular ducts (also called granular convoluted tubules) which contain few, if any, basal striations and numerous apically located secretion granules. In the rat submandibular gland, granular ducts are absent at birth, do not begin to develop until approximately the sixth week when acinar development is complete, and are often incompletely developed at four months of age (Srinivasan and Chang, 1975). Granules within granular duct cells have been shown to contain a number of biologically active substances such as renin (Gresik et al., 1978), nerve growth factor (Levi-Montalcini et al., 1972), and epidermal growth factor (Cohen, 1962; Roberts, 1977; Gresik and Barka, 1978). Whether these substances are formed or merely stored here is unknown. Striated ducts are generally confined to lobules and converge into two or more generations of interlobular excretory ducts which are lined by a simple columnar epithelium.

The term excretory duct refers to all ducts lying between striated ducts and the orifice to the main duct, without regard

to their morphology or topography. By further convergence, the excretory ducts form a main excretory duct, which usually singly terminates in the oral cavity. Proximally, the epithelium of excretory ducts is tall, simple columnar which changes to stratified columnar epithelium near the oral cavity. Three distinct cell types, light, dark, and basal cells, occur throughout the excretory duct system including the main excretory duct of the rat (Shackleford and Schneyer, 1971).

The secretory endpieces in the parotid gland of the rat are composed of all serous acini. Shackleford and Wilborn (1968) proposed the term serous cell to denote those cells which lack mucosubstances in the secretory granules. Neutral glycoproteins are present in the secretion granules of the parotid gland. Acinar cells are arranged about an irregular, centrally placed lumen. The cells are pyramidal in shape, and microvilli may be present on the luminal surface. A few microvilli are present along the basal plasmalemma. Serous cells are characterized by the presence of abundant granular endoplasmic reticulum in the basal cytoplasm (Parks, 1961). The Golgi complex, which may show variability in location and orientation, consists of stacks of saccules, numerous peripheral vesicles, and condensing vacuoles (Hand, 1971). The morphology of the mature secretory granules is highly variable from species to species but in the rat is generally uniformly electron-opaque. The functional significance of the substructure of granules is not understood. It may reflect enzymatic sequestration and segregation with the secretory granules (Tandler, 1972). Discharge of the

secretory granules is accomplished by fusion of the granular membrane with the luminal plasmalemma followed by extrusion of the granule content (Amsterdam et al., 1969).

Another cell type found within the secretory endpieces is the non-secretory myoepithelial cell. In most species, these cells are alkaline phosphatase- and ATPase-positive (Shear, 1966). Myoepithelial cells are stellate in shape, and their cell body is located near the junction of the secretory endpiece and intercalated duct. These cells with their long tapering processes are situated between the basal lamina and the acinar cells (Tandler, 1965). Numerous similarities between myoepithelial cells and smooth muscle cells are evident, and myoepithelial cells are generally considered to be contractile. While myoepithelial cell contraction undoubtedly causes a transitory increase in the rate of delivery of saliva to the oral cavity, the primary function of these cells appears to be that of prevention of distension of the endpieces during secretion. This is accomplished by providing support for the endpieces and by widening and shortening the intercalated ducts so that outflow resistance is lowered (Young and van Lennep, 1977).

Salivary glands arise from the oral epithelium as small buds or cords of cells that grow downward from the epithelium. The anlage of the parotid gland of the rat appears in the buccal wall near the angle of the mouth on the fourteenth day <u>in utero</u>. This is one day later than the beginning of the primordium of the submandibular gland. By the fifteenth day, the parotid anlage proliferates and forms a narrow cord, the future main excretory duct, terminating in a cluster

of cells. By the sixteenth day in utero, the terminal cluster migrates to the area of the developing mandibular ramus where it ramifies into several branches, each with its own terminal cluster. The epithelial cells are surrounded by a capsule of mesenchyme at the time of initial branching. Terminal clusters proliferate and remold to form a number of terminal clusters which are the primitive endpieces and probably also future intercalated ducts. Luminization of the main excretory duct commences on day 17 in utero just proximal to the body of the gland, but the ductal system is not patent until the twentieth day in utero. At birth, the parotid gland consists largely of small patent ducts and solid terminal clusters. No PAS positive material is present in parenchymal cells prior to birth. Secretions of the parotid gland cannot enter the oral cavity until about two days after birth. Both the submandibular and sublingual glands are at a more advanced developmental stage than the parotid gland at birth (Redman and Sreebny, 1970a).

During the first 24 hours after birth, amylase activity begins to increase above that of other tissues, and a few secretory granules appear in a number of the terminal cluster cells. PAS positive material appears in the terminal clusters and ductal lumina, indicating initiation of differentiation of secretory endpieces and secretory activity. Rapid growth and development of the gland occurs between one and 12 days after birth. Gland weight, amylase, and nucleic acid content increase during this period. There is also a progressive increase in the number and size of secretory granules, rough endoplasmic reticulum, and Golgi apparatus. Cell size remains

essentially constant between 3 and 18 days of age but enlarges markedly between 18 and 25 days (Redman and Sreebny, 1971). Numerous mitotic figures are present through the 25th day, but then mitotic activity diminishes to nearly zero with increasing age. Cellular proliferation is a characteristic feature of the early stages of parotid gland development, but both hyperplasia and hypertrophy occur between days 18 and 25. Thereafter, increase in cell size and number continue but at a greatly reduced rate. At 25 days, the parotid gland resembles that of the adult in all respects except size and weight (Schneyer and Hall, 1969; Redman and Sreebny, 1970b, 1971).

The secretory units or salivons of salivary glands consist of both ducts and secretory endpieces. Until recently the main secretory function was attributed to acinar cells, and the ducts were considered to be essentially conduits for conduction of the secretory products to the oral cavity. In recent years a secretory function for the ducts, involving sodium reabsorption and potassium secretion by active processes, has been elucidated. The perfused duct of the submandibular gland of the rat has served as the principal structure used for identification of ductal secretory functions (L. H. Schneyer, 1969, 1970, 1974, 1976, 1977). Secretory endpieces elaborate a precursor secretion which contains virtually all the proteins and water which are ultimately secreted into the oral cavity. The osmotically important ions, sodium (Na^+), potassium (K^+), chloride (Cl^-), and bicarbonate (HCO_3^-), are present in the precursor fluid

in essentially the same concentrations as that of plasma; hence, the fluid within the secretory endpieces and intercalated ducts is isotonic. Sodium and chloride are reabsorbed within the striated and excretory ducts. At the same time, potassium and bicarbonate are secreted into the lumina of ducts but this occurs to a lesser extent than does the reabsorption of sodium and chloride. Movement of sodium and potassium is accomplished by active transport while that of chloride is by passive processes. The ducts are essentially impermeable to movements of water across their walls. As a result, the final saliva contains concentrations of potassium which are usually much lower than those of serum. Saliva is generally of low osmolality and hypotonic with respect to plasma at low flow rates but at least for some glands, approaches isotonicity at high flow rates (Schneyer and Schneyer, 1967; Schneyer et al., 1972).

In many animals, both sympathetic and parasympathetic nerve fibers can be found innervating the three major salivary glands. Preganglionic sympathetic fibers arise in the intermedio-lateral cell column of the first and second thoracic segments of the spinal cord and ascend in the sympathetic trunk to synapse in the superior cervical ganglia. Postganglionic sympathetic fibers are distributed to the salivary glands along with the arteries which supply the glands. The nerve cell bodies of origin for the preganglionic parasympathetic fibers are located in the superior and inferior salivatory nuclei located at caudal pons and rostral medulla levels, respectively (Wang, 1964). Axons destined for the parotid gland arise in the inferior salivatory nucleus and accompany the glosso-

pharyngeal nerve to synapse in the otic ganglion. From this ganglion, postganglionic fibers pass to the auriculotemporal nerve and from there to the parotid gland. Some fibers also reach the parotid gland via the facial and chorda tympani nerves. Preganglionic parasympathetic fibers to the submandibular and sublingual glands arise in the superior salivatory nuclei, leave the cranium in the facial nerve and run with the chorda tympani nerve which joins the lingual nerve to synapse in the submandibular ganglion located within or near the submandibular and sublingual glands (Crosby et al., 1962; Wang, 1964; Hollinshead, 1967; Hellekant and Hagstrom, 1974). Hellekant and Kasahara (1973) have shown that some fibers which evoke secretion also run in the lingual branch of the trigeminal nerve and do not come entirely from the chorda tympani nerve.

In most salivary glands, it is the secretory endpieces which are most densely innervated with cholinergic fibers and also with adrenergic fibers when these are present. Neuroeffector junctions consist of short, varicose segments of the nerve terminal, bare of Schwann cell covering, at which there is an accumulation of cytoplasmic vesicles containing a neurotransmitter substance. Nerve terminals are naked axons that have left unmyelinated nerves in the connective tissue to penetrate the basal lamina of the acinar cells and terminate at the base of secretory cells, in the intercellular spaces between secretory cells, or between secretory cells and myoepithelial cell processes. The pattern of glandular innervation is variable among species, among glands within a single species, and at different stages of development within a single gland (Young and

van Lennep, 1977, and book in press). Endpieces in all glands are innervated by cholinergic fibers, but in some species endpieces lack appreciable adrenergic supply. In some glands the nerve fibers are of the hypolemmal type (i.e., they penetrate the basal lamina and lie within 20 nm of the acinar cell membrane) while in others they are of the epilemmal type (i.e., they lie outside the basal lamina) (Young and van Lennep, 1977).

Both adrenergic and cholinergic terminals have been identified in the rat parotid gland. No membrane specializations occur at the contact area, but a subsurface cistern of endoplasmic reticulum generally parallels the cell membrane at the contact area. Both adrenergic and cholinergic nerve terminals have been observed to make close contact with the same cell (Hand, 1972a). The parotid gland is composed of a single type of secretory cell, so cholinergic and adrenergic nerves must influence the same cells in different secretory ways (Garrett, 1974).

Secretory activity of salivary glands is generally elicited by stimulation of the neural pathways to these organs. While stimulation of either sympathetic or parasympathetic nerves to the glands induces secretion, it is the parasympathetic innervation that is responsible for elaboration of copious quantities of a watery fluid. Secretion elaborated by stimulation of the sympathetic innervation, on the other hand, is viscous and sparse, and until recently it was not considered to be physiologically important (Schneyer and Schneyer, 1967; Schneyer and Hall, 1967; Schneyer et al., 1972; Emmelin et al., 1973; Schneyer and Emmelin, 1974). However, it has now been shown

that the sympathetic innervation exerts a significant role in regulation of secretion of intrinsic proteins such as amylase. Saliva evoked by parasympathetic stimulation of the parotid gland is low in amylase, but sympathetic saliva is rich in amylase (Schneyer et al., 1972; C. A. Schneyer, 1974b). The fact that sympathetic secretomotor nerves are not ubiquitous, however, also indicates that this branch is generally of less importance (Garrett, 1974; Young and van Lennep, 1977, and book in press).

Although salivary secretion is usually induced only when the autonomic innervation is stimulated, in some species, such as sheep and cattle, a spontaneous isotonic saliva is produced by the parotid gland (Coats and Wright, 1957; Kay, 1960). The sublingual glands of the cat and dog and the submandibular gland of the rabbit also secrete spontaneously (Schneyer and Schneyer, 1967; Schneyer and Emmelin, 1974). In addition, a new group of polypeptide substances, including the Leeman factor, physalaemin (Schneyer and Hall, 1968a), and eledoisin, have also been found to evoke secretion. Peripheral neural factors are not involved in this secretion since blockade of receptor sites in the salivary gland with drugs has no effect on the secretory influences of these agents (Schneyer et al., 1972).

The autonomic innervation to the glands not only induces secretomotor activity but also regulates vasomotor activity (Emmelin, 1964) and maintains the structural and functional status of the glands of adult and immature animals (Schneyer and Hall, 1966b, 1967a; Schachter, 1967; Schneyer, 1972; Schneyer and Emmelin, 1974). Growth and differentiation of glands is also influenced by the

autonomic innervation in immature animals (Schneyer, 1972). The link between secretory activity and the effect on structure is virtually inseparable. Augmentation or diminution of autonomic activity to the glands results in an increase or decrease in size, growth changes, and functional activity (Schneyer and Hall, 1964, 1967a; Wells, 1967). Furthermore, not only the level but the kind (parasympathetic or sympathetic) and the duration of neural stimulation modifies these aspects (Schneyer and Hall, 1966b; Schneyer, 1972).

Although both autonomic branches are required to maintain normal gland structure and function, it is the parasympathetic innervation that is the principal regulator of structure and function of the parotid gland in the adult and immature rat (Schneyer and Hall, 1967a, b, 1970). In adult rats, parasympathetic denervation results in a 30-35% reduction in weight of the parotid gland, a 35-45% decrease in the size of acinar cells, and a 40-55% decrease in concentration and total amount of amylase in the gland (Schneyer and Hall, 1964, 1967a). Total DNA is reduced about 20% two weeks following parasympathectomy, whereas total RNA is decreased approximately 50% (Schneyer, 1973b). Cell number is permanently reduced following parasympathectomy. Although the gland is composed of numerous secretory units or salivons, it is primarily the secretory endpieces which are modified morphologically by parasympathectomy. Striations seen in striated ducts are also less evident (Schneyer and Hall, 1967a). Resection of the auriculotemporal nerve in immature animals does not eliminate the usually observed changes in postnatal growth, but the magnitude of these growth changes is reduced. Total cell

number, acinar cell size, gland size, total RNA, and amylase concentration are 16-50% less than in the normally innervated contralateral control gland (Schneyer and Hall, 1967b, 1970) at all ages during postnatal development. Glandular activity mediated by the parasympathetic innervation is thus partly responsible for regulation of cell number and size in developing parotid gland (Schneyer and Hall, 1970). Sodium concentration in saliva is increased following parasympathectomy, and potassium is lowered in immature rats (Schneyer and Hall, 1967b).

The sympathetic innervation appears to have little influence in regulation of gland size, mitotic activity (C. A. Schneyer, 1974a), or postnatal growth changes (Schneyer and Hall, 1970). Extirpation of superior cervical ganglia results in only small changes in gland weight, cell size, cell number, or amylase (Schneyer and Hall, 1966b). One to four days following sympathectomy parotid gland size is increased, partially due to increased mitotic activity and total DNA. Two weeks following sympathectomy total DNA has returned to normal levels, and gland weight, cell size, and total RNA are reduced only 10-14% (Schneyer, 1973b). In developing parotid glands sympathectomized at 8 days of age, no changes in gland weight, cell size, or total RNA are seen prior to 32 days of age, and these changes are only slight. Cell number and mitotic index are not altered by sympathectomy at any age (Schneyer and Hall, 1970). Sodium concentration in saliva is unchanged, and potassium concentration is slightly elevated in immature animals following sympathectomy (Schneyer and Hall, 1967b). In adult rats, superior cervical ganglionectomy

produces striking morphological changes in acinar cells, but other portions of the secretory units are apparently unaffected. Some acinar cells are smaller than normal, others appear larger than normal, and approximately 20% are the same size as in control glands. Secretory granules contain material of uneven density. Changes seen following sympathectomy may be due to impairment of the synthetic portion of the secretory process or to prolonged storage (Wilborn and Schneyer, 1972).

In addition to chemical or surgical denervation, the autonomically mediated activity to the salivary glands can be reduced by substitution of the normal chow diet with an all-liquid diet (Hall and Schneyer, 1964). The innervation to the glands remains intact, but reflexly mediated activity of the glands is markedly reduced. Liquid diet thus provides a more physiological means of reducing autonomically mediated glandular activity (Schneyer and Hall, 1966b; C. A. Schneyer, 1974a). There is a reduction in the masticatory activity of the animal, and subsequently parotid gland size is reduced by 39-52%. Acinar cells of the parotid gland are decreased to approximately half the size of acinar cells in control glands, and this reduction in acinar cell size is responsible for the decreased gland weight. Ducts are not altered. Reduction in gland size occurs rapidly and reaches a maximum within four days of initiation of the all-liquid diet (Hall and Schneyer, 1964). Amylase concentration of the gland is reduced 70%, while concentration in saliva is decreased 40%. Total amylase is also reduced by liquid diet (C. A. Schneyer, 1974b). Accompanying these changes in gland and acinar cell size is

an approximate 50% decrease in total RNA in the parotid gland two weeks following initiation of an all-liquid diet, but total DNA is unchanged (C. A. Schneyer, 1970, 1973b). No appreciable change in the concentration of sodium in saliva occurs, but potassium concentration is elevated in adult rats (Schneyer and Hall, 1966b).

Liquid diet results in major alterations of acinar cells, but other parenchymal components are not affected. Ultrastructural modifications in acinar cells include cellular atrophy, accumulation of lipid droplets in the basal portion of the cells, reduction in quantity of secretory granules, smaller intercellular canaliculi, and less extensive Golgi apparatus. The secretory process is apparently impaired by liquid diet, and the gland is physiologically less active (Wilborn and Schneyer, 1970).

Within 48 hours after the reintroduction of solid food there is a sharp burst of mitotic activity (from 0.0 in controls to 22.3 mitoses per 1000 acinar cells in adult animals) and a return to normal cell size within 7 days (C. A. Schneyer, 1970). The induction of mitotic activity indicates an increase in glandular activity. This provides additional support for the view that dietary consistency, and hence masticatory activity, alters the level of autonomically mediated reflex stimulation of the gland. The mitotic burst which occurs following reintroduction of chow diet is neurally mediated since it can be greatly reduced by prior introduction of either atropine or propranolol (C. A. Schneyer, 1970). Both autonomic branches are involved in regulation of mitosis and cell size, but the primary effect of the liquid diet is mediated through the

parasympathetic innervation to the glands (Hall and Schneyer, 1973; C. A. Schneyer, 1974a; Schneyer and Hall, 1975).

If immature animals are maintained on a liquid diet past weaning, parotid gland weight, acinar cell size, and total RNA continue to increase, but the extent of these increases is considerably less than in chow fed controls. Thus, there is not a cessation of growth, but glands in animals on liquid diet are smaller and contain fewer mitotic figures, smaller acinar cells, and less total DNA and RNA. Growth of the parotid gland is inhibited by liquid diet due to diminished physiological activity of the gland. While an intact innervation is not the prerequisite for normal glandular growth, it appears that physiological activity mediated through the autonomic innervation is required in order to regulate growth of the developing gland (Hall and Schneyer, 1969; Schneyer, 1972).

Enhancement of glandular activity and consequently gland size can be accomplished by excessively stimulating neural pathways by dietary means, by administration of specific autonomic agents, or by use of specific surgical manipulations that result in increased autonomic activity. Chronic incisor amputation (Wells and Munson, 1960; Wells, 1967), feeding of raw pancreas (Ershoff and Levin, 1962), papain or bulk diet (Wells, 1967; Wells and Peronace, 1967; Schneyer and Hall, 1976) result in enhanced neural stimulation to the glands. When isoproterenol, an agonist that acts primarily at beta-adrenergic receptors, is administered in pharmacological doses over a period of 5-7 days, it causes a 3-5 fold increase in the size

of the mitotically stable parotid gland of adult rats and also an accompanying increase in the size of the acinar cells (Brown-Grant, 1961; Selye et al., 1961; Schneyer, 1962). This effect can be prevented by administering beta-adrenergic antagonists such as propranolol (Barka, 1965a; Pohto, 1968). The parotid gland undergoes the greatest degree of enlargement of all major salivary glands receiving chronic isoproterenol treatment (Schneyer, 1962). Reversal of gland enlargement can be achieved by cessation of isoproterenol treatment. Marked reduction in cell size occurs by 14 days, and reversal is complete after 50 days (Schneyer, 1962; Schneyer et al., 1967).

Mitotic index increases sharply 2-3 days after initiation of isoproterenol injections but drops shortly thereafter. This suggests that cellular proliferation plays a role in increasing gland size during the early interval after isoproterenol initiation but is probably not involved in gland enlargement after chronic isoproterenol treatment. Acinar cell hypertrophy contributes to gland enlargement during the entire period that isoproterenol is administered (Schneyer et al., 1967). A burst of DNA synthesis occurs within 18 hours after initiation of the isoproterenol regimen further indicating that hyperplasia is an early component of gland change (Barka, 1965b; C. A. Schneyer, 1969). The increase in total DNA may be only partially a reflection of increase in cell number since nuclear size is increased and extensive polyploidy has been recorded in parotid (Schneyer et al., 1967; C. A. Schneyer, 1969) as well as submandibular glands (Radley, 1967) treated chronically with this amine.

Synthesis of RNA and protein is also enhanced by administration of isoproterenol in both submandibular and parotid glands. Elevated RNA synthesis precedes the stimulation of DNA synthesis (Barka, 1966, 1968, 1970b; Ekfors and Barka, 1971; Lillie and Han, 1973).

A single injection of isoproterenol acts as a potent sialagogue and essentially depletes the parotid and submandibular glands of their zymogen granules and amylase (Schneyer, 1962; Radley, 1969; Lillie and Han, 1973; Cope and Williams, 1973) and reduces gland content of secretory enzymes to less than 5% of the original amount (Byrt, 1966; Pohto, 1966; Sreebny et al., 1971; Lillie and Han, 1973; Robinovitch, 1973). With chronic isoproterenol treatment the sialagogue effect is less for the enlarged glands, and the reduction of gland amylase and secretory granules is less (Byrt, 1966). In fact, some cells appear unaffected and remain engorged with granules (Radley, 1969).

In spite of the several-fold increases in gland weight and abundance of secretory granules within acinar cells, the effect of chronic isoproterenol administration on the content of secretory proteins in the parotid gland is less dramatic. Schneyer (1962) reported that a five-fold increase in the weight of the parotid gland was accompanied by only a 40% increase in total gland amylase. Barka (1970a) showed a five-fold increase in gland weight but only a 70% increase in total gland activity of ribonuclease, another secretory protein of the rat parotid gland. Recently, Robinovitch et al. (1977) reported that parotid gland weight was increased four-fold, RNA five-fold, and DNA and protein three-fold. Although acinar

cells were filled with granules, total gland amylase was not changed. The amylase to protein ratio was reduced from 35% in control animals to only 16% in saliva from animals chronically treated with isoproterenol. The secretory enzymes, amylase, ribonuclease, and deoxyribonuclease, were reduced, and new components appeared in several chromatographic fractions. It appears that all the new secretory components may be proline-rich (Robinovitch et al., 1977). Other investigators have also reported the appearance of new secretory proteins and glycoproteins in parotid saliva (Fernandez-Sorenson and Carlson, 1974), in whole saliva (Menaker, Sheetz, Cobb, and Navia, 1974; Sheetz, 1976), and in a secretory granule fraction isolated from submandibular glands (Menaker, Cobb and Taylor, 1974) of rats chronically treated with isoproterenol.

Maturation and differentiation of the incompletely developed parotid gland of the very young rat pup can be accelerated when isoproterenol is administered over a 7 day period during early postnatal development (Schneyer and Shackleford, 1963; C. A. Schneyer, 1973a, 1977). The effects of the agent depend on the age of the animal at the time isoproterenol administration is initiated. If isoproterenol is administered prior to weaning when mitotic index is high, mitotic activity is reduced and total DNA of the gland decreases, but parotid gland weight, acinar cell size, and total RNA increase (C. A. Schneyer, 1973a, 1977). Definitive acini form earlier, and striated ducts become more abundant (Schneyer and Shackleford, 1963). Multiple injections of isoproterenol in the preweanling rat alter the parotid acinar cell cycle and the labelling

and mitotic indices. Total cell cycle time is unaltered so these changes represent a manipulation of events within the normal cycle. The G_1 phase is lengthened, and the S phase is shortened. Mitotic index is decreased, whereas the labelling index is increased by isoproterenol treatment. Isoproterenol may stimulate cells from the G_2 period to enter into cell division, and it may be these G_2 cells which produce the compensatory hypertrophy induced by isoproterenol. Non-cycling G_0 cells are also present in the neonatal parotid gland, but their role in the normal differentiation process is obscure (Klein et al., 1976). If, however, isoproterenol treatment is begun after weaning when cellular proliferation has diminished, the response of the gland is similar to that which occurs in adult rats. Acinar cells enlarge to the same maximum as do adults, total DNA and RNA in the gland increase, and mitotic index increases markedly (C. A. Schneyer, 1973a, 1977).

Removal of the superior cervical ganglia from adult rats does not alter the response of the submandibular gland to isoproterenol and suggests that an intact neural pathway is not essential to induce the changes caused by isoproterenol (Barka, 1967). Likewise, neonatal sympathectomy causes no apparent effect on the development of the submandibular gland. However, after isoproterenol treatment these sympathectomized glands show greater gland weight, total DNA and RNA, and protein than corresponding neurally intact glands in the same animal (Gresik and Barka, 1977).

When the bulk of the diet of the animal is increased by mixing inert cellulose with the normal chow diet (50:50), the masticatory

activity of the animal is increased, resulting in enhancement of neurally mediated glandular activity. Gland size, acinar cell size, and total RNA are increased with bulk diet, and these increases are mediated chiefly through the parasympathetic innervation to the glands (Wells, 1967; Wells and Peronace, 1967; Schneyer and Hall, 1976). Cell number is also increased, since DNA levels are approximately 17% higher in parotid glands of bulk-fed rats than in chow-fed rats. Denervation studies indicate that the parasympathetic innervation plays the major role in mediating the increased levels of DNA (Wells, 1967; Schneyer and Hall, 1976). Within two days after initiation of the bulk diet, a marked decrease in the mitotic index is observed (from 0.04 ± 0.03 in controls to 12 ± 2 mitotic figures per 1000 acinar cells in experimental animals) (C. A. Schneyer, 1974b). Within four days, mitotic levels drop to nearly zero in the animals fed bulk diet. Surgical denervation of the parotid gland prior to introduction of the bulk diet modifies the burst of mitotic activity observed with bulk diet alone. Mitotic index in sympathectomized parotid glands of animals on bulk diet is 67% less than in innervated glands of rats fed bulk diet and 80% less than in parasympathectomized, bulk diet-fed animals (Schneyer and Hall, 1976).

Another means of inducing an increase in salivary gland size is by removal of some of the gland pairs. This partial sialoadenectomy appears to cause an increase in size of the remaining glands, because the functional work load on them is increased. Work by Bizzozera in 1903 (according to a discussion by Alho, 1961) showed that unilateral extirpation of the submandibular or parotid gland of rabbits results

in mitotic proliferation but no weight gain in the contralateral gland. Using rats and mice from which one submandibular gland was removed, Alho reported increased mitotic activity in acinar cells of the contralateral gland; gland weight and nucleic acids were inconstantly modified. In studies on dental caries resulting from extirpation of various combinations of salivary glands, Schwartz and Shaw (1955) reported that compensatory enlargement occurs in the remaining gland pairs. Removal of one or two gland pairs, such as the parotid gland, caused an increase in weight of the remaining glands (submandibular and sublingual). Extirpation of only the sublingual gland caused an enlargement in the parotid gland but no change in the submandibular gland. Until recently the mechanism for this enlargement was unknown, and some authors attributed it to humoral factors since ablation of a single member of a gland pair led to compensatory enlargement not only of the other member of the pair but also to the remaining unrelated salivary glands (Alho, 1961).

Recent work by Hall and Schneyer (1977, 1978) on adult rat showed that the compensatory response induced by ablation of the submandibular and sublingual glands four weeks earlier involved an increase in acinar cell size, gland size, mitotic activity, and nucleic acid content. The neural mediation of this compensatory response of the parotid gland of adult rat following ablation of the submandibular and sublingual glands was demonstrated using liquid diet and bulk diet. Gland enlargement is abolished when liquid diet is used, thus showing that humoral influences do not mediate the compensatory response. Bulk diet combined with gland ablation more than

doubled the increase in acinar cell size induced by partial desalivation alone (36% increase) or bulk diet alone (36% increase). A similar pattern is also observed for gland size and cell number (Hall and Schneyer, 1977). Both autonomic pathways to the parotid gland must be intact in order for the maximum compensatory enlargement to occur. In neurally intact animals, parotid gland size increases 34%, acinar cells enlarge 17%, total DNA increases about 18%, and total RNA increases 38% following partial desalivation of adult rats. Sympathectomy and gland ablation together result in a 6% increase in gland size, a 9% decrease in cell size, and an 18% increase in cell number as measured by total DNA, and a 35% increase in RNA. The increase in RNA is due to the increase in cell number since cell size is unchanged. Parasympathectomy combined with ablation of the submandibular-sublingual complex prevents the occurrence of compensatory changes. The same reduction in parotid gland weight (40%), acinar cell size (20%), total DNA (16%), and total RNA (44%) occurs following parasympathectomy and gland ablation as occurs following parasympathectomy alone. An apparent synergism between the parasympathetic and sympathetic innervation exists and seems to mediate compensatory enlargement, but the parasympathetic division appears to be the principal regulator of gland size and cell number (Hall and Schneyer, 1978).

Two distinct growth phases (preweanling and postweanling) exist in the developing rat parotid gland between birth and approximately six weeks of age (Schneyer and Schneyer, 1961; Schneyer and Hall, 1969). At birth the parenchyma of the parotid gland is rudimentary

with respect to acini, cells are small and undifferentiated, and cellular proliferation is marked. Prior to five days of age, developing acini are difficult to distinguish from developing intercalated ducts (Schneyer and Schneyer, 1961; Schneyer and Hall, 1969, 1970; Redman and Sreebny, 1971). Only small quantities of parotid gland are present before the tenth day, but gland size greatly increases between days 12 and 15 (Epstein et al., 1970). Definitive acini are not evident prior to day 15, and a copious amount of saliva is not secreted before this time (Schneyer and Schneyer, 1967). Between the sixteenth and twenty-fifth day, cellular proliferation and some cellular hypertrophy occur. With weaning, mitotic activity falls precipitously, and cell size increases markedly (Schneyer and Hall, 1969). The preweanling phase of development is relatively insensitive to neural factors, whereas the postweanling phase is acutely sensitive to neural regulation (Hall and Schneyer, 1964, 1969; Schneyer and Hall, 1970).

Because the incompletely differentiated parotid gland exhibits predictable phases of growth and predictable responses to neural influences during these phases, it is an ideal model for studying cellular mechanisms in compensatory responses. For example, the immature salivary gland has a phase where marked proliferative activity is a part of the growth changes; the adult gland, conversely, displays a virtually quiescent mitotic status (Alho, 1961; Winick and Noble, 1965; Schneyer and Hall, 1969; Schneyer, 1972). Immature glands exhibit pronounced changes in cell size and differentiation during the postweanling phase of development. Again, the adult

gland displays little change in cell size under normal physiological conditions (Schneyer, 1972). Thus, compensatory hypertrophy in the developing gland may differ markedly from that of the adult and help to elucidate the factors contributory to compensatory growth responses generally.

Proposal

Compensatory enlargement of various adult organs is a frequent subject of investigation (Tomanek, 1975). Although some work has been done on compensatory enlargement of salivary glands of adult animals, there are no reported studies on compensatory changes in salivary glands of immature animals. Thus, prior to this work, it has not been established whether compensatory enlargement occurs in developing salivary glands. Because two growth phases exist in the developing gland, the influence of partial desalivation on these two phases of development was first investigated. One, two, and three week intervals following submandibular-sublingual extirpation were used to ascertain whether there is an optimal interval required to elicit compensatory changes in the parotid gland or whether gradually increasing compensatory responses continue indefinitely with no finite plateau. Changes in parotid gland weight, acinar cell size, and mitotic index were assessed. Analysis of amylase activity provided evidence for functional developmental status of parotid glands and allowed for correlation of morphological changes with these. Some glands were stimulated with pilocarpine to assess other

functional modalities, such as sodium and potassium, associated with secretory activity.

An important point investigated was the determination of whether glandular enlargement occurs during the period of high mitotic activity, and, if so, whether this enlargement is the consequence of further increases in mitotic index or of premature increases in cell size, or to both processes.

The autonomic nervous system regulates normal postnatal growth, differentiation, secretion, and structure of salivary glands. Neural influences are minimal prior to weaning but considerable after weaning (Hall and Schneyer, 1964, 1969; C. A. Schneyer, 1970, 1972; Schneyer and Hall, 1970). Therefore, the influence of such factors in mediating compensatory responses in incompletely developed parotid glands was investigated. The role of the autonomic innervation in the regulation of the compensatory response imposed by partial desalivation was delineated by parasympathetic and/or sympathetic postganglionic denervation of intact and partially desalivated rats.

MATERIALS AND METHODS

Experimental Animals

Long-Evans rats (Rattus norvegicus) of both sexes varying in age from 8-42 days were used in these experiments. The animals were kept with their mothers until 22 days of age after which time they were maintained on standard lab chow and water ad libitum. Alternating dark and light cycles of 12 hours duration each were electronically regulated.

To induce compensatory enlargement of the parotid gland, the submandibular and sublingual glands were removed bilaterally under light ether anesthesia from animals which were 8, 10, 15, 17, 21, or 22 days of age. From some of the animals the superior cervical ganglia or segments of the auriculotemporal nerves were also removed bilaterally. Unoperated or sham operated animals which were neurally intact served as controls. Additionally, animals in which the parotid gland was sympathectomized or parasympathectomized only were used for controls.

One, two, or three weeks following surgery the parotid glands were removed or stimulated and examined for structural and functional changes. Preweanling rats were removed from their mother 6 to 8 hours prior to gland removal; postweanling animals were

fasted 12 to 14 hours. The animals were anesthetized by intraperitoneal injection of Nembutal at a dose of 50 milligrams per kilogram of body weight.

Determination of Amylase Activity

Parotid glands were rapidly removed from the anesthetized animal and weighed on a torsion balance to determine total gland weight. When gland weight exceeded 100 milligrams, the gland was divided until approximately 100 milligrams were obtained. This sample was then placed in a small container to which a few drops of 0.9% saline were added to maintain amylase activity, frozen, and stored at -15°C until amylase activity could be determined.

Amylase activities were assayed by the method of Myers et al. (1944), modified for a micromethod by Marsters et al. (1960). Frozen parotid glands were ground in a mortar to which a few grains of sand and a few milliliters of phosphate-buffered saline (0.05 M pH 7) were added. Then the ground gland mixture was diluted to 10 milliliters with phosphate-buffered saline and centrifuged at 5°C for 20 minutes to separate the insoluble fraction. Fifty microliters of the supernatant were diluted to 5 milliliters with phosphate-buffered saline, and then was further diluted with the same buffer to yield Klett readings ranging from 100 to 300 units. One half milliliter of the final gland dilution was incubated at 37°C in 2 milliliters of 4 milligrams per milliliter of soluble starch in phosphate-buffered saline for exactly 15 minutes. Dry picric acid in sufficient quantity to saturate the reaction mixture

was used to stop the reaction, and the precipitate was filtered. Next, 1 milliliter of 15% sodium carbonate was added to 1 milliliter of reaction mixture filtrate in a Klett tube, and the tube was placed in a boiling water bath for 20 minutes. The solution was then diluted with distilled water to 10 milliliters, mixed well, and read on a Klett photoelectric colorimeter with a No. 54 green filter having a transmittance of 520 to 580 mμ. A picric acid blank was used to set the instrument at zero. Amylase activity was expressed as milligrams of reducing substance (glucose) per milligram of gland wet weight. Values were calculated from a standard curve which related Klett units to glucose concentration. The slope of this standard curve was equivalent to 0.002822 milligram of glucose per Klett unit with a standard deviation of ± 0.0002 mg/Klett unit. Thus the value of 0.0028 milligram of glucose per Klett unit was used to calculate all amylase activities.

Histological Preparations

Parotid glands were quickly dissected free with a minimum of handling and placed in either 10% neutral buffered formalin or Bouin's fixative (Humason, 1967). Following dehydration in graded alcohol series and clearing, the glands were embedded in paraffin. The glands were sectioned at a thickness of 6 micrometers and stained with hematoxylin and eosin (Preece, 1972), periodic acid Schiff (PAS) reagent (Humason, 1967), or periodic acid Schiff reagent following prior digestion with diastase (Preece, 1972).

Acinar cell size was estimated from the sections stained with hematoxylin and eosin by counting the number of nuclei within a calibrated area containing acinar cells only. Because nuclear size remained constant, cell size is inversely related to the number of nuclei counted (Schneyer and Hall, 1969). A total of 10-50 calibrated areas was counted. The number of mitotic figures observed in the 50 calibrated areas was recorded, and mitotic index was expressed as the number of mitotic figures per 1000 acinar cells.

Other glands were finely minced and fixed in cold (0-4°C) 4% glutaraldehyde in Clark's buffer (Clark, 1963). After 4 hours in the fixative the tissue was washed in Clark's buffer (pH 7.4) until the next day. The tissue was postfixed for 2 hours in 2% osmium tetroxide (OsO_4) buffered with Clark's buffer. Tissues were dehydrated in a graded series of ethanol and propylene oxide and embedded in Dow epoxy resin (DER) 334 (Winborn, 1965). Thin sections (600-800 Å) were cut with glass knives on a Sorvall MT-2b ultramicrotome, mounted on uncoated, 300 mesh copper grids, stained first with uranyl acetate (Watson, 1958) and then with lead citrate (Venable and Coggeshall, 1965), and examined with a Phillips EM 200 electron microscope at 60 Kv. For correlative light microscopy, one micrometer thick sections of the DER embedded tissue were placed on glass slides and stained with 1% toluidine blue (Richardson et al., 1960) or double stained with 1% toluidine blue followed by 0.1% basic fuchsin (Sheetz, 1976). Light microscopic sections were examined and photographed with a Leitz Orthoplan Photomicroscope.

Determination of Sodium and Potassium Ion Concentration

After removal of one parotid gland for histology or amylase determination, the remaining parotid gland was stimulated to secrete by intraperitoneal injection of the parasympathomimetic, pilocarpine hydrochloride, at a dose of 5 milligrams per kilogram of body weight. The main parotid duct was dissected free from investing connective tissue and cut. Three microliters of parotid saliva were collected directly from the cut end of the duct with a micropipette (Schneyer and Schneyer, 1961) and placed in 2 milliliters of lithium (15 milliequivalents per liter). Values for sodium and potassium were determined by flame photometer (Instrumentation Laboratory, Inc., model 143) and expressed as milliequivalents per liter.

The time required to collect the saliva was also recorded. Salivary flow rate was computed and expressed as microliters per minute per gram of wet tissue.

Treatment of Data

Calculated values for each parameter for each rat were averaged, and standard deviation and standard error were computed. Using student's t test the difference between control and experimental means was considered significant when the probability that means differed from each other was less than 0.05 (Lacey, 1960).

RESULTS

Evidence of Compensatory Changes One Week following Gland Ablation

Preweanling Animals

Submandibular and sublingual glands were removed from preweanling animals when they were 8 or 15 days of age. With few exceptions, pups partially desalivated at 8-12 days of age failed to survive. As indicated by the data in Table I, only three animals of a total of 17 rats partially desalivated (SMSLX) at 8 days of age survived to 16 days. Tissues were removed from these animals only for assessment of gland weight and histological characteristics. Cell size and mitotic index were unchanged in these three animals.

The photomicrograph presented in Figure 1 is representative of one micrometer, plastic embedded sections from parotid glands of 8-day old control animals and demonstrates that in these glands the size of acinar cells is small. Abundant connective tissue cells are located in the relatively large amounts of interparenchymal space, and the gland is relatively undifferentiated. Figure 2 is representative of parotid glands from 16-day old control animals and shows a pattern similar to that observed in the 8-day old animals. Size of acinar cells is increased only very little from that of 8-day old rats. Sections from animals partially desalivated

Table I. Gland Weight, Acinar Cell Size, and Mitotic Index of Parotid Gland from Immature Rat One Week following Bilateral Extirpation of the Submandibular and Sublingual Glands

Condition	Age of Animals in Days†	Parotid Gland Wet wt (mg) / Body wt (g)	No. of Nuclei / Area	Mitotic Index
CONTROL	16	0.630 (1)	75.50±2.90 (2)	0±0 (2)
SMSLX	8→16		78.80 (1)	0 (1)
CONTROL	22	1.701±.04 (5)	51.98±2.17 (5)	1.43±1.050 (5)
SMSLX	15→22	1.826±.07 (6)	49.30±2.30 (5)	0.40±0.400 (5)
CONTROL	29-31	1.383±.04 (10)	49.21±1.11 (9)	1.20±0.638 (9)
SMSLX	22→29	1.804±.07 (6)*	41.56±1.55 (9)*	0.73±0.727 (9)

Values represent the mean ± the standard error of the mean. SMSLX indicates bilateral extirpation of submandibular and sublingual glands on all tables. *Indicates difference between control and SMSLX values is significant ($P < 0.05$, student's t-test). Numbers in parentheses indicate number of animals. †In Table I and all succeeding tables, the age of the experimental animals (given in column 2) at the time of gland extirpation or other surgery is given first, followed by an arrow that points to a second number which indicates age at the time of sacrifice.

one week earlier (Figure 3) are not recognizably different from control animals.

When the submandibular-sublingual complex was removed at 15 days of age, almost all of the animals survived. When parotid glands were examined one week after removal of the other major salivary glands for evidence of structural and functional alterations resulting from the gland ablation, gland weight, acinar cell size, and mitotic index were not statistically different ($P > 0.05$) from control values (Table I). Amylase concentration of parotid gland and total amylase of the gland did not differ from controls (Table II). Furthermore, no change in sodium or potassium concentration or flow rate of pilocarpine-evoked parotid saliva was found (Table III).

Histological sections of parotid glands from rats partially sialoadenectomized at 17 days of age and sacrificed at 24 days showed that the secretory granules within the secretory endpieces are enlarged, stain intensely with basic fuchsin (Figure 5), and have become electron lucent (Figure 7). Secretory endpieces in control glands contain a combination of the toluidine blue-staining (Figure 4), electron dense (Figure 6) secretory granules as well as the basic fuchsin-staining granules.

Postweanling Animals

Rats sialoadenectomized at weaning and sacrificed one week later showed statistically significant increases in several parameters when comparison was made with controls. Weight of the parotid gland, size of its acinar cells (Table I), and total amylase of the gland

Table II. Amylase Concentration and Total Amylase of Parotid Gland from Immature Rat One Week following Bilateral Extirpation of the Submandibular and Sublingual Glands

Condition	Age of Animals in Days	Amylase Concentration (mg RS/mg wet wt)	Total Gland Amylase (mg RS/gland)
CONTROL	22	179±33.70 (5)	11,135± 475 (5)
SMSLX	15→22	162±15.37 (6)	8,737± 993 (6)
CONTROL	29-31	291±25.05 (9)	25,995±2978 (9)
SMSLX	22→29	340±29.30 (5)	40,426±3234 (5)*

Values represent the mean ± the standard error of the mean.
*Indicates difference between control and SMSLX values is significant ($P < 0.05$, student's t-test). Numbers in parentheses indicate number of animals.

Table III. Sodium and Potassium Concentration and Flow Rate of Pilocarpine-Evoked Saliva from Denervated Rat Parotid Gland One Week following Bilateral Extirpation of the Submandibular and Sublingual Glands

Condition	Age of Animals in Days	Na^+ (mEq/l)	K^+ (mEq/l)	Flow Rate (µl/min/g)
CONTROL	22	17.80± 5.24 (5)	42.60±2.38 (5)	26.23±5.701 (5)
SMSLX	15→22	18.20± 3.33 (6)	46.30±3.14 (6)	20.54±2.779 (5)
CONTROL	29-31	96.00±10.33 (5)	25.46±3.41 (5)	30.98±7.880 (5)
SMSLX	22→29	102.00± 6.76 (5)	17.80±0.80 (5)	39.91±8.982 (5)

Values represent the mean ± the standard error of the mean. Numbers in parentheses indicate number of animals. Pilocarpine was administered intraperitoneally in a single dose of 5 mg/kg body weight.

(Table II) were all increased. Gland weight was increased 30% ($P < 0.01$) over that of controls, cell size was enlarged 18% ($P < 0.01$), and total gland amylase was 56% higher ($P < 0.01$) than that of controls. Mitotic index was not changed, nor were amylase concentration of the gland, sodium and potassium concentration of saliva, and flow rate of saliva (Tables I-III).

Evidence of Compensatory Changes Two Weeks following Gland Ablation

Preweanling Animals

Desalivation of animals in the 7-10 day old range was again attempted. Death previously occurred when glands were removed at this young age. The cause of this mortality was ascribed to the failure of the rats to suckle adequately, since absence of milk in the stomach was routinely observed. By coating the pup's mouth hourly with vaseline the rats were able to survive and gain weight at approximately the same rate as controls. However, body weight of these partially desalivated animals tended to be somewhat less than that of control animals. Vaseline was applied to the oral orifice of control and partially desalivated animals from 8:00 a.m. to 10 p.m. during the period between gland ablation and sacrifice.

When the submandibular and sublingual glands were extirpated at 7, 8, or 10 days of age and the parotid glands were examined at 20-24 days of age, there were no statistically significant changes in any of the parameters examined with the exception of amylase concentration in the parotid gland. Amylase concentration showed

a 55% decrease ($P < 0.01$) from controls in these rats partially desalivated at 7-8 days and sacrificed two weeks later (at 21-22 days of age). Because the other rats of similar age did not show this change in amylase concentration, the decreased concentration of amylase in the 21-22 day old rats is probably not truly indicative of amylase concentration in this age group.

Histological sections from these experimental animals reveal a change in the size and staining intensity of secretory granules within some secretory endpieces. In one micrometer sections stained with toluidine blue and basic fuchsin, control glands from weanling animals contain a variety of dense, blue staining granules and enlarged, red staining granules (Figure 8). In the parotid glands of the partially desalivated animals, more granules are found that are enlarged and stain with basic fuchsin than are found in control glands (Figure 9). Electron micrographs from 20-day old control animals reveal the presence of two types of secretory granules (Figure 10). One type is electron dense, and the other is more electron lucent. Both types may be present within the same cell. Additionally some of these electron dense granules contain a more electron dense rim. Granules of this last type predominate in secretory endpieces of parotid glands from partially desalivated animals (Figure 11). Electron lucent granules containing flocculent material are also present. Granules with reduced opacity stained with basic fuchsin.

Postweanling Animals

If the two week period following sialoadenectomy extended beyond weaning, increases in cell size, gland weight, and total gland amylase were observed. When the submandibular-sublingual complex was removed at 15 days of age, the weight of the parotid gland in the experimental animals after a two week period was 58% ($P < 0.01$) greater than that of controls, and acinar cell size was 23% ($P < 0.02$) larger (Table IV). (Cell size is inversely related to the number of nuclei per calibrated area; thus, fewer nuclei per area indicate increased cell size). Data in Table V indicate that total gland amylase of experimental glands was increased 64% ($P < 0.01$) over control values. Amylase concentration of the gland was unaltered. Sodium and potassium concentration and flow rate of pilocarpine-evoked saliva did not differ from control values during this period (Table VI). Increased acinar cell size and secretory granule enlargement were evident in histological sections of parotid glands from partially desalivated rats. In the control gland secretory granules stain intensely with toluidine blue (Figure 12), whereas those in the SMSLX gland were enlarged and stain only slightly with toluidine blue (Figure 13).

The most pronounced changes in the parotid gland were found when ablation of the submandibular and sublingual glands occurred at 22 days of age. Two weeks following gland removal (36 days of age), parotid glands of partially desalivated rats weighed 23% ($P < 0.01$) more than control glands, and parotid acinar cells were 32% ($P < 0.01$) larger (Table IV) than those of controls. Mitotic index did not differ statistically from control values, but there was a suggestion

Table IV. Gland Weight, Acinar Cell Size, and Mitotic Index of Parotid Gland from Immature Rat Two Weeks following Bilateral Extirpation of the Submandibular and Sublingual Glands

Condition	Age of Animals in Days	Parotid Gland Wet wt (mg) / Body wt (g)	No. of Nuclei / Area	Mitotic Index
CONTROL	20	1.645±.06 (6)	44.10 (1)	6.80 (1)
SMSLX	8→20	1.241±.19 (5)	55.03±5.06 (3)	23.14±10.853 (3)
CONTROL	21,22	1.300±.13 (4)	63.83±4.19 (6)	7.37± 1.676 (6)
SMSLX	7,8→21,22	1.390±.12 (8)	69.43±5.96 (9)	9.31± 3.046 (9)
CONTROL	23,24	1.596±.35 (3)	48.02 (1)	1.67 (1)
SMSLX	10→23,24	2.377±.16 (3)	36.30±5.81 (2)	4.45± 2.786 (2)
CONTROL	28	1.285±.04 (9)	52.89±1.51 (8)	1.76± 0.713 (8)
SMSLX	15→28	2.034±.11 (5)*	43.00±3.09 (5)*	0.63± 0.626 (5)
CONTROL	36-38	1.320±.03 (13)	53.28±1.56 (12)	0.46± 0.311 (10)
SMSLX	22→36,37	1.629±.06 (9)*	40.43±2.67 (11)*	2.06± 0.879 (9)

Values represent the mean ± the standard error of the mean. *Indicates difference between control and SMSLX values is significant ($P < 0.05$, student's t-test). Numbers in parentheses indicate number of animals.

Table V. Amylase Concentration and Total Amylase of Parotid Gland from Immature Rat Two Weeks following Bilateral Extirpation of the Submandibular and Sublingual Glands

Condition	Age of Animals in Days	Amylase Concentration (mg RS/mg wet wt)	Total Gland Amylase (mg RS/gland)
CONTROL	20	148±22.50 (5)	8,092±1337 (5)
SMSLX	8→20	132±31.95 (2)	6,230±2030 (2)
CONTROL	21,22	172±15.60 (3)	7,140±2062 (3)
SMSLX	7,8→21,22	78±17.90 (7)*	3,183± 754 (7)
CONTROL	23,24	253±43.10 (3)	20,510±5600 (3)
SMSLX	10→23,24	230±25.34 (3)	25,445±5708 (3)
CONTROL	28	213±24.07 (8)	16,169±1499 (8)
SMSLX	15→28	237±15.19 (5)	26,578±2855 (5)*
CONTROL	36-38	247±21.94 (13)	36,794±4423 (13)
SMSLX	22→36,37	291±21.68 (9)	52,986±3828 (9)*

Values represent the mean ± the standard error of the mean. *Indicates difference between control and SMSLX values is significant ($P < 0.05$, student's t-test). Numbers in parentheses indicate number of animals.

Table VI. Sodium and Potassium Concentration and Flow Rate of Pilocarpine-Evoked Saliva from Denervated Rat Parotid Gland Two Weeks following Bilateral Extirpation of the Submandibular and Sublingual Glands

Condition	Age of Animals in Days	Na^+ (mEq/l)	K^+ (mEq/l)	Flow Rate (µl/min/g)
CONTROL	20	43.98± 9.65 (4)	39.20±4.26 (4)	28.91± 6.831 (4)
SMSLX	8→20	63.07±10.34 (3)	44.20±2.98 (3)	24.95± 3.073 (3)
CONTROL	21,22	33.95±12.24 (4)	47.70±2.25 (4)	25.80±10.214 (3)
SMSLX	7,8→21,22	27.43± 2.41 (6)	53.38±1.47 (6)	18.29 (1)
CONTROL	23,24	9.16± 2.50 (2)	35.45±1.85 (2)	40.46±21.460 (2)
SMSLX	10→23,24	9.49± 0.50 (2)	44.29±7.99 (2)	21.43± 0.590 (2)
CONTROL	28	68.67± 2.75 (6)	28.50±1.38 (6)	27.61± 7.080 (6)
SMSLX	15→28	82.60± 9.63 (5)	29.40±2.01 (5)	18.87± 4.100 (4)
CONTROL	36-38	75.00± 6.64 (10)	23.50±1.39 (10)	22.82± 1.634 (10)
SMSLX	22→36,37	66.70± 6.63 (7)	20.00±0.77 (7)*	23.20± 4.187 (7)

Values represent the mean ± the standard error of the mean. *Indicates difference between control and SMSLX values is significant ($P < 0.05$, student's t-test). Numbers in parentheses indicate number of animals. Pilocarpine was administered intraperitoneally in a single dose of 5 mg/kg body weight.

of increased mitotic activity in the parotid glands of the desalivated animals. A 44% ($P < 0.02$) increase in total gland amylase was found in parotid glands of 36- or 37-day old experimental animals (Table V), but concentration of amylase did not differ from controls. In Table VI potassium concentration in saliva from pilocarpine stimulated glands was decreased 15% ($P < 0.05$) in the partially desalivated animals. Sodium concentration and flow rate were unchanged.

Paraffin embedded sections stained with hematoxylin and eosin from these animals desalivated at weaning and sacrificed at 34 days of age clearly demonstrate that acinar cells of parotid glands from desalivated rats are enlarged (Figure 15) when compared with those of control glands (Figure 14). Parotid acinar cells in SMSLX animals have a foamy appearance which is not apparent in control glands. It was from sections similar to these that acinar cell nuclei were counted and mitotic index was determined. Plastic embedded sections stained with toluidine blue from 36-day old control rats (Figure 16) show numerous dark blue staining secretory granules within the acinar cells. Parotid glands from desalivated animals of the same age primarily contain enlarged, pale blue staining secretory granules within secretory endpieces (Figure 17). In electron micrographs of control parotid glands secretory granules are electron dense (Figure 18). Acinar cells of glands from SMSLX animals contain enlarged, electron lucent secretory granules (Figure 19) some of which appear to be coalescing. Electron dense granules like those

seen in control acinar cells are occasionally scattered among the enlarged lucent granules.

At higher magnification, it is evident that the membranes surrounding the secretory granules in the glands from partially desalivated animals (Figure 20) are not continuous. No changes in any of the ductal elements were observed.

Evidence of Compensatory Changes Three Weeks following Gland Ablation

Removal of the submandibular-sublingual complex at 17 days of age followed by sacrifice three weeks later resulted in statistically significant changes in acinar cell size, total glandular amylase, sodium concentration, and flow rate. As indicated by the data in Table VII, parotid gland weight of partially desalivated animals was 23% ($P > 0.05$) greater and acinar cell size was 32% larger ($P < 0.01$) than that of controls. Mitotic index did not differ from control values. Total amylase of the parotid gland increased 38% ($P < 0.05$) in experimental animals (Table VIII), but the concentration of amylase in the gland was unchanged. Examination of saliva for changes in electrolytes revealed that sodium concentration in partially desalivated rats was 37% ($P < 0.02$) less than control values but that potassium concentration in experimental and control animals was identical (Table IX). Flow rate of saliva diminished 33% ($P < 0.01$) in experimental animals.

When the rats were sialoadenectomized at weaning and sacrificed three weeks later (43 days of age), statistically significant changes

Table VII. Gland Weight, Acinar Cell Size, and Mitotic Index of Parotid Gland from Immature Rat Three Weeks following Bilateral Extirpation of the Submandibular and Sublingual Glands

Condition	Age of Animals in Days	Parotid Gland Wet wt (mg) / Body wt (g)	No. of Nuclei / Area	Mitotic Index
CONTROL	35-38	1.299±.03 (16)	53.28±1.56 (12)	0.39±0.262 (12)
SMSLX	17→38	1.593±.15 (7)	40.45±3.07 (4)*	0±0 (5)
CONTROL	43	1.073±.06 (5)	43.62±2.87 (6)	0±0 (6)
SMSLX	22→43	1.410±.07 (6)*	39.52±1.81 (6)	0±0 (6)

Values represent the mean ± the standard error of the mean. *Indicates difference between control and SMSLX values is significant ($P < 0.05$, student's t-test). Numbers in parentheses indicate number of animals.

Table VIII. Amylase Concentration and Total Amylase of Parotid Gland from Immature Rat Three Weeks following Bilateral Extirpation of the Submandibular and Sublingual Glands

Condition	Age of Animals in Days	Amylase Concentration (mg RS/mg wet wt)	Total Gland Amylase (mg RS/gland)
CONTROL	35-38	261±19.59 (16)	37,154±3681 (16)
SMSLX	17→38	290±38.12 (5)	51,424±5640 (5)*
CONTROL	43	267±27.18 (5)	39,712±3489 (5)
SMSLX	22→43	277±20.09 (5)	53,242±3419 (5)*

Values represent the mean ± the standard error of the mean.
*Indicates difference between control and SMSLX values is significant ($P < 0.05$, student's t-test). Numbers in parentheses indicate number of animals.

Table IX. Sodium and Potassium Concentration and Flow Rate of Pilocarpine-Evoked Saliva from Denervated Rat Parotid Gland Three Weeks following Bilateral Extirpation of the Submandibular and Sublingual Glands

Condition	Age of Animals in Days	Na^+ (mEq/l)	K^+ (mEq/l)	Flow Rate (µl/min/g)
CONTROL	36-38	75.00±6.64 (10)	23.50±1.39 (10)	22.82±1.634 (10)
SMSLX	17→38	47.25±7.09 (4)*	23.50±1.55 (4)	15.24±0.915 (4)*
CONTROL	43	101.20±6.89 (5)	19.00±2.41 (5)	45.81±9.246 (6)
SMSLX	22→43	91.50±6.58 (6)	18.20±2.33 (6)	52.73±7.505 (5)

Values represent the mean ± the standard error of the mean. *Indicates difference between control and SMSLX values is significant ($P < 0.05$, student's t-test). Numbers in parentheses indicate number of animals. Pilocarpine was administered intraperitoneally in a single dose of 5 mg/kg body weight.

only in gland weight and total gland amylase were observed. Parotid gland weight in the partially desalivated animals was 31% ($P < 0.01$) greater than that of controls (Table VII), and total gland amylase was 34% ($P < 0.05$) (Table VIII) higher. Other measured parameters were not statistically different from control values (Tables VII-IX).

Only paraffin embedded sections which were used to ascertain cell size were made of parotid glands from these two groups of animals. No glands were embedded in plastic for electron microscopy.

Evidence for Neural Regulation of Compensatory Enlargement

In the following experiments the submandibular and sublingual glands were extirpated bilaterally, and the parotid glands were denervated bilaterally in some of the partially desalivated animals. The denervation and partial desalivation were accomplished during the same surgical operation with the exception of one experimental group.

One Week Denervation and Desalivation

In the first experiments combining denervation with gland ablation, the animals were operated on at weaning and sacrificed one week later. Two different groups of animals combining several litters were used. Partial desalivation of the animals at 21 days of age resulted in the same previously reported, statistically significant increase in parotid gland weight, acinar cell size, and total gland amylase (Tables X and XI). These three parameters were also increased when the submandibular-sublingual complex was ablated

Table X. Gland Weight, Acinar Cell Size, and Mitotic Index of Denervated Parotid Gland from Immature Rat One Week following Bilateral Extirpation of the Submandibular and Sublingual Glands

Condition	Age of Animals in Days	Parotid Gland Wet wt (mg) / Body wt (g)	No. of Nuclei / Area	Mitotic Index
CONTROL	28	1.285±.04 (9)	52.89±1.51 (8)	1.76±0.713 (8)
SMSLX	21→28	1.833±.09 (5)*	38.95±4.65 (2)*	1.15±1.145 (2)
PX	21→28	0.911±.05 (5)*	62.37±3.90 (3)*	3.04±1.334 (3)
PX-SMSLX	21→28	1.331±.06 (4)	43.90±1.55 (2)*	1.10±1.100 (2)
SX-SMSLX	21→28	1.664 (1)	53.00 (1)	0 (1)
CONTROL	29-31	1.383±.04 (10)	49.21±1.11 (9)	1.20±0.638 (9)
SMSLX	22→30	1.483±.12 (3)	41.56±1.55 (9)*	0.73±0.727 (9)
SX	22→30	1.314±.04 (5)	50.40±1.87 (3)	1.91±1.070 (3)
SX-SMSLX	22→30	1.635±.07 (5)*	51.13±1.33 (3)	0±0 (3)

Values represent the mean ± the standard error of the mean. *Indicates difference between control and experimental values is significant ($P < 0.05$, student's t-test). Numbers in parentheses indicate number of animals. In Table X and all succeeding tables, SMSLX indicates bilateral extirpation of submandibular and sublingual glands; PX - bilateral resection of auriculotemporal nerves; PX-SMSLX - parasympathectomy combined with gland ablation; SX - bilateral removal of superior cervical ganglia; and SX-SMSLX - sympathectomy combined with gland ablation.

Table XI. Amylase Concentration and Total Amylase of Denervated Parotid Gland from Immature Rat One Week following Bilateral Extirpation of the Submandibular and Sublingual Glands

Condition	Age of Animals in Days	Amylase Concentration (mg RS/mg wet wt)	Total Gland Amylase (mg RS/gland)
CONTROL	28	213±24.07 (8)	16,169±1499 (8)
SMSLX	21→28	314±11.39 (5)	27,132±1847 (5)*
PX	21→28	191±45.08 (4)	9,520±1432 (4)*
PX-SMSLX	21→28	280±39.42 (3)	15,913±1801 (3)
SX-SMSLX	21→28	446 (1)	32,620 (1)
CONTROL	30	187± 2.95 (2)	14,140± 560 (2)
SMSLX	22→30	253±40.92 (3)	24,558±3431 (3)
SX	22→30	329±24.21 (5)*	29,396±3328 (5)*
SX-SMSLX	22→30	338±23.09 (5)*	35,962±2113 (5)*

Values represent the mean ± the standard error of the mean. *Indicates difference between control and experimental values is significant ($P < 0.05$, student's t-test). Numbers in parentheses indicate number of animals.

at 22 days of age and the animals sacrificed at 30 days, but only acinar cell size differed statistically from control values (Tables X and XI). As in previous one week experiments with this age group, mitotic index, amylase concentration of the gland, sodium and potassium concentration in saliva, and flow rate of saliva did not differ from control values (Tables X-XII).

Parotid gland weight increased 43% ($P < 0.01$) in the SMSLX rats sacrificed at 28 days but only 7% ($P > 0.05$) in the SMSLX rats sacrificed at 30 days. Small sample size in the latter group may account for this discrepancy. Additionally, the extent of enlargement one week following gland ablation is apparently more variable than after two weeks. Removal of a section of the auriculotemporal nerve (PX) resulted in a 29% ($P < 0.01$) decrease in the weight of the parotid gland. When parasympathectomy was combined with gland ablation (PX-SMSLX) or when superior cervical ganglia (SX) were removed, no changes in gland weight occurred. However, when sympathectomy was combined with gland ablation (SX-SMSLX) gland size increased 29% in the one animal sacrificed at 28 days and 18% ($P < 0.01$) in those animals sacrificed at 30 days.

Based on counts of nuclei, parotid acinar cell size increased 36% ($P < 0.05$) in SMSLX animals sacrificed at 28 days and 18% ($P < 0.01$) in SMSLX animals sacrificed at 30 days of age (Table X). Parasympathectomy resulted in an 15% ($P < 0.05$) decrease in cell size. Combining gland ablation with parasympathectomy caused a 20% increase ($P < 0.01$) in cell size when compared to control values and a 42% increase when compared with PX alone.

Table XII. Sodium and Potassium Concentration and Flow Rate of Pilocarpine-Evoked Saliva from Denervated Rat Parotid Gland One Week following Bilateral Extirpation of the Submandibular and Sublingual Glands

Condition	Age of Animals in Days	Na^+ (mEq/l)	K^+ (mEq/l)	Flow Rate (µl/min/g)
CONTROL	28	68.67± 2.75 (6)	28.50±1.38 (6)	27.61± 7.080 (6)
SMSLX	21→28	71.70± 8.69 (3)	22.30±2.44 (3)	25.64±11.135 (3)
PX	21→28	122.30± 4.06 (3)*	26.00±1.00 (3)	85.97±30.085 (3)
PX-SMSLX	21→28	103.70± 5.24 (3)*	22.00±1.00 (3)*	46.96± 1.120 (2)
SX-SMSLX	21→28	98.00 (1)	20.00 (1)	54.64 (1)
CONTROL	29-31	96.00±10.33 (5)	25.46±3.41 (5)	30.98± 7.880 (5)
SMSLX	22→30	77.70±16.59 (3)	24.73±3.08 (3)	22.53± 3.941 (3)
SX	22→30	104.70± 6.48 (3)	20.48±1.53 (4)	41.10±15.755 (4)
SX-SMSLX	22→30	83.50± 4.99 (4)	23.20±1.25 (4)	18.52± 3.355 (4)

Values represent the mean ± the standard error of the mean. *Indicates difference between control and experimental values is significant ($P < 0.05$, student's t-test). Numbers in parentheses indicate number of animals. Pilocarpine was administered intraperitoneally in a single dose of 5 mg/kg body weight.

Mitotic index (Table X) did not differ statistically from controls in any of the experimental animals. A suggestion of increased mitotic activity was observed in PX glands.

Amylase concentration of the parotid gland was changed in only the SX and SX-SMSLX animals (Table XI). Sympathectomy produced a 76% increase in amylase concentration ($P < 0.01$). Combining sympathectomy with partial desalivation elicited an 81% ($P < 0.01$) increase in concentration in the 30-day old experimental animals when compared with controls, but concentration in these glands did not differ from that of SX glands. Concentration in the one SX-SMSLX animal sacrificed at 28 days was increased 109%.

Total amylase in the parotid gland (Table XI) of the SMSLX animals increased 68% ($P < 0.01$) in the animals sacrificed when 28 days old and 74% ($P > 0.05$) in the 30-day old animals. A 41% ($P < 0.01$) decrease in total gland amylase was observed following parasympathectomy, but no change in amylase levels was seen when parasympathectomy was combined with gland ablation. Removal of the superior cervical ganglia resulted in a 108% increase ($P < 0.01$) in total gland amylase. In 30-day old SX-SMSLX animals total gland amylase was elevated 154% ($P < 0.01$) over controls and 22% over that of SX alone. It was increased 99% over control values in the one 28-day old SX-SMSLX rat.

The only statistically different changes in sodium concentration of saliva were observed in PX and PX-SMSLX animals (Table XII). Concentration increased 66% ($P < 0.01$) in saliva from parasympathectomized

glands and 51% ($P < 0.01$) when parasympathectomy was combined with gland ablation.

Potassium concentration in pilocarpine-evoked saliva decreased 23% ($P < 0.01$) in PX-SMSLX rats (Table XII), but other experimental values did not differ from those of controls. The single value obtained from the 28-day old SX-SMSLX rat also indicates a decrease in potassium concentration, but this decrease is not evident in the 30-day old animals. No statistically different changes in flow rate of saliva occurred.

Two Week Denervation and One Week Desalivation

The next group of animals was denervated at 16 days of age and sacrificed at 31 days (Tables XIII-XV). Originally, 12 animals were both desalivated and denervated at 16 days of age and 8 were denervated only. However, all animals in which both desalivation and denervation were performed died by the twenty-second day of age. In an attempt to salvage the experiment, the submandibular and sublingual glands were ablated from the denervated animals on the twenty-fourth day. Some of the control animals were partially desalivated at this time.

Parotid gland weight increased 74% ($P < 0.01$) in the SMSLX animals and 19% ($P < 0.01$) in the SX-SMSLX animals (Table XIII). A slight decrease in gland weight occurred when parasympathectomy and gland ablation were combined. Parotid acinar cell size enlarged 72% ($P < 0.01$) in the animals from which the submandibular-sublingual complex was ablated. A 40% increase ($P < 0.01$) in cell size was

Table XIII. Gland Weight, Acinar Cell Size, and Mitotic Index of Denervated Parotid Gland from Immature Rat One Week following Bilateral Extirpation of the Submandibular and Sublingual Glands

Condition	Age of Animals in Days	Parotid Gland $\frac{\text{Wet wt (mg)}}{\text{Body wt (g)}}$	$\frac{\text{No. of Nuclei}}{\text{Area}}$	Mitotic Index
CONTROL	29-31	1.383±.04 (10)	49.21±1.11 (9)	1.20±0.638 (9)
SMSLX	24→31	2.407±.16 (3)*	28.60±2.75 (3)*	6.34±4.433 (3)
SX-SMSLX	16→24→31	1.645±.05 (4)*	48.73±1.19 (4)	4.02±2.407 (4)
PX-SMSLX	16→24→31	1.199±.08 (4)	35.05±1.11 (4)*	1.44±0.838 (4)

Values represent the mean ± the standard error of the mean. *Indicates difference between control and experimental values is significant ($P < 0.05$, student's t-test). Numbers in parentheses indicate number of animals.

Table XIV. Amylase Concentration and Total Amylase of Denervated Parotid Gland from Immature Rat One Week following Bilateral Extirpation of the Submandibular and Sublingual Glands

Condition	Age of Animals in Days	Amylase Concentration (mg RS/mg wet wt)	Total Gland Amylase (mg RS/gland)
CONTROL	29-31	291±25.05 (9)	25,995±2978 (9)
SMSLX	24→31	319±21.00 (3)	55,451±3667 (3)*
SX-SMSLX	16→24→31	436±36.23 (4)*	50,615±2918 (4)*
PX-SMSLX	16→24→31	389± 9.00 (2)*	32,830±5530 (2)

Values represent the mean ± the standard error of the mean. *Indicates difference between control and experimental values is significant ($P < 0.05$, student's t-test). Numbers in parentheses indicate number of animals.

Table XV. Sodium and Potassium Concentration and Flow Rate of Pilocarpine-Evoked Saliva from Denervated Rat Parotid Gland One Week following Bilateral Extirpation of the Submandibular and Sublingual Glands

Condition	Age of Animals in Days	Na^+ (mEq/l)	K^+ (mEq/l)	Flow Rate (µl/min/g)
CONTROL	29-31	96.00±10.33 (5)	25.46±3.41 (5)	30.98± 7.880 (5)
SMSLX	24→31	91.50±10.50 (2)	23.95±0.65 (2)	9.78± 3.490 (2)
SX-SMSLX	16→24→31	102.50± 6.55 (4)	19.05±2.44 (4)	22.70± 4.277 (3)
PX-SMSLX	16→24→31	128.25± 4.11 (4)*	23.30±1.33 (4)	65.30±18.222 (3)

Values represent the mean ± the standard error of the mean. *Indicates difference between control and experimental values is significant ($P < 0.05$, student's t-test). Numbers in parentheses indicate number of animals. Pilocarpine was administered intraperitoneally in a single dose of 5 mg/kg body weight.

seen in PX-SMSLX rats. Sympathectomy combined with gland extirpation resulted in no change in cell size. Mitotic index in the three experimental groups was not statistically different from control values, but an increase in mitotic activity was suggested by the SMSLX and SX-SMSLX data.

The data for amylase concentration of the gland indicated a 50% increase ($P < 0.01$) in concentration in glands from SX-SMSLX animals and a 34% increase ($P < 0.01$) in glands from PX-SMSLX animals (Table XIV). Concentration was unchanged in glands from SMSLX rats. Total amylase in the parotid gland in partially desalivated animals increased 113% ($P < 0.01$). A 95% elevation ($P < 0.01$) of total gland amylase resulted following sympathectomy and gland ablation. No change in quantity of amylase was observed following parasympathectomy and gland ablation.

No changes in concentration of sodium or potassium in saliva were observed except in the PX-SMSLX animals where a 34% increase ($P < 0.05$) in sodium occurred. Salivary flow rate decreased in SMSLX animals and increased in PX-SMSLX animals, but these changes did not differ statistically from control values.

Two Week Denervation and Desalivation Studies on Preweanling Animals

Three litters of preweanling animals were denervated and desalivated when 10 days old. Vaseline was applied to the oral orifice of these pups from the time of surgery until sacrifice as previously described. Using this technique, 24 of the 31 rats were successfully maintained until they were sacrificed at 23 or 24 days of age. All

but one of the pups which died were with the same female rat. This female rat ate at least three of the pups, appeared not to have the best maternal instincts, and failed to nurse the pups as frequently as the other two lactating females.

Of the eight parameters measured on these animals only sodium concentration of the PX-SMSLX animals was statistically different from control values (Tables XVI-XVIII). A six-fold increase in sodium concentration ($P < 0.01$) in saliva from PX-SMSLX animals occurred. Because of smaller gland size in these young animals, the parotid glands could not be divided for amylase and histological examination; therefore, the whole gland had to be used for examination of one parameter or the other. Hence, only one gland was embedded in paraffin, and statistical analysis of cell size and mitotic index could not be done. An increase in acinar cell size in the SMSLX animals and a decrease in cell size in PX animals was indicated from the number of nuclei per area in the hematoxylin and eosin stained sections (Table XVI).

Representative light level, plastic embedded histological sections of the various partial desalivation and denervation states of this experiment are found in Figures 21-26. The control section (Figure 21) reveals the presence of well formed, definitive secretory endpieces containing numerous dark blue staining secretory granules. A few acinar cells containing basic fuchsin-staining granules are also evident (see also Figure 4). In Figure 22 from a partially desalivated animal, the secretory granules have enlarged and stain bright red with basic fuchsin. A few small, dark blue staining

Table XVI. Gland Weight, Acinar Cell Size, and Mitotic Index of Denervated Parotid Gland from Immature Rat Two Weeks following Bilateral Extirpation of the Submandibular and Sublingual Glands

Condition	Age of Animals in Days	Parotid Gland Wet wt (mg) / Body wt (g)		No. of Nuclei / Area		Mitotic Index	
CONTROL	23,24	1.596±.35	(3)	48.02	(1)	1.67	(1)
SMSLX	10→23,24	2.377±.16	(3)	36.39±4.11	(2)	4.45±1.970	(2)
SX	10→23,24	1.692±.23	(3)				
SX-SMSLX	10→23,24	1.895±.11	(6)	51.20	(1)	5.47	(1)
PX	10→23,24	1.271±.21	(3)	64.42	(1)	5.90	(1)
PX-SMSLX	10→23,24	1.663±.13	(6)	52.00	(1)	3.46	(1)
CONTROL	36-38	1.320±.03	(13)	53.28±1.56	(12)	0.46±0.031	(10)
SMSLX	22→36,37	1.629±.06	(9)*	39.50±2.92	(10)*	2.06±0.879	(9)
SX	22→36,37	1.278±.09	(3)	44.20±0.79	(3)*	2.11±0.119	(3)*
SX-SMSLX	22→36,37	1.318±.07	(6)	50.50±3.11	(4)	2.41±1.473	(4)
PX	22→36,37	0.972±.06	(4)*	57.01±4.03	(3)	1.63±0.254	(3)*
PX-SMSLX	22→36,37	0.912±.08	(5)*	45.04	(1)	0.44	(1)

Values represent the mean ± the standard error of the mean. *Indicates difference between control and experimental values is significant ($P < 0.05$, student's t-test). Numbers in parentheses indicate number of animals.

Table XVII. Amylase Concentration and Total Amylase of Denervated Parotid Gland from Immature Rat Two Weeks following Bilateral Extirpation of the Submandibular and Sublingual Glands

Condition	Age of Animals in Days	Amylase Concentration (mg RS/mg wet wt)	Total Gland Amylase (mg RS/gland)
CONTROL	23,24	253±43.10 (3)	20,610±5600 (3)
SMSLX	10→23,24	230±25.34 (3)	25,445±5708 (3)
SX	10→23,24	310± 4.00 (3)	21,257±12,273 (3)
SX-SMSLX	10→23,24	304±34.16 (6)	22,097±3827 (6)
PX	10→23,24	249±31.09 (3)	13,907±2957 (3)
PX-SMSLX	10→23,24	263±26.53 (6)	15,377±2259 (6)
CONTROL	36-38	247±21.94 (13)	36,794±4423 (13)
SMSLX	22→36,37	291±21.68 (9)	52,986±3828 (9)*
SX	22→36,37	420± 6.06 (3)*	70,146±3957 (3)*
SX-SMSLX	22→36,37	338±24.62 (6)*	49,230±6132 (6)
PX	22→36,37	298±37.59 (4)	42,039±6663 (4)
PX-SMSLX	22→36,37	310±11.56 (5)*	30,628±4058 (5)

Values represent the mean ± the standard error of the mean. *Indicates difference between control and experimental values is significant ($P < 0.05$, student's t-test). Numbers in parentheses indicate number of animals.

Table XVIII. Sodium and Potassium Concentration and Flow Rate of Pilocarpine-Evoked Saliva from Denervated Rat Parotid Gland Two Weeks following Bilateral Extirpation of the Submandibular and Sublingual Glands

Condition	Age of Animals in Days	Na^+ (mEq/l)	K^+ (mEq/l)	Flow Rate (µl/min/g)
CONTROL	23,24	9.16± 2.50 (2)	35.45±1.85 (2)	40.46±21.46 (2)
SMSLX	10→23,24	9.49± 0.50 (2)	44.29±7.99 (2)	21.43± 0.59 (2)
SX	10→23,24	28.31 (1)	38.96 (1)	33.14 (1)
SX-SMSLX	10→23,24	10.52± 2.13 (5)	44.37±5.10 (5)	29.24± 3.89 (4)
PX	10→23,24	53.80±17.80 (2)	37.95±2.65 (2)	38.76±24.41 (2)
PX-SMSLX	10→23,24	68.27±10.82 (5)*	33.89±3.57 (5)	43.28± 8.98 (5)
CONTROL	36-38	75.00± 6.64 (10)	23.50±1.39 (10)	22.82± 1.63 (10)
SMSLX	22→36,37	66.70± 6.63 (7)	20.00±0.76 (7)*	23.20± 4.19 (7)
SX	22→36,37	122.38± 0.83 (2)*	19.96±1.64 (2)*	70.25±10.73 (2)*
SX-SMSLX	22→36,37	94.08± 9.08 (4)	20.39±2.78 (4)	42.66± 5.45 (4)*
PX	22→36,37	96.57 (1)	23.98 (1)	154.37 (1)
PX-SMSLX	22→36,37	82.84±13.95 (4)	25.80±2.12 (4)	55.92±24.65 (4)

Values represent the mean ± the standard error of the mean. *Indicates difference between control and experimental values is significant ($P < 0.05$, student's t-test). Number in parentheses indicate number of animals. Pilocarpine was administered intraperitoneally in a single dose of 5 mg/kg body weight.

granules are also evident. Sympathectomy results in a similar pattern to that seen in the control sections, but the secretory granules appear denser, somewhat larger, and stain purplish (Figure 23). Figure 24 from an SX-SMSLX animal contains both the toluidine blue-staining secretory granules similar to those seen in control and SX animals and the enlarged basic fuchsin-staining granules like those found in the SMSLX animals. Parasympathectomized glands contain a combination of small dense red staining secretory granules and enlarged red staining granules (Figure 25). Similarly, sections from PX-SMSLX rats contain both kinds of secretory granules (Figure 26), but the intensity of the red stain is diminished and the enlarged, lucent granules are more numerous.

Figures 27-32 are representative electron micrographs of parotid glands from the same six groups of animals just described at the light microscopic level. In Figure 27, a section from a 24-day old control animal, the secretory granules are primarily electron dense (see also Figure 6). Some of these granules contain a rim of more electron opaque material. Acinar cells in the micrograph (Figure 28) of parotid gland from a 24-day old animal that was partially desalivated at 10 days of age are filled with enlarged, electron lucent granules. Electron dense granules are found in some cells. Figure 29 is a micrograph of parotid gland from a sympathectomized animal in which the secretory granules have an electron dense rim and an electron lucent center. Some of these granules have a stippled appearance. Granules similar or identical to these were also seen in glands from sympathectomized-desalivated animals. Additionally,

electron lucent granules like those in Figure 30 were present. Electron lucent and electron dense granules, as well as granules of intermediate density, are found in Figure 31, a micrograph taken of a parasympathectomized parotid gland. When parasympathectomy was combined with gland ablation, secretory granules ranging from electron opaque to electron lucent were present within the acinar cells (Figure 32).

Two Week Denervation and Desalivation Studies on Postweanling Animals

In this experiment the rats were partially desalivated and denervated at weaning and sacrificed between 36 and 38 days of age (Tables XVI-XVIII). Of all the animals originally included in this experiment only one PX-SMSLX animal died prior to the sacrifice date. Parotid gland weight was increased 23% ($P < 0.01$) in the partially desalivated rats but was decreased 26% ($P < 0.01$) with parasympathectomy and 31% ($P < 0.01$) when parasympathectomy was combined with gland ablation (Table XVI). Sympathectomy alone or together with gland ablation did not alter gland weight.

Acinar cells were 35% larger ($P < 0.01$) in partially desalivated animals than in control rats and were 21% larger ($P < 0.01$) in sympathectomized glands (Table XVI). Cell size in the one PX-SMSLX gland embedded in paraffin was 18% greater than in control glands and 27% larger than parasympathectomized glands. No change in cell size was observed in SX-SMSLX or PX animals.

Mitotic index differed statistically from controls in both sympathectomized ($P < 0.01$) and parasympathectomized ($P < 0.02$)

glands (Table XVI). Other values were not statistically different from controls, but a suggestion of increased mitotic activity is apparent in the SMSLX and SX-SMSLX animals.

Increased amylase concentration in the parotid gland occurred in SX, SX-SMSLX, and PX-SMSLX animals (Table XVII). Concentration in these three animal groups was increased 70% ($P < 0.01$), 37% ($P < 0.02$), and 26% ($P < 0.05$), respectively, over control values. Other parameters did not differ from control values. With respect to the total quantity of amylase in the whole gland, increases were observed only in SMSLX and SX rats. Total gland amylase was elevated 44% ($P < 0.02$) in glands from partially desalivated rats and increased 91% ($P < 0.01$) in sympathectomized parotid glands.

In Table XVIII sodium concentration in saliva of postweanling animals did not differ from control values in any of the experimental groups except the SX animals. Sodium concentration in these animals was increased 63% ($P < 0.01$). Concentration of potassium in saliva from both SMSLX and SX rats showed a 15% decrease ($P < 0.05$). Other potassium values did not differ from those of controls. Flow rate of pilocarpine-evoked saliva was enhanced in the SX and SX-SMSLX animals. A 208% ($P < 0.01$) increase in flow rate was measured in SX animals, and an 87% ($P < 0.01$) increase was recorded from SX-SMSLX animals. Certainly flow rate from the one parasympathectomized gland was greatly increased, but statistics could not be done on this single sample. Increased flow rate is suggested by the data for the PX-SMSLX animals, but a wide standard deviation existed.

Figures 33-38 are representative of one micrometer sections stained with toluidine blue and basic fuchsin from these six post-weanling animal groups which were operated on at weaning and sacrificed at 36-38 days of age. Numerous dense, toluidine blue-staining secretory granules within secretory endpieces are apparent in Figure 33 from a control parotid gland. Only connective tissue in the interparenchymal space shows any indication of basic fuchsin staining. Acinar cells in parotid glands from partially desalivated rats contain numerous enlarged, basic fuchsin-staining secretory granules (Figure 34). These granules vary in size, and the intensity of the red staining diminishes as the granules increase in size. A few dark staining secretory granules are sometimes seen in the acinar cells. The fact that parotid acinar cell size has enlarged in the SMSLX animals is readily apparent. Removal of the superior cervical ganglia results in the presence of numerous dense secretory granules which stain purple (Figure 35). These granules appear to be somewhat larger than those in control glands. An almost identical pattern to that of sympathectomized glands is seen in Figure 36 which is a section from a sympathectomized-desalivated animal. Parasympathectomy results in the presence of numerous dense, basic fuchsin-staining granules (Figure 37). When parasympathectomy is combined with gland ablation (Figure 38) the secretory granules enlarge, become lucent, and stain intensely with basic fuchsin. A few toluidine blue-staining granules are present in intercalated ducts.

Representative electron micrographs of glands from these six postweanling animal groups are found in Figures 39-44. In Figure 39 from a control gland the secretory granules are electron dense. Some lipid is evident in an infranuclear position. Extirpation of the submandibular and sublingual glands results in compensatory enlargement of acinar cells and secretory granules (Figure 40). The secretory granules have enlarged and become electron lucent, their membranes are not continuous, and some granules coalesce. Coalescence of granules is common in mucous containing cells of the submandibular gland but not in the parotid gland. Some of the acinar cells also contain a few electron dense secretory granules like those seen in control glands scattered among the lucent granules. No alteration in the endoplasmic reticulum or Golgi apparatus is apparent. Figures 41 and 42 illustrate the identical appearance of acinar cells in parotid glands from SX and SX-SMSLX animals. The secretory granules are electron dense but have a stippled appearance. Some granules have an electron dense rim and a less electron dense center. In parasympathectomized glands (Figure 43) the secretory granules resemble those seen in controls, but the granules are not quite as electron dense. Some granules also contain a ring of electron dense material at the periphery. Combining parasympathectomy with gland ablation (Figure 44) results in an enlargement of the secretory granules and a greater decrease in density than was seen in PX glands. The extent of changes in these granules appears to be intermediate to that seen in partially desalivated animals and control animals. No

change in any duct cells were observed in these various denervation and desalivation states.

Other Stains

Parotid glands from most experimental groups were also stained by the periodic acid Schiff (PAS) reaction. Some of these sections were digested with diastase prior to staining with PAS. Persistence of PAS positive staining material in the glands following diastase digestion indicated that the PAS reaction was staining something other than glycogen or starch, such as glycoproteins. All glands contained PAS positive material in the secretory endpieces, but secretory granules present in intercalated ducts exhibited the greatest intensity of staining. Parotid glands from partially desalivated, postweanling animals showed an increase in size of acinar cells and stained less intensely than control glands. These changes were similar to those seen in the sections stained with hematoxylin and eosin (Figures 14 and 15). No correlation between the PAS stained sections and toluidine blue-basic fuchsin-stained sections could be made.

DISCUSSION

The fact that 7-10 day old preweanling, partially desalivated animals almost never survived suggests an important role for saliva in the young animal. A lack of milk in the stomach of these young rats following submandibular and sublingual gland ablation indicated that they died due to lack of nutritive substance. The expedient of applying vaseline to the oral orifice of the young pups permitted them to survive. This suggests that the saliva produced by the submandibular and sublingual glands acts as a lubricant and sealant for the young pup to attach to its mother's teats. Submandibular and sublingual glands produce a seromucous and mucous secretion. Without these glands and their secretion, the very young animal cannot survive. The development of the parotid gland lags behind that of the submandibular and sublingual glands, and the parotid gland does not contribute greatly to the total saliva in the rat prior to the fifteenth day of postnatal life (Epstein, 1970). The lack of parotid secretion prior to this time apparently is not important for the survival of the young animal. Since the parotid secretion is serous, it contains little mucoid substance to aid in teat attachment.

By 15 or 16 days of age the animals can survive after removal of the submandibular-sublingual complex, but they do not gain weight and grow as rapidly as do unoperated controls. The innervation to the

parotid gland may also be interrupted at this age or earlier without threatening the animal's life. If parotid denervation of either autonomic branch is combined with partial desalivation at this time all of the animals die. By 15 days of age the contribution of saliva made by the parotid is of some significance, since presence of the intact (but not denervated and therefore inactive) parotid can at least prevent death of the desalivated (SMSLX) rat. Denervation of the parotid in partially desalivated young animals prevents the production of any saliva, and again, the animals are without lubricating fluid for attachment to teats of the mother. Lubricating the rat's mouth with vaseline enables them to suckle, survive, and grow. This further stresses the importance of the salivary glands during postnatal development.

Entire litters of rats, including both males and females, were used in these experiments. It has previously been shown that no sex related differences are present in parotid glands of immature rats (Schneyer and Hall, 1969; Klein and Harrington, 1976).

The data clearly indicate that compensatory enlargement of the parotid gland of the immature rat occurs following removal of the other major salivary glands. However, this response becomes evident only when the period following partial desalivation extends beyond weaning. Compensatory enlargement consists of increased gland weight, increased size of individual acinar cells and size of secretory granules. Ductal cells are not affected. Mitotic index is not influenced by the compensatory process either when gland ablation is performed in the preweanling or postweanling animals. Thus, it is

concluded that the enlargement of the parotid gland is due to cellular hypertrophy and not to cellular hyperplasia.

These experiments in the immature animal were deliberately undertaken to delineate a possible influence of mitosis on the glandular enlargement, because the developing gland is characterized by two distinct phases of growth. An early phase occurs prior to weaning and exhibits pronounced proliferative activity and little change in either size of cells or degree of differentiation. During a later phase following weaning, mitosis drops precipitously (reaching low levels of 3-4/1000 by 32 days of age (Schneyer and Hall, 1969), and cell size and differentiation increase markedly. These latter processes become complete within 6-7 weeks after birth (Schneyer and Hall, 1969; Redman and Sreebny, 1970b and 1971). It was therefore significant that early proliferative activity is virtually unaltered during the change in glandular size. The entire enlargement of the parotid gland that occurs when the compensatory process is followed to a point beyond weaning (when mitotic index is still considerable in magnitude) is attributed to increase in size of acinar cells only.

Until weaning, the animals are on an all-liquid diet, and the absence of glandular enlargement is not unexpected. This situation is physiologically normal, and glandular activity is low as a consequence of absence of neurally-mediated glandular activity. This situation can be experimentally induced in the adult by introduction of an all-liquid diet. The consequence of this diet in adult partially desalivated animals is an absence of compensatory

enlargement of the parotid even though ablation of the submandibular and sublingual glands has occurred at least one week prior to this (Hall and Schneyer, 1977). Compensatory enlargement in the immature animal only occurs when a solid diet has been introduced (e.g., that which occurs normally at weaning), and neurally-mediated glandular activity is thereby increased. Removal of the parotid parasympathetic innervation at the time of removal of the submandibular-sublingual complex effectively eliminates any excessive glandular activity normally induced in the remaining glands.

It is particularly interesting that hyperplasia is not involved in any of the changes in the young animal since under other conditions of increased physiological load, this process is also affected. Chronic administration of large doses of isoproterenol affect mitotic index, but this change occurs only in the postweanling gland or gland of the adult rat when mitotic activity is normally low (C. A. Schneyer, 1973a, 1977). In the preweanling rat, the normal proliferative activity is in fact inhibited by the administration of isoproterenol over a period of 7-8 days (C. A. Schneyer, 1973a, 1977). A clear dissociation between events of hyperplasia and hypertrophy is thus evident with both compensatory enlargement and isoproterenol treatment.

In the present study functional changes associated with compensatory enlargement are concerned almost exclusively with synthesis of the digestive enzyme amylase which is normally produced by the acinar cells of the parotid gland. A progressive increase in total amylase activity as well as concentration occurs in rat parotid

gland during normal postnatal development (Redman and Sreebny, 1971; Schneyer and Hall, 1972). However, in the current investigation only total amount of amylase of the gland increased, and concentration did not. Since the entire increase in gland size can be attributed only to increase in cytoplasmic content of cells, and not to increase in number of cells, it is apparent that the larger cells are producing more amylase, because there is a greater amount of cytoplasmic volume. The fact that concentration does not change indicates that the rate of formation of amylase is increased in the enlarged cells, and the amount per unit of cytoplasm increases to keep pace with the increase in total amount of cytoplasm. Again, this same phenomenon has been previously seen in glands of immature rats treated with isoproterenol (C. A. Schneyer, 1977). The mechanism of the change in amylase under these conditions of isoproterenol treatment of the young animal differs sharply from that of the adult or the postweanling animal. When isoproterenol is administered to preweanling rats total amylase is doubled, amylase per unit of cytoplasm is unchanged, and amylase per microgram of DNA is tripled. Gland weight is doubled in isoproterenol-treated animals, but total DNA is decreased. Therefore, the increase in total gland amylase is due entirely to cellular hypertrophy induced by isoproterenol and not to increased cell number. In postweanling animals, total gland amylase doubles, but a decrease in concentration of amylase per unit of cytoplasm is observed, and this suggests that the increase in rate of formation of amylase does not proceed apace with the increase in cytoplasmic volume (C. A. Schneyer, 1977).

The other functional parameters examined, i.e., sodium, potassium, and flow rate of pilocarpine-evoked saliva, are not changed when glands are enlarged by compensatory hypertrophy. This is not unexpected in light of the fact that no ductal changes are seen morphologically. A suggestion of a decrease in flow rate did occur but was not statistically significant. These findings are consistent with the fact that ductal changes do not accompany the hypertrophy induced by isoproterenol administration (Schneyer, 1962), and again point to the fact that in both cases, only the acinar cells and not the ducts are affected by the enlargement process. Modifications of sodium and potassium concentration are not observed following removal of the submandibular-sublingual complex. The only observed changes that do occur are seen with parasympathectomy. Premature age-related increases in sodium concentration are observed with parasympathectomy, and potassium concentration is prematurely reduced from the high levels normally seen in the immature rat. This fact has been reported previously (Schneyer and Hall, 1967b, 1968b), but present data provide additional confirmatory evidence. This suggests that denervation affects the electrolyte fluxes and leads to premature acceleration of a process that occurs normally. Since sodium concentration is greater in the final secretion and potassium concentration less, it appears that less sodium reabsorption and less potassium secretion occur in the absence of the innervation in the immature animal. It must be stressed that the major change must nonetheless involve the acini since only acini and not ducts appear morphologically modified. This points to the fact that the

precursor fluid formed at the acinar-intercalated duct complex is probably the site where alteration in electrolyte concentrations with denervation occurs, and that subsequent modifications throughout the duct system occur normally. Compensatory hypertrophy is evident after only one week of desalivation, but it becomes accentuated when the period between gland extirpation and sacrifice persists for two weeks. The three week data presented here are suggestive of a decrease in the extent of the compensatory response. Working with adult rats, Hall and Schneyer (1977, 1978) have described a 20-30% increase in cell size and gland weight after a four week period. Recently, Wilborn (1977) has confirmed this response in adult rats after a three month period.

Compensatory responses are lacking in parotid glands of pre-weanling animals and occur only when the period of desalivation extends beyond weaning. This suggests that the change in dietary consistency and the age of the animals are critical factors in the induction of the compensatory response. The change from an all-liquid to a solid diet is in turn associated with the degree of neurally mediated glandular activity. Prior to weaning, neural influences are minimal. With the introduction of solid food just prior to or at weaning, reflexly mediated glandular activity is greatly increased due to onset of mastication (C. A. Schneyer, 1970, 1972, 1974b, 1977; Hall and Schneyer, 1969). Removal of the submandibular and sublingual glands results in a functional overload on the remaining parotid glands after weaning, causing excessive neural stimulation of the glands and resulting in the compensatory response.

Weaning itself imposes a new functional increase in neural activity in the salivary glands. The cytological changes observed at weaning are primarily under sympathetic control and are similar to changes seen when neurally mediated glandular activity is enhanced by other means (Hall and Schneyer, 1964, 1969, 1973; C. A. Schneyer, 1970, 1974a, b; Schneyer and Hall, 1972b, 1976). Thus, any change from one level of neurally mediated glandular activity to another provides the stimulus for enhanced growth responses. Some of the changes occurring in control rats at weaning that are particularly suggestive of this involve changes in size and staining characteristics of secretory granules. These resemble changes induced when isoproterenol or gland ablation has been employed to enhance growth responses, although the extent of this change at weaning is not as great.

Another finding that is fairly consistent is the occurrence of basally located lipid droplets in acinar cells of parotid glands of animals in which reflexly mediated glandular activity has been decreased over a prolonged time interval. This was seen in the present work whenever a period of fasting was required prior to sacrifice. The most conspicuous accumulation of lipid was seen in preweanling animals. The accumulation of lipid that is apparently related to decreased glandular activity had earlier been reported in glands of adult rats maintained on liquid diet (Wilborn and Schneyer, 1970) and in glands of fasted adult rats (Hand, 1972b).

The dependence of the compensatory response induced by partial desalivation on neurally mediated glandular activity was assessed by

the simultaneous removal of the submandibular-sublingual complex and denervation of the autonomic pathways to the parotid gland. In preweanling animals (treated with vaseline), no statistically significant changes were observed in any of the measured parameters. The only instances of any change were with parasympathectomy alone or with partial desalivation alone, where a small change, of opposite direction, was observed in acinar cell size. In postweanling animals, 21 days of age or older, the compensatory parotid enlargement did not occur in the absence of an intact parasympathetic pathway. Gland weight was in fact decreased in most parasympathectomized-desalivated animals and in all parasympathectomized glands. No change in gland weight occurred following sympathectomy, but when ganglionectomy was combined with desalivation gland weight increased. Acinar cell size was decreased in all parasympathectomized glands. However, in the animals in which parasympathectomy was combined with gland ablation, cell size was significantly increased over control values, but this increase was not as great as that seen in the partially desalivated, neurally intact animals. Generally, cell size was not changed in the sympathectomized glands or in glands from sympathectomized-desalivated animals. Thus, it appears that the increase in gland weight that accompanies compensatory enlargement of the parotid gland is dependent on an intact parasympathetic pathway, but increases in size of acinar cells depend on a functioning sympathetic pathway.

The functional status of the partially desalivated, sympathectomized, and sympathectomized-desalivated animals is altered, and

the increased levels of total amylase in parotid glands of these three groups is attributed to a role of the parasympathetic innervation in regulating synthesis of amylase. The amount of amylase synthesized increases as a consequence of increased amount of cytoplasm in acinar cells. Each unit of acinar cytoplasm in parotid glands of partially desalivated animals was capable of producing the same amount of amylase as control glands. Amylase concentration was increased in sympathectomized, sympathectomized-desalivated, and parasympathectomized-desalivated groups but was decreased in parasympathectomized glands. Schneyer and Hall (1972b) reported a decrease in both concentration and total amylase of the parotid gland and also in cell number following parasympathectomy and attributed the decreases to a reduction of cell mass.

The sympathetic innervation exerts the primary role in the enlargement of secretory granules and the change in staining intensity of these granules. This role becomes more prominent when parasympathectomy is accompanied by desalivation. Enlarged, electron lucent, basic fuchsin-staining, granules were observed only when the sympathetic innervation was intact. A preponderance of this type of granule exists in parotid glands of partially desalivated animals or glands in which desalivation is combined with parasympathectomy. Some of the enlarged, electron lucent granules appear to have defects in or a complete absence of membranes. This could be due to tangential sectioning or to inadequate or inappropriate fixation of these membranes. However, a portion of the intervening membranes is

believed to disappear where fusion between adjacent granules occurs (Martinez-Hernandez et al., 1972).

Secretory granules in sympathectomized glands are identical to those which were described in the adult parotid gland by Wilborn and Schneyer (1972). They described two cell types: dark cells and light cells. Only an occasional light cell was seen in these young animals. The uneven electron density of secretory granules in sympathectomized glands of adult animals was attributed either to prolonged storage of granules or to alterations in the synthetic stage of the secretory process (Wilborn and Schneyer, 1972). Work by Garrett and Thulin (1975) on sympathectomized rat parotid gland indicates that secretion of secretory granules is primarily controlled by the sympathetic innervation. Amylase is thought to be prepackaged in the secretory granules and released with sympathetic stimulation (Schneyer and Hall, 1972a, b; Sreebny et al., 1971; Garrett and Thulin, 1975). Autonomic stimulation results in release of secretory granules, but stimulation of the parasympathetic innervation fails to cause secretory granule release (Garrett and Thulin, 1975). Amylase content in saliva is high following sympathetic stimulation but low following parasympathetic stimulation (Schneyer and Hall, 1972a, b; C. A. Schneyer, 1974a). This correlates well with the data presented herein that indicate an increase in both concentration and total amylase of the parotid gland following sympathectomy. Without an intact sympathetic innervation the granules remain densely packed in the acinar cells and undergo changes which are probably due to prolonged storage.

The role of the sympathetic innervation in maintenance of granule size and density is further demonstrated by experiments involving parasympathectomy. In these cases where the sympathetic pathway was intact, the granules showed less electron density; the variations in granule density and size were related to the age of the animals and experimental manipulation employed.

Sheetz (1976) described a change in size and staining characteristics of secretory granules in the submandibular gland of immature, isoproterenol-treated rats that was almost identical to that observed in parotid glands from partially desalivated animals. Electron micrographs from the parotid gland of isoproterenol-treated adult rats also reveal the presence of enlarged secretory granules (Robinovitch et al., 1977). The nature of the biochemical reaction occurring in the granules has not been delineated. It is possible that the changes in size and staining of secretory granules reflect changes in the amount of amylase present or the presence of other biochemical moieties, such as glycoproteins.

Since the introduction of basic dyes for staining of epoxy-embedded sections by Richardson et al. (1960), a number of such stains have been employed, but there is little understanding of how they act. Most methods used for staining one micrometer thick sections from glutaraldehyde-OsO_4-fixed, epoxy-embedded tissue employ single or closely related basic dyes. These dyes generally produce a monochromatic stain that lacks contrast. Therefore, a number of investigators have used two or more dyes to gain greater differentiation of tissue constituents (Huber et al., 1968; Sievors,

1971). The combination stain (toluidine blue-basic fuchsin) used in this investigation was developed by Sheetz (1976) and was used by him to recognize functionally different cells in the submandibular gland of the immature rat.

Prostaglandin E_1 has recently been shown to cause changes in parotid gland secretory granules similar to those observed following gland ablation. Secretory granules become electron lucent for at least 5 hours after injection of prostaglandin E_1. Amylase levels are also altered, and levels in the plasma rise. This is probably due to increased secretion from the parotid gland (Lillie, 1974). These effects are similar to beta-adrenergic influences and point to a modulating role of prostaglandin E_1 in beta-receptor activity.

Although the extent of parotid gland and acinar cell enlargement is approximately five times greater in isoproterenol-treated rats than the hypertrophy induced by partial sialoadenectomy, there appears to be a similarity in the two responses in immature animals (Morgan et al., 1978). The secretory granules in both instances enlarge, become electron lucent, and stain red with basic fuchsin. Because isoproterenol is a beta-adrenergic agonist, this similarity of response suggests that the increased cellular size and enlarged secretory granules seen in parotid glands of partially desalivated animals may be due to stimulation of beta-adrenergic receptors. Thus, the data presented herein suggest an important role for the sympathetic as well as for the parasympathetic innervation in the development of any induced changes in the parotid gland of the immature rat.

SUMMARY

Gland ablation has for the first time been employed to induce compensatory responses in the parotid gland of immature rats. The use of the immature rat provided a system that exhibited two distinct phases of growth and differentiation. One phase occurs prior to weaning and is characterized by the presence of small, undifferentiated endpieces, abundant interparenchymal space, and marked mitotic activity. The second phase is initiated by the event of weaning and is characterized by increase in size and differentiation of acinar cells, and a sharp drop in mitotic activity. The change from liquid to solid diet at weaning introduces neurally mediated activity to the glands. Prior to weaning such influences are virtually absent. These conclusions are based on the following findings. The parotid gland of the preweanling rat exhibits no changes in size or level of differentiation if submandibular and sublingual glands are extirpated at 8 or 16 days of age and parotid glands are examined prior to or at weaning. However, there is an enlargement of secretory granules in parotid glands of partially desalivated animals during weaning. When the submandibular and sublingual glands are removed at weaning, or any time thereafter, there are marked compensatory increases in size of the parotid gland as well as its constituent acinar cells. This

compensatory hypertrophy occurs as early as one week following gland ablation and is maximal two weeks after ablation. The secretory granules in the postweanling glands are enlarged and stain with basic fuchsin, but the intensity of staining of these larger granules is less than in granules in glands of partially desalivated animals examined at weaning.

Functional parameters examined include amylase levels in the gland, concentration of sodium and potassium, and flow rate of parotid saliva. In the preweanling animals no changes occurred in any of these as a result of partial desalivation. In the postweanling animals, concentration of amylase was unchanged, but total amylase of the gland was increased 50-100% greater than that of controls. Concentration of electrolytes and flow rate of saliva were not altered in partially desalivated postweanling animals when comparison was made with controls.

The compensatory changes induced by gland ablation in the postweanling animals appear to be neurally mediated. The parasympathetic innervation is largely responsible for the increases in gland size and amylase, and to some extent cell size. The sympathetic innervation is largely responsible for the increased size of acinar cells and secretory granules. Although mitotic index appeared uninfluenced by these manipulations, it is possible that the increased gland size is to some extent caused by an increase in number of cells.

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ILLUSTRATIONS

PLATE I

Figure 1. Light micrograph of parotid gland from 8-day old control rat. Acinar cells (arrow) are small, but some cells contain secretory granules at this age. Numerous ducts (d) are present. Relatively large amounts of interparenchymal space, filled with connective tissue cells are evident. Stained with toluidine blue-basic fuchsin (TB-BF). X 750.

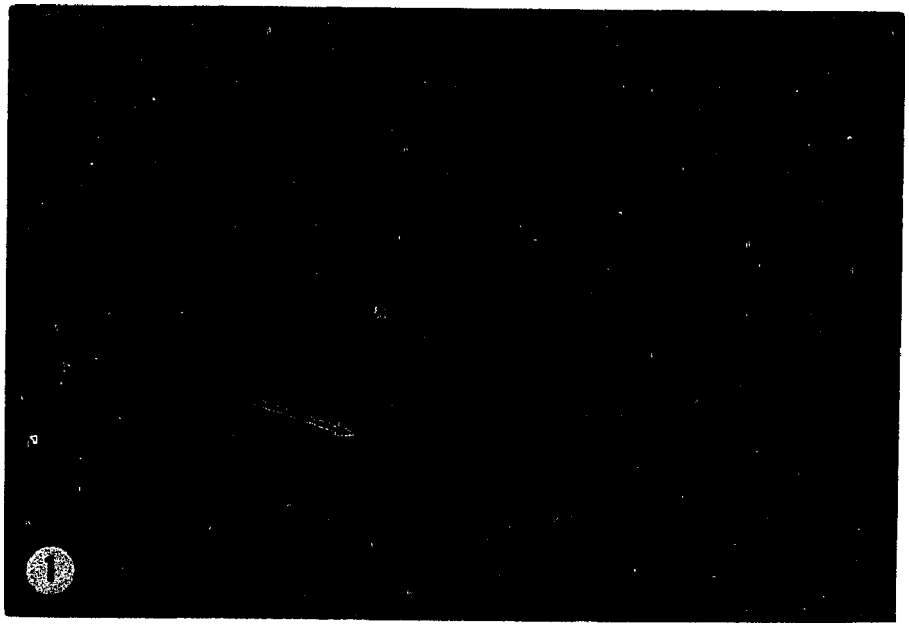
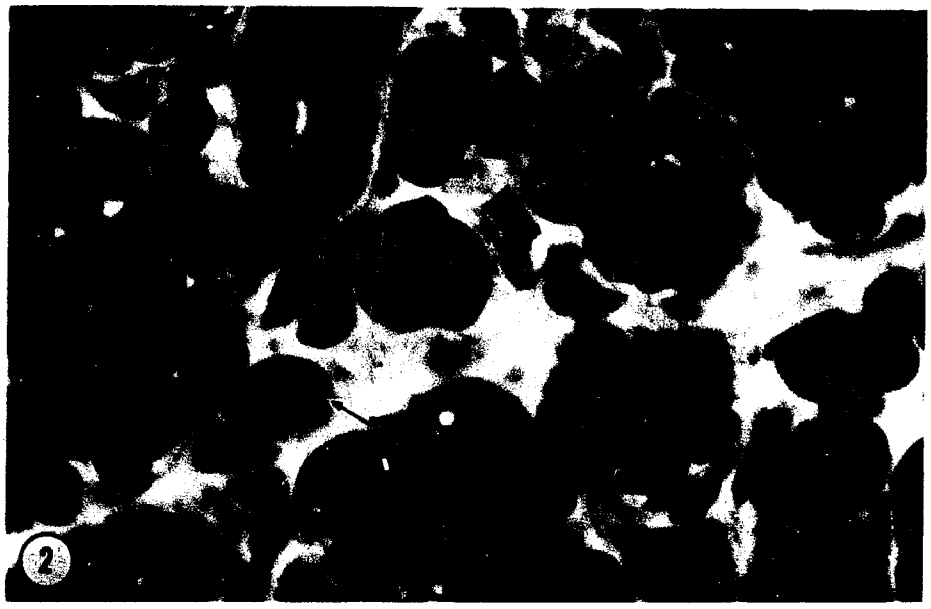


PLATE II

Figure 2. Light micrograph of parotid gland from 16-day old control rat. Acinar cells are small and are still incompletely differentiated. Several mitotic figures (m) are visible, and secretory granules are present in these dividing cells. Lipid droplets (arrow) are evident in the basal portion of most acinar cells. Stained with TB-BF. X 1,180.

Figure 3. Light micrograph of parotid gland from 16-day old animal from which submandibular and sublingual glands were removed one week earlier. No changes are evident following gland ablation. Acinar cells are small, ducts are prominent, and numerous connective tissue cells fill the relatively large amounts of interparenchymal space. Stained with TB-BF. X 1,180.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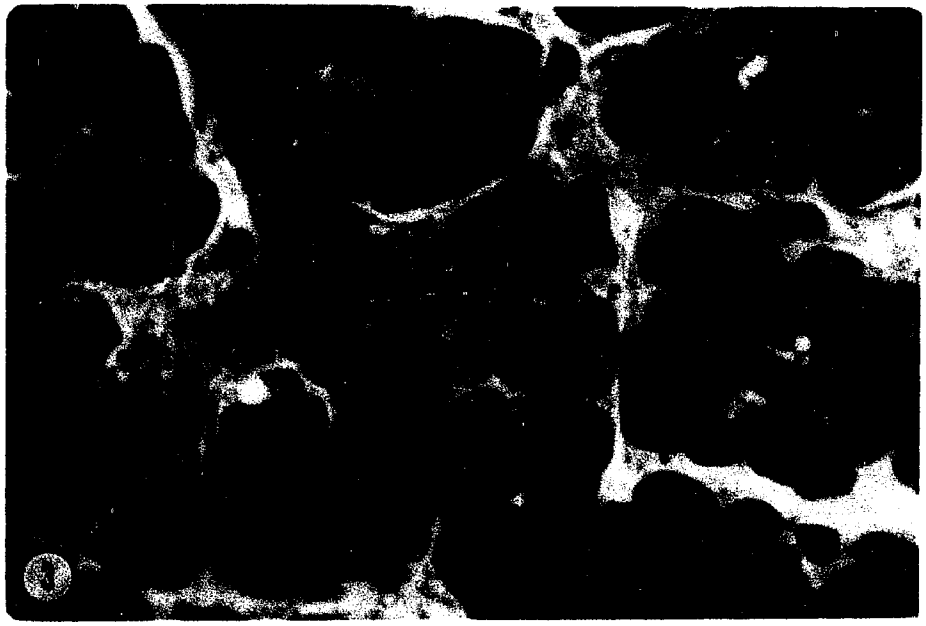
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PLATE III

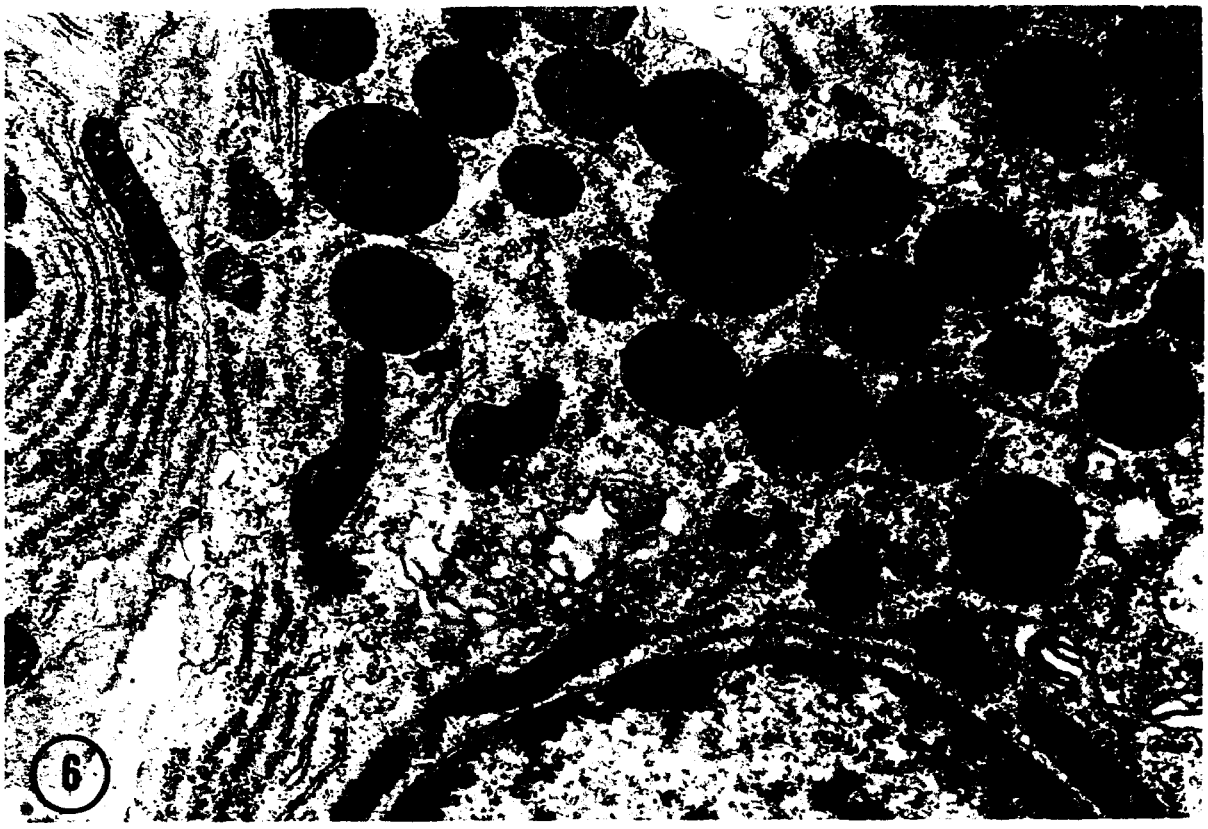
Figure 4. Light micrograph of parotid gland from 24-day old control animal. Secretory endpieces are filled with both toluidine blue-staining and basic fuchsin-staining secretory granules. Some of the red staining granules are enlarged. A striated duct (SD) is present. Stained with TB-BF. X 1,180.

Figure 5. Light micrograph of parotid gland from 24-day old rat that was partially desalivated at 17 days of age. Almost all the secretory granules are enlarged and stain red with basic fuchsin. No toluidine blue-staining granules are evident. Stained with TB-BF. X 1,180.

PLATE IV

Figure 6. Electron micrograph of a portion of an acinar cell in a parotid gland from a 24-day old control animal. Numerous uniformly-opaque secretory granules (SG) are present. The Golgi apparatus (G) and rough endoplasmic reticulum (RER) are indicative of synthetic processes. Typical mitochondria (m) are also present. X 20,550

Figure 7. Electron micrograph of acinar cell from parotid gland of a 24-day old animal that was partially desalivated at 17 days of age. The electron density of the secretory granules (SG) is markedly reduced compared to that of the granules in Fig. 6. Condensing granules (CG) which have just formed in the Golgi apparatus are evident. X 20,550.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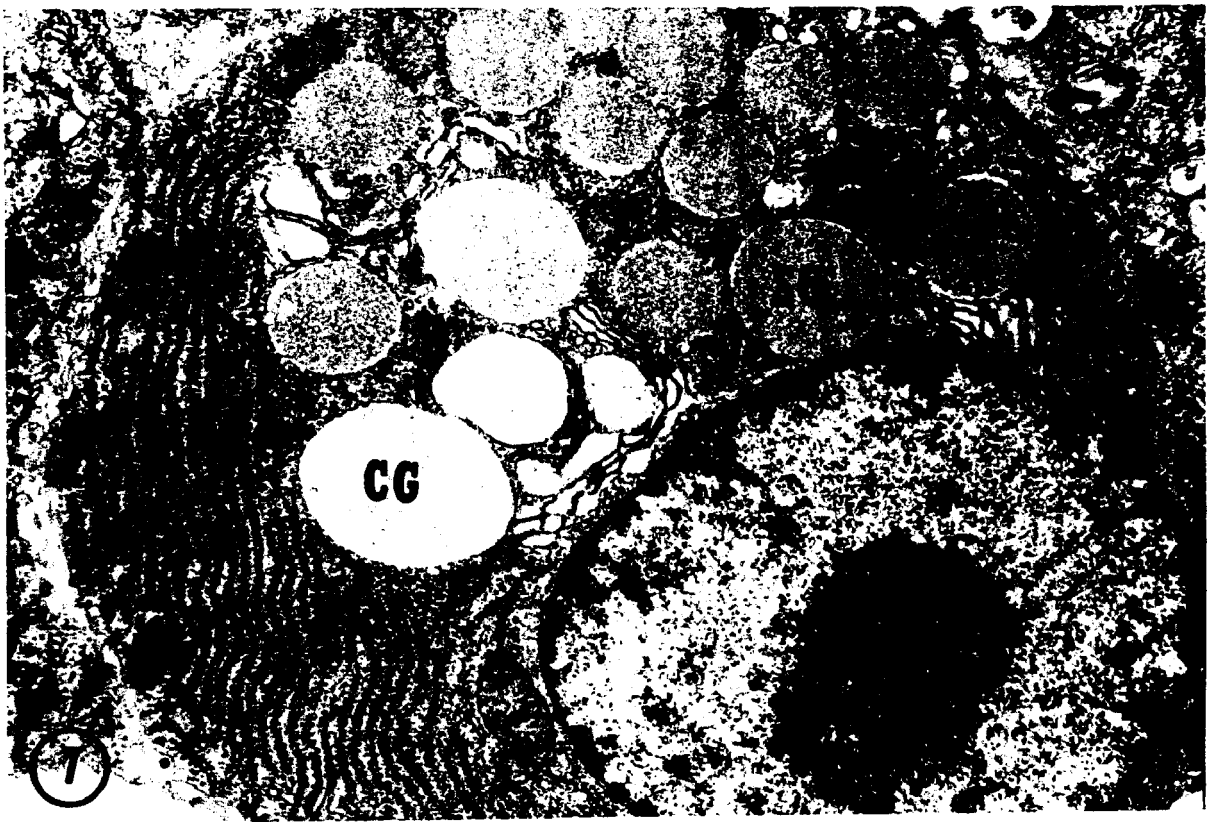

CG
7

PLATE V

Figure 8. Light micrograph of parotid gland from 22-day old control rat. A combination of toluidine blue-staining and basic fuchsin-staining secretory granules occurs at weaning. Stained with TB-BF. X 1,180.

Figure 9. Light micrograph of parotid gland from 22-day old animal from which the submandibular-sublingual complex was extirpated at 8 days of age. All secretory granules in the secretory endpieces are enlarged and stain red. Stained with TB-BF. X 1,180.

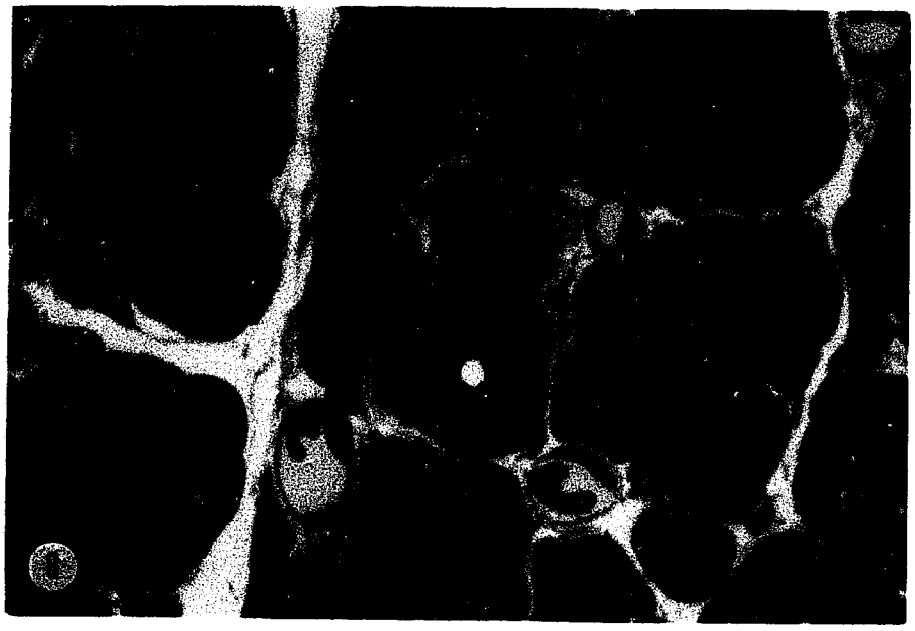
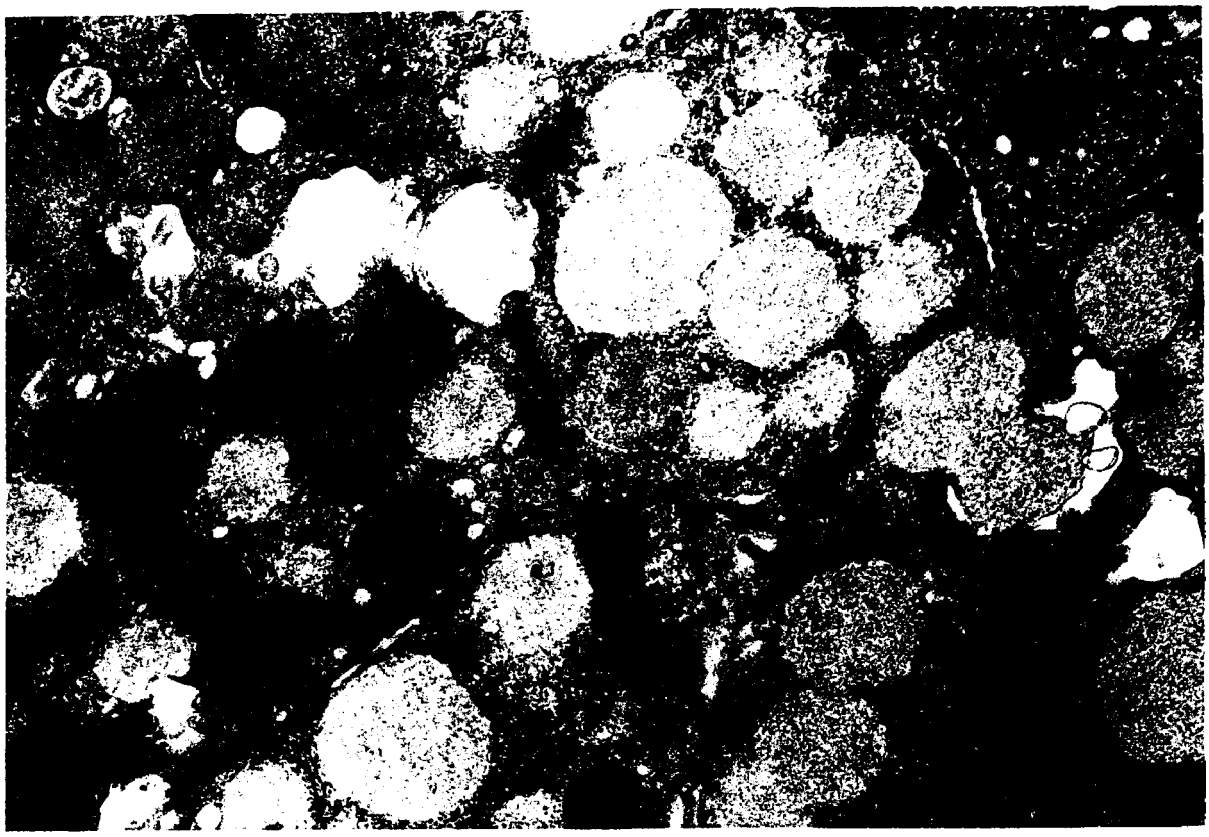


PLATE VI

Figure 10. Electron micrograph showing secretory granules in the apical portion of several acinar cells in the parotid gland of a 20-day old control rat. A variety of electron dense and electron lucent secretory granules is present. Both types of granules may be present within the same cell. X 20,550.

Figure 11. Electron micrograph of secretory granules within acinar cells from a 20-day old animal partially desalivated at 8 days of age. Most of the granules have an electron dense rim and an electron lucent center. Other granules are electron lucent, and some of these contain material of slightly greater density. X 20,550.



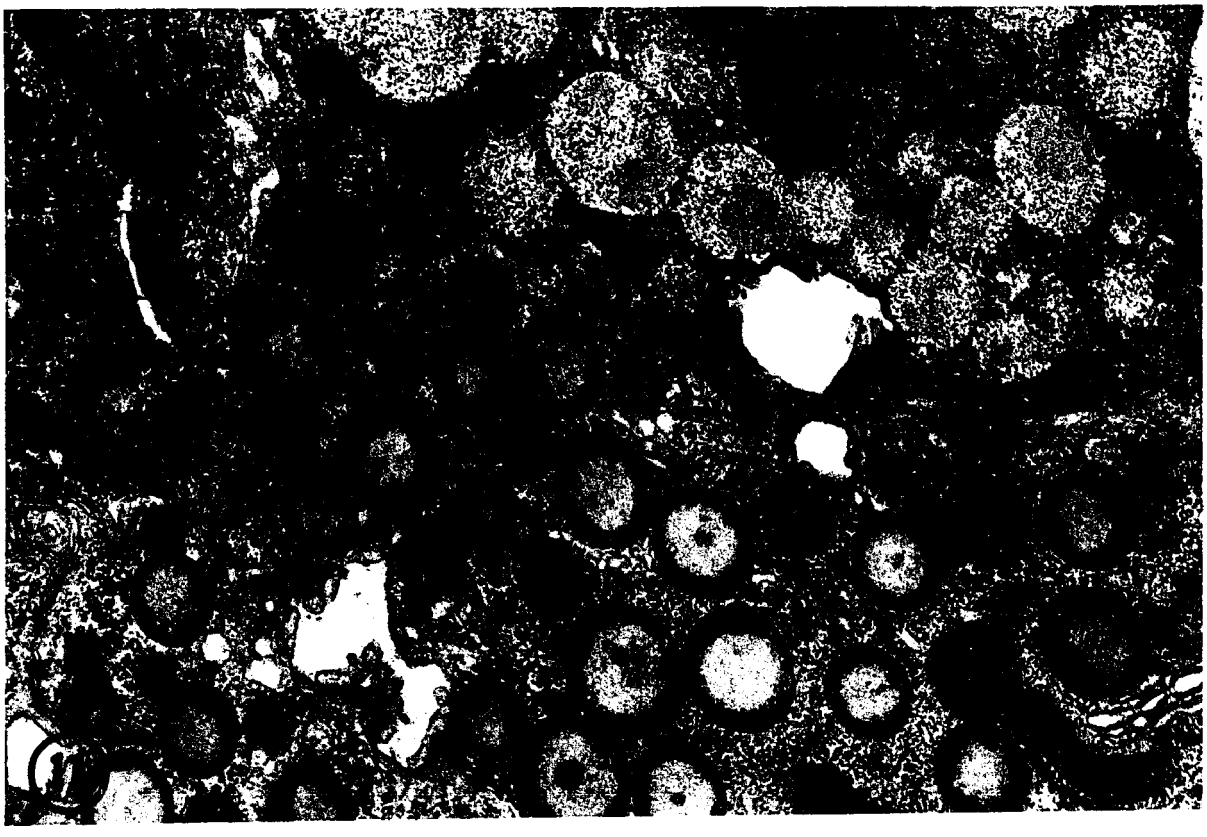


PLATE VII

Figure 12. Light micrograph of parotid gland from a 28-day old control rat. Acinar cells contain toluidine blue-staining secretory granules. Droplets of lipid (arrow) are located in the basal portion of many acinar cells. Stained with TB-BF. X 1,180.

Figure 13. Light micrograph of parotid gland from a 28-day old animal that was partially desalivated at 15 days of age. All the secretory granules are enlarged and stain very little with either dye. Stained with TB-BF. X 1,180.

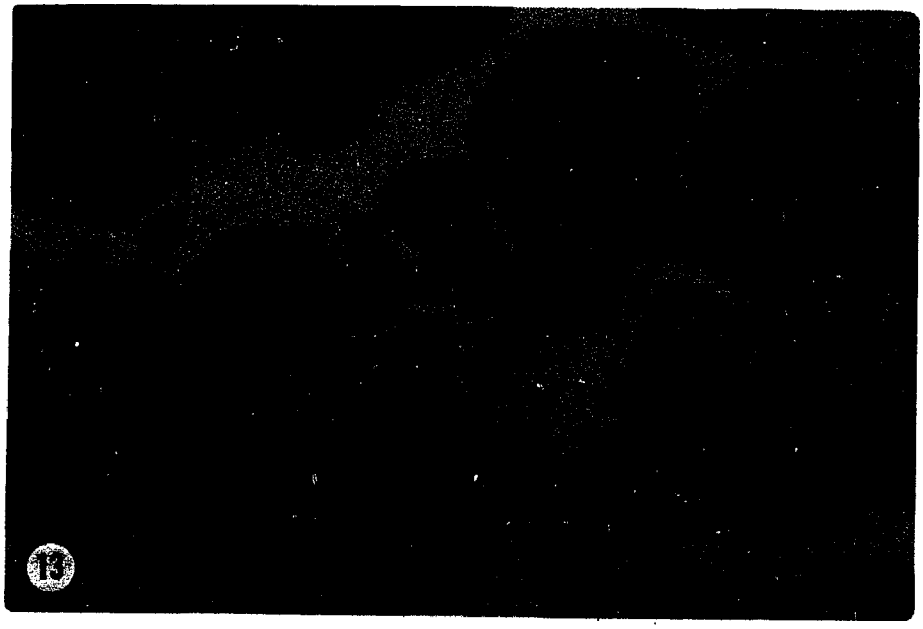


PLATE VIII

Figure 14. Light micrograph of parotid gland from a 34-day old animal. Stained with hematoxylin and eosin. X 750.

Figure 15. Light micrograph of parotid gland from a 34-day old rat from which the submandibular and sublingual glands were removed at 20 days of age. Acinar cells are enlarged and the cytoplasm has a foamy appearance. Stained with hematoxylin and eosin. X 750.

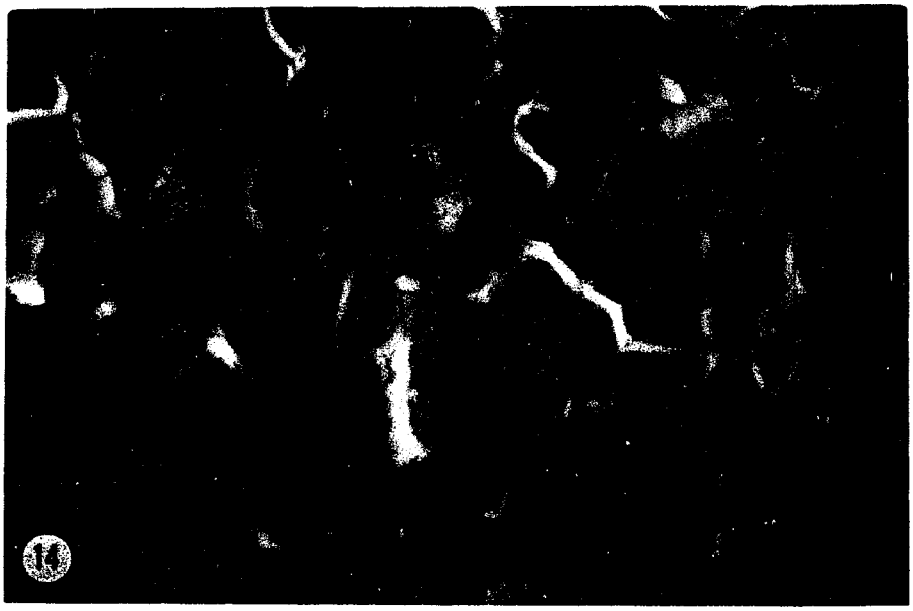

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PLATE IX

Figure 16. Light micrograph of parotid gland from a 36-day old control animal. Acinar cells are filled with dark blue secretory granules. Stained with toluidine blue. X 750.

Figure 17. Light micrograph of parotid gland from a 35-day old animal that had the submandibular-sublingual complex removed at 21 days of age. Secretory granules are enlarged and stain only faintly. Coalescence of granules is evident. The fact that the secretory endpieces have increased in size is also apparent. Stained with toluidine blue. X 750.


16

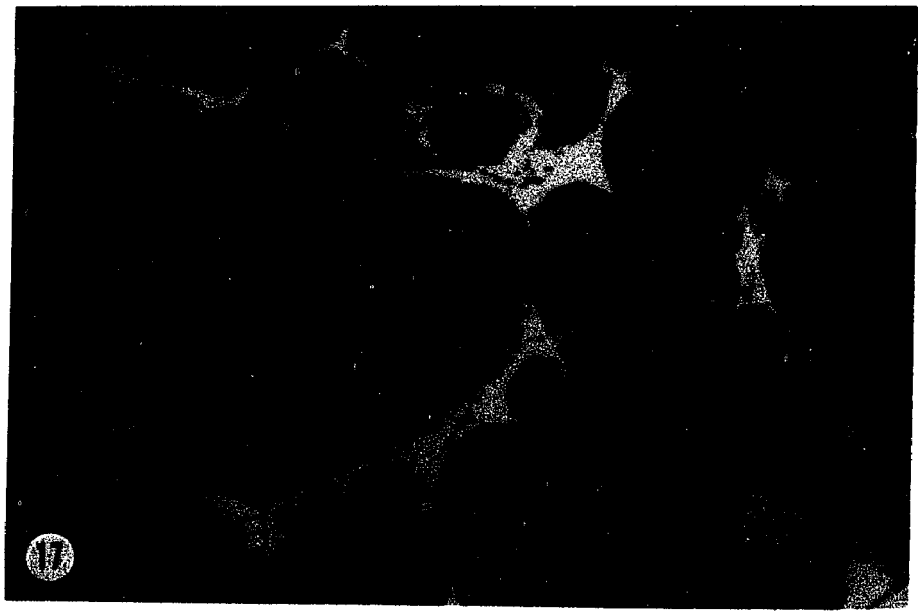
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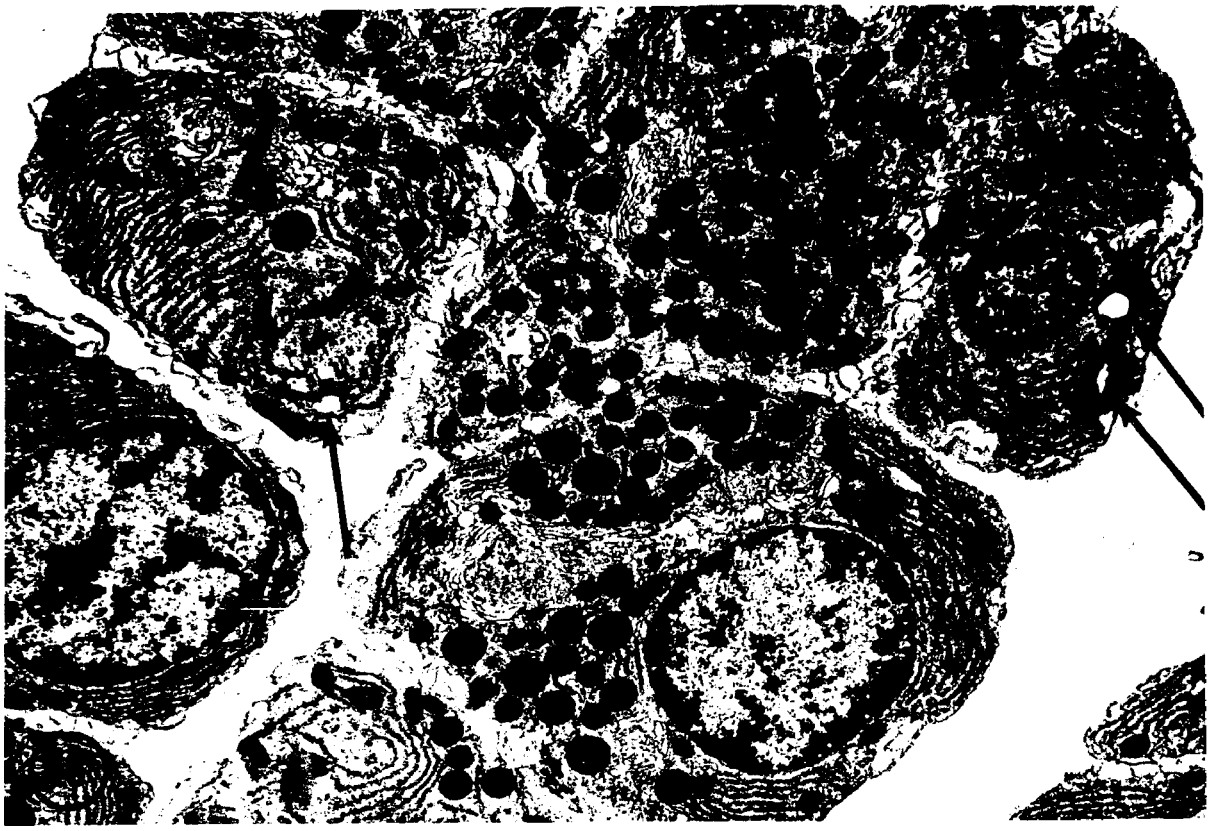
PLATE X

Figure 18. Electron micrograph of acinar cells in parotid gland from a 36-day old control animal. Secretory granules are uniformly opaque. Abundant rough endoplasmic reticulum and several Golgi complexes are visible. Lipid droplets (arrow) are present in several of the cells. X 7,500.

Figure 19. Electron micrograph of parotid gland from a 35-day old rat that was partially desalivated at 21 days of age. Acinar cells are filled with electron lucent secretory granules. X 9,000.



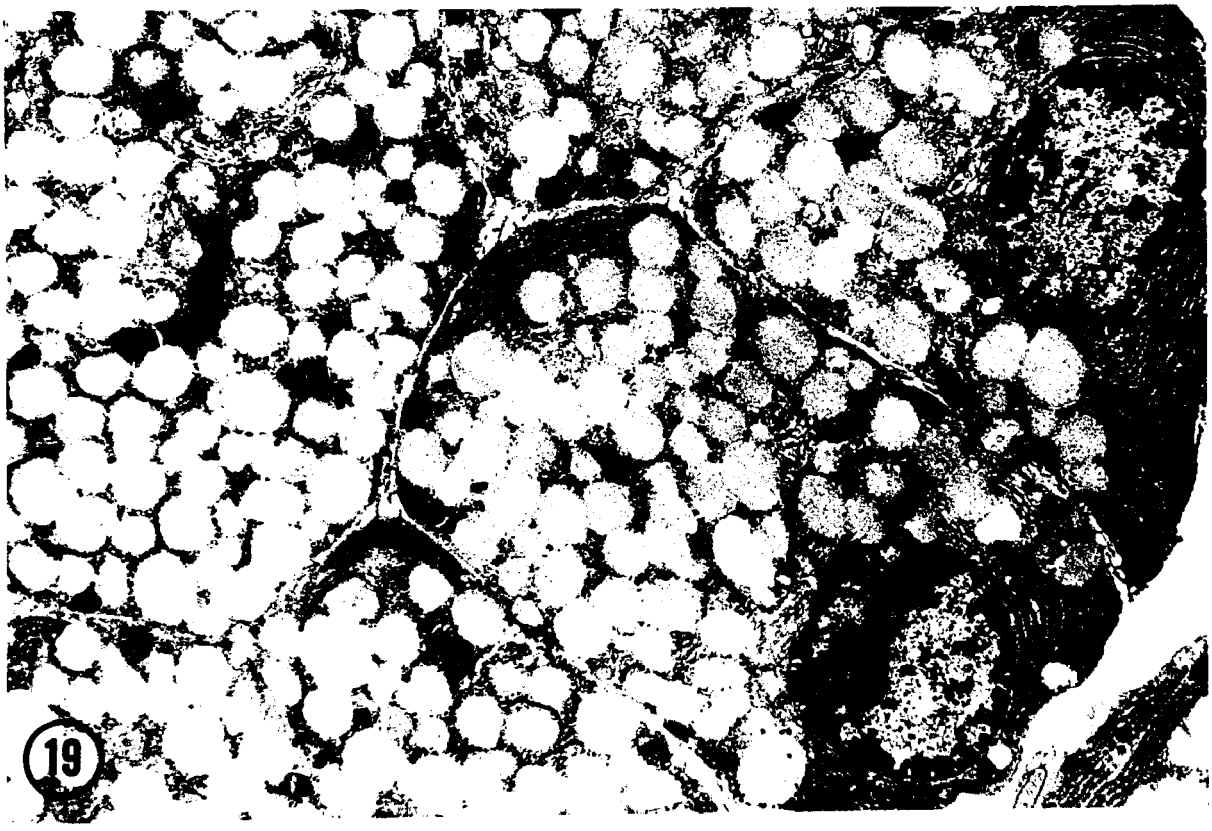


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PLATE XI

Figure 20. Higher magnification electron micrograph of a portion of an acinar cell from same animal as in Fig. 19. Membranes surrounding the enlarged, electron lucent secretory granules (L) are not continuous and are absent between coalescing granules. Secretory granules (D) similar to those seen in normal glands are scattered among the electron lucent granules. Mitochondria (M) are also present. X 30,300.

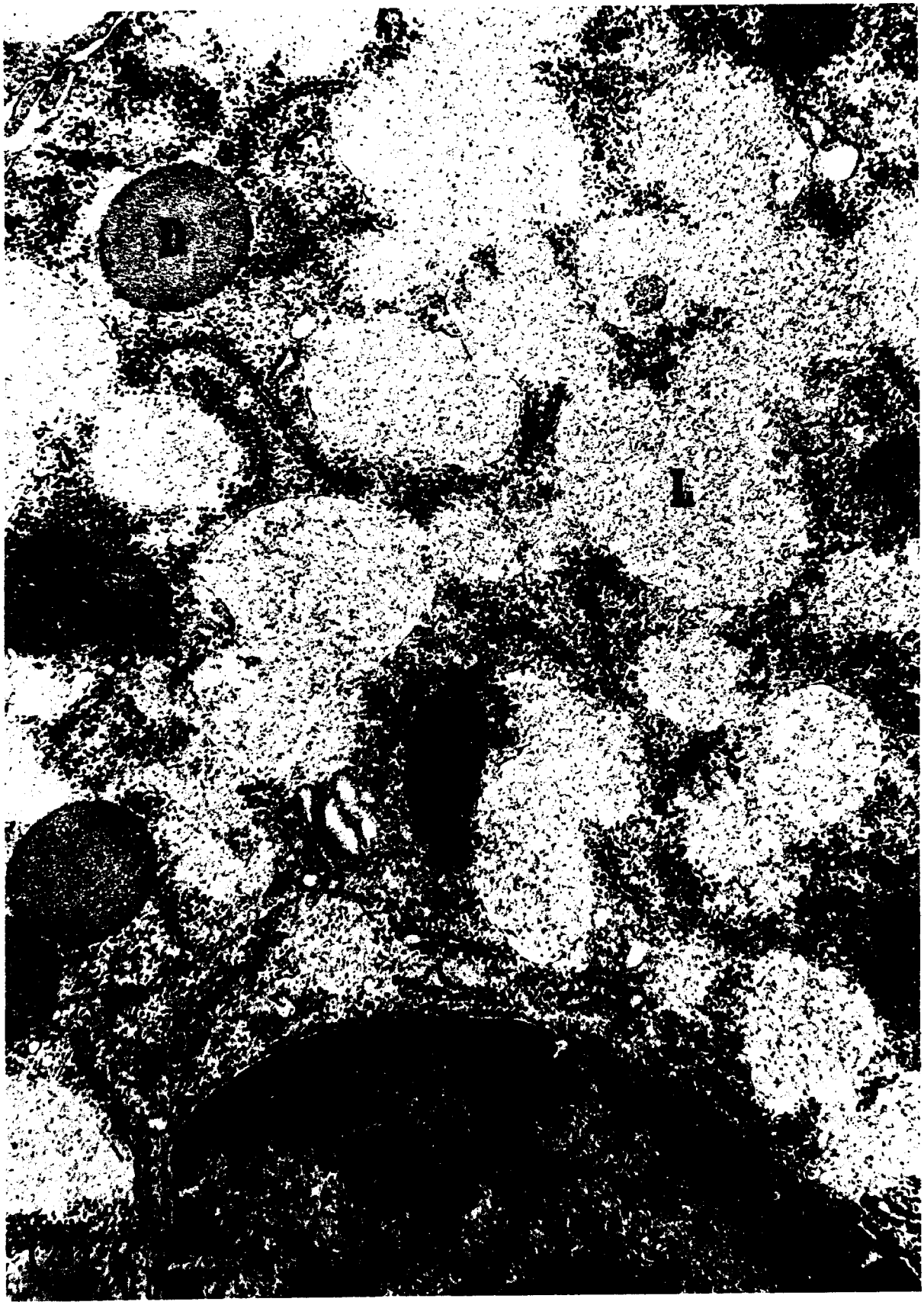


PLATE XII

Figure 21. Light micrograph of parotid gland from 24-day old control animal. Secretory endpieces contain toluidine blue-staining secretory granules and only a few basic fuchsin-staining granules. Stained with TB-BF. X 1,180.

Figure 22. Light micrograph of parotid gland from a 24-day old animal partially desalivated at 10 days of age. Secretory granules are enlarged and stain intensely with basic fuchsin. Some granules are larger and more lucent than others. Toluidine blue-staining granules are located in intercalated duct cells (I). Stained with TB-BF. X 1,180.

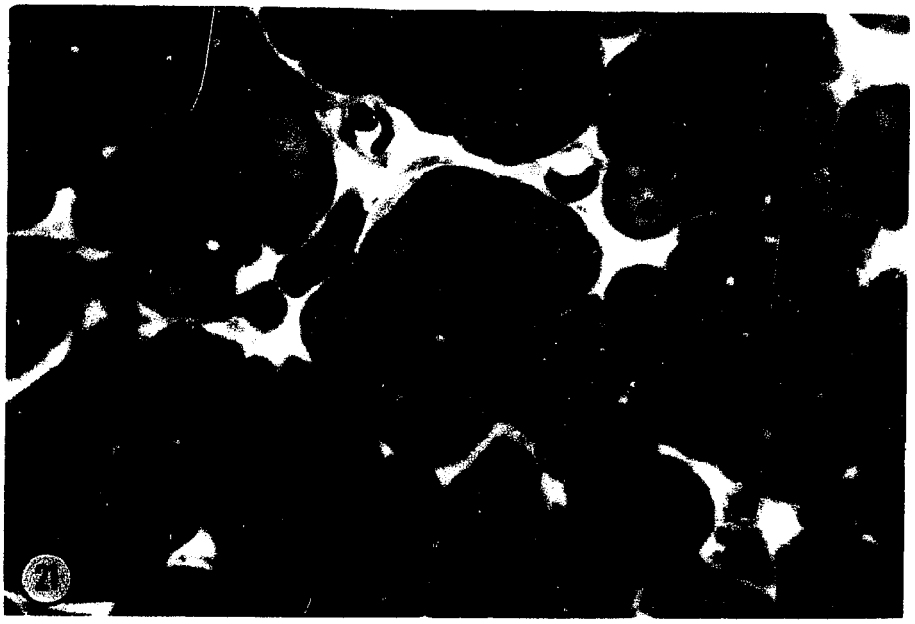
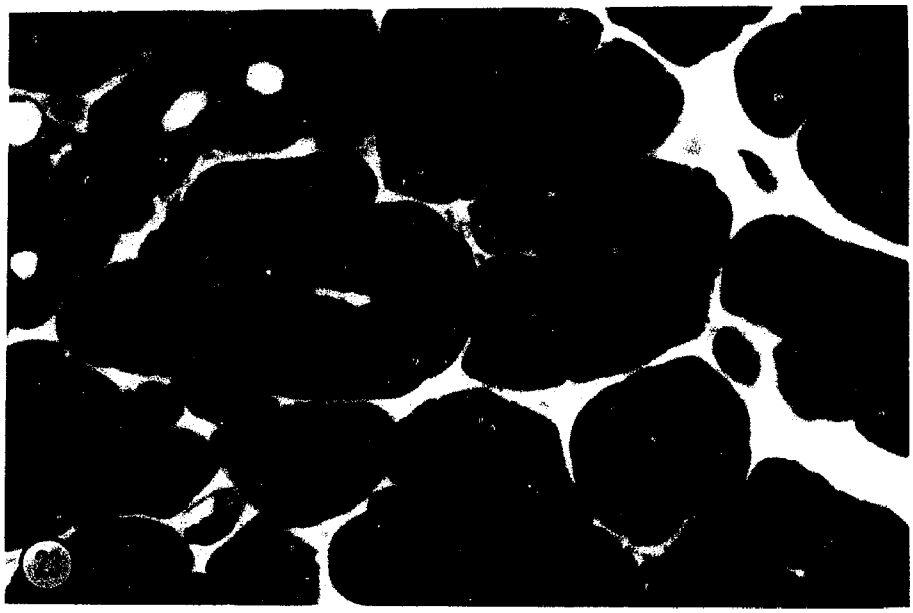


PLATE XIII

Figure 23. Light micrograph of parotid gland from 24-day old rat sympathectomized at 10 days of age. Acinar cells are completely filled with secretory granules. Most granules are stained by both dyes resulting in the purple color. Stained with TB-BF. X 1,180.

Figure 24. Light micrograph of parotid gland from 24-day old rat partially desalivated and sympathectomized at 10 days of age. A combination of enlarged, red granules, purple, and blue granules is visible. Lipid droplets are present in the basal region of several of the secretory endpieces. Two cells are undergoing mitosis (arrows). Stained with TB-BF. X 1,180.



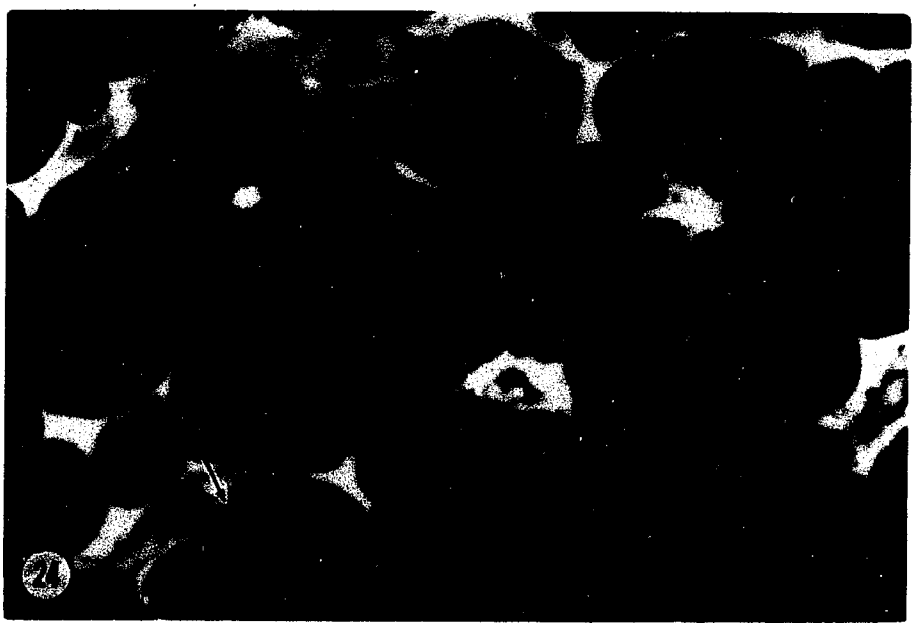
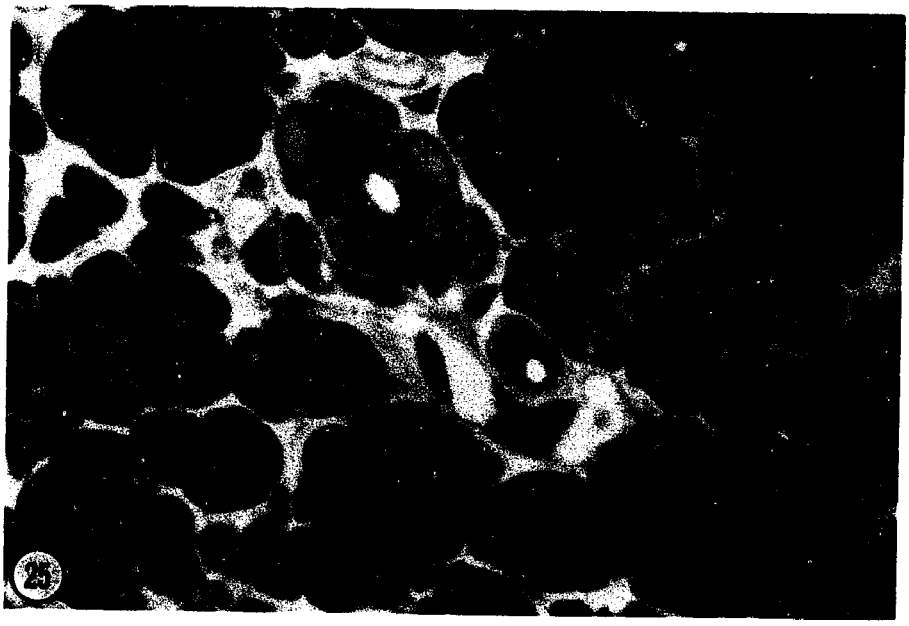


PLATE XIV

Figure 25. Light micrograph of parotid gland from 24 day-old animal parasympathectomized at 10 days of age. Secretory granules stain primarily with basic fuchsin and vary in size and staining intensity. The fact that cell size is reduced is apparent when acini are compared to those in Fig. 21. A striated duct (d) is seen in the middle of the section. Stained with TB-BF. X 1,180.

Figure 26. Light micrograph of parotid gland from 24-day old animal partially desalivated and parasympathectomized at 10 days of age. Secretory granules vary in size, color, and staining intensity. Stained with TB-BF. X 1,180.



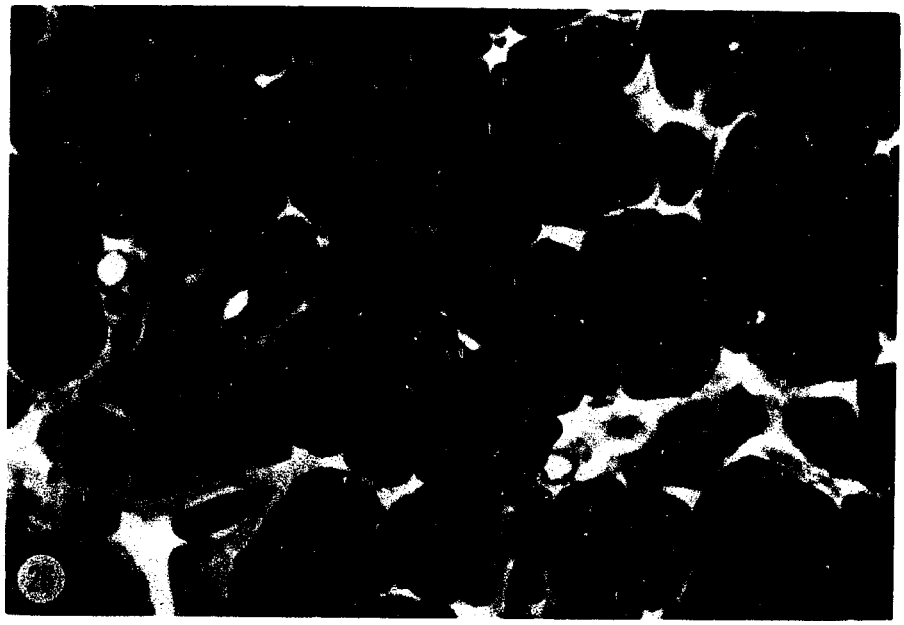
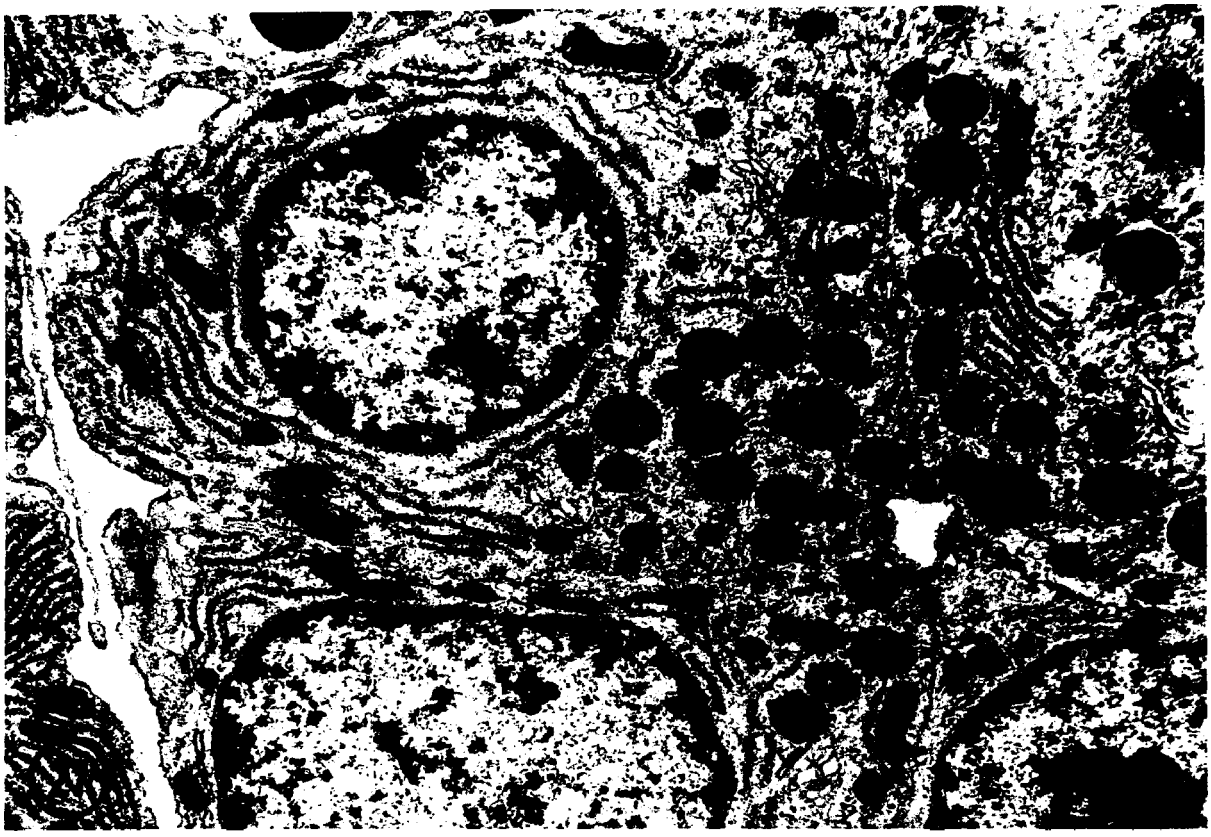


PLATE XV

Figure 27. Electron micrograph of parotid gland from 24-day old control animal. Secretory granules are electron dense and some granules contain less electron dense material in the center. X 12,200.

Figure 28. Electron micrograph of parotid gland from 24-day old animal partially desalivated at 10 days of age. Both electron lucent and electron dense secretory granules are present, but the electron lucent granules predominate. A few of the electron lucent granules appear to be fusing (arrow). X 12,200.



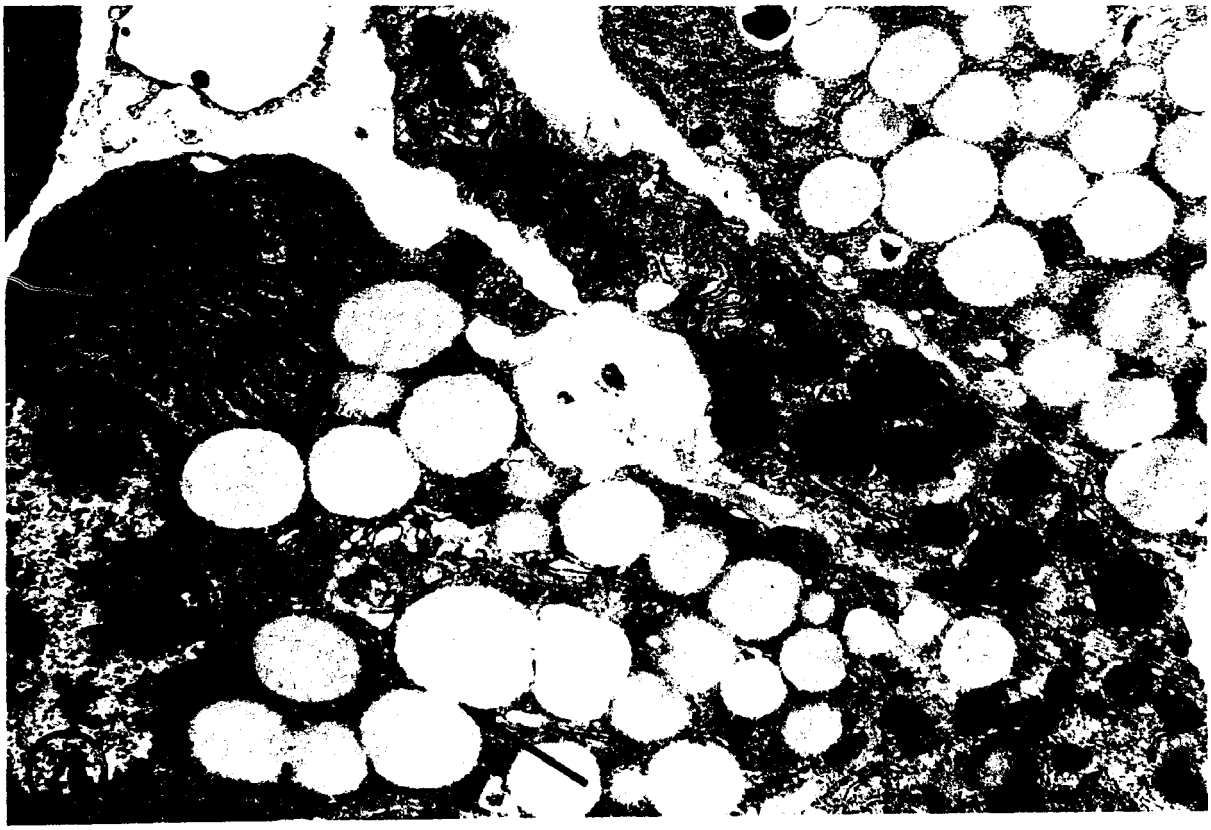
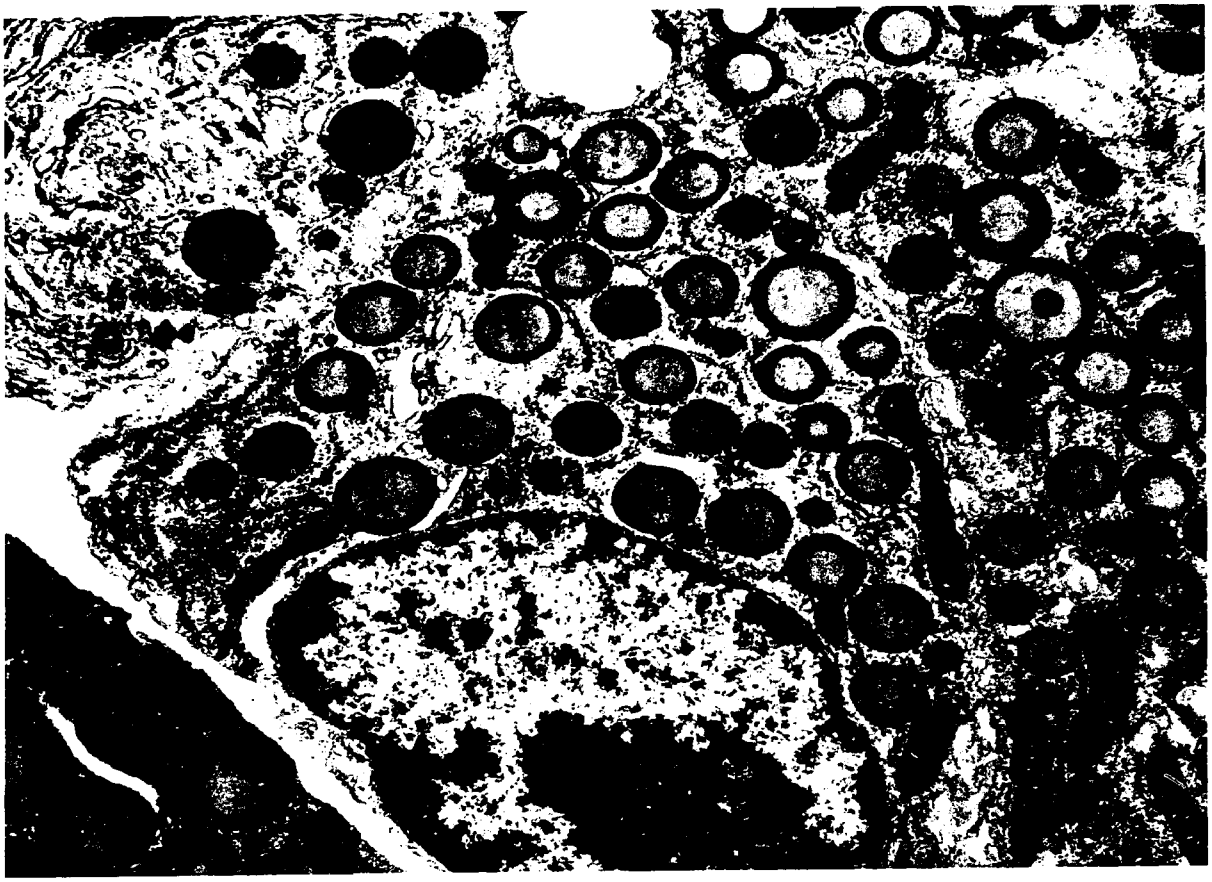


PLATE XVI

Figure 29. Electron micrograph of parotid gland from 24-day old animal sympathectomized at 10 days of age. Acinar cells are filled with secretory granules that have an electron dense rim and a core of reduced density. X 12,200.

Figure 30. Electron micrograph of parotid gland from 24-day old animal partially desalivated and sympathectomized at 10 days of age. Secretory granules in this acinar cell are uniformly electron lucent. X 12,200.



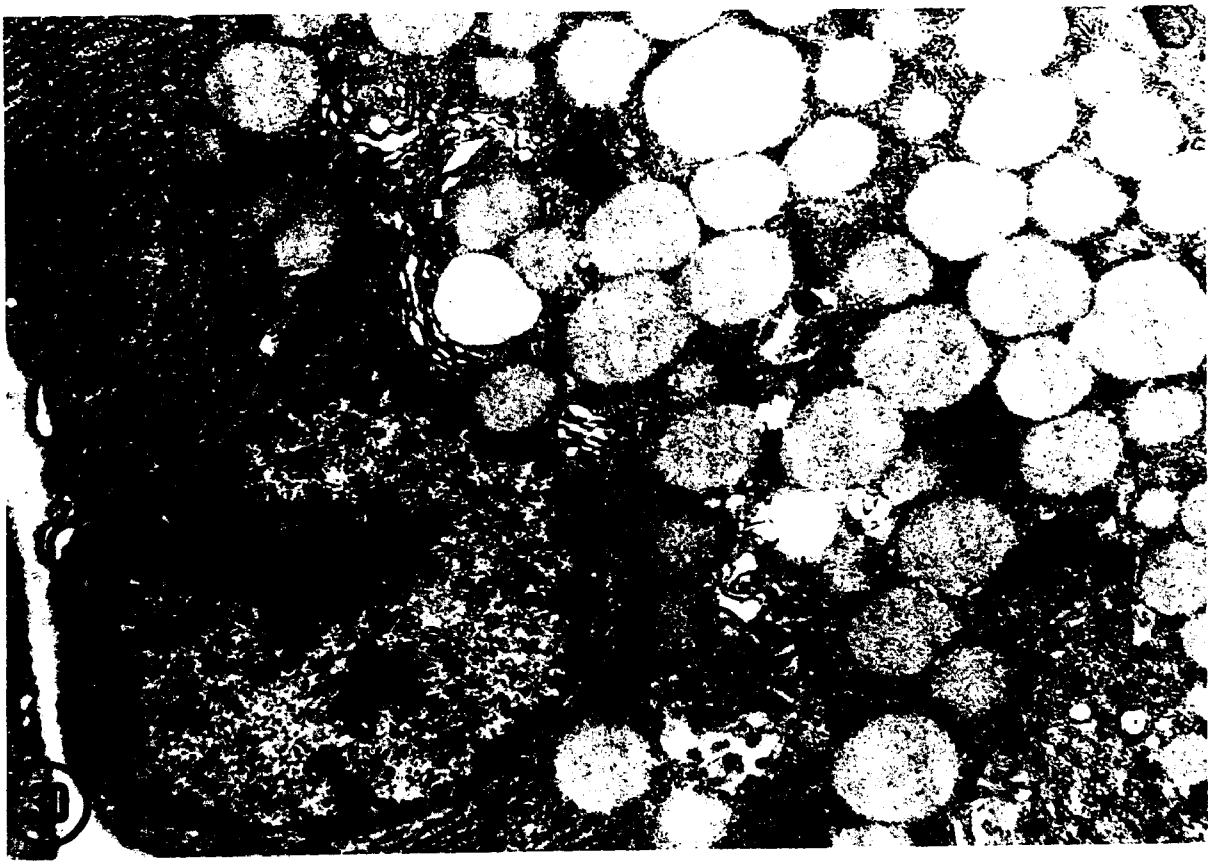
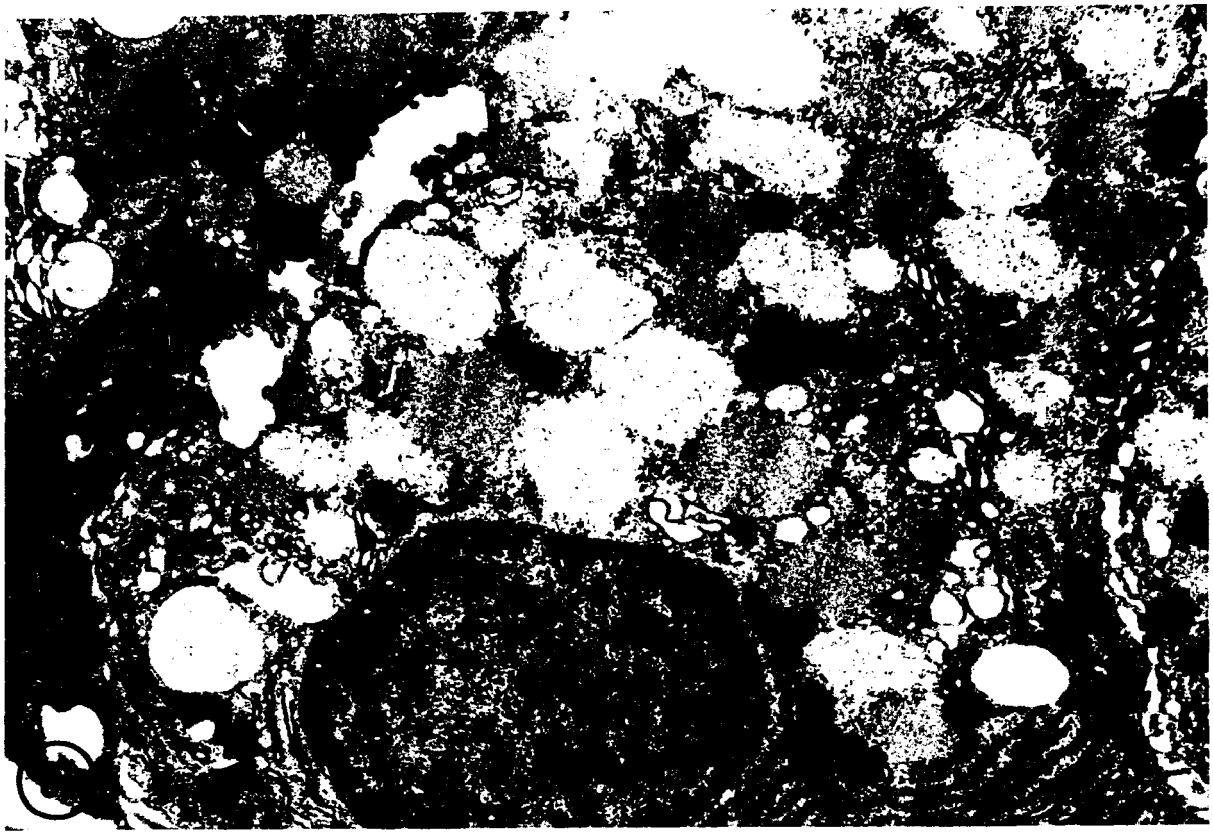


PLATE XVII

Figure 31. Electron micrograph of parotid gland from 24-day old rat that was parasympathectomized at 10 days of age. Secretory granules range from electron lucent to electron dense. Both electron lucent and electron dense granules may be present within the same acinar cell. X 12,200.

Figure 32. Electron micrograph of parotid acinar cells from 24-day old animal that was partially desalivated and parasympathectomized at 10 days of age. A variety of secretory granules ranging from electron opaque to electron lucent are visible. X 12,200.



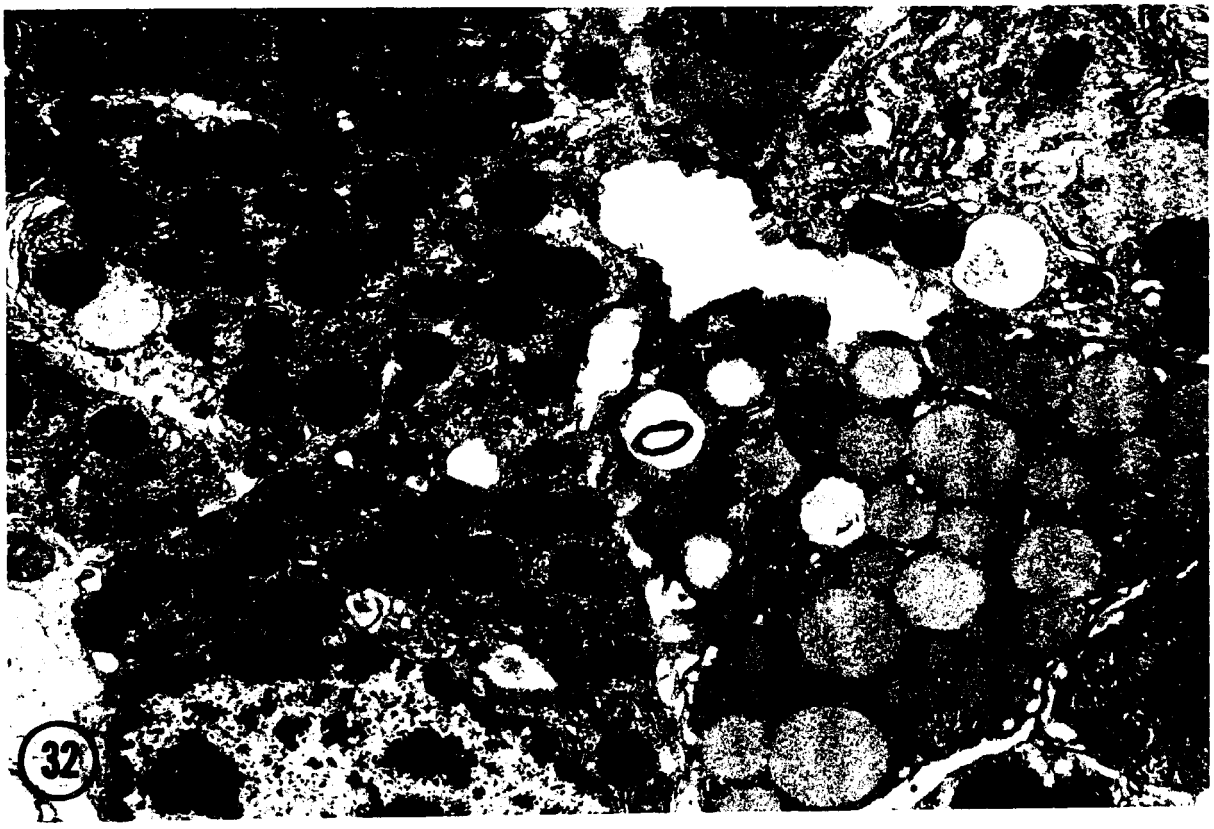

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PLATE XVIII

Figure 33. Light micrograph of parotid gland from 36-day old control animal. Toluidine blue-staining secretory granules are located in the cells of the secretory endpieces. Stained with TB-BF. X 1,180.

Figure 34. Light micrograph of parotid gland from 37-day old rat that was partially desalivated at 22 days of age. Acinar cell size is increased, and the secretory granules are enlarged and stain red with basic fuchsin. Stained with TB-BF. X 1,180.


33

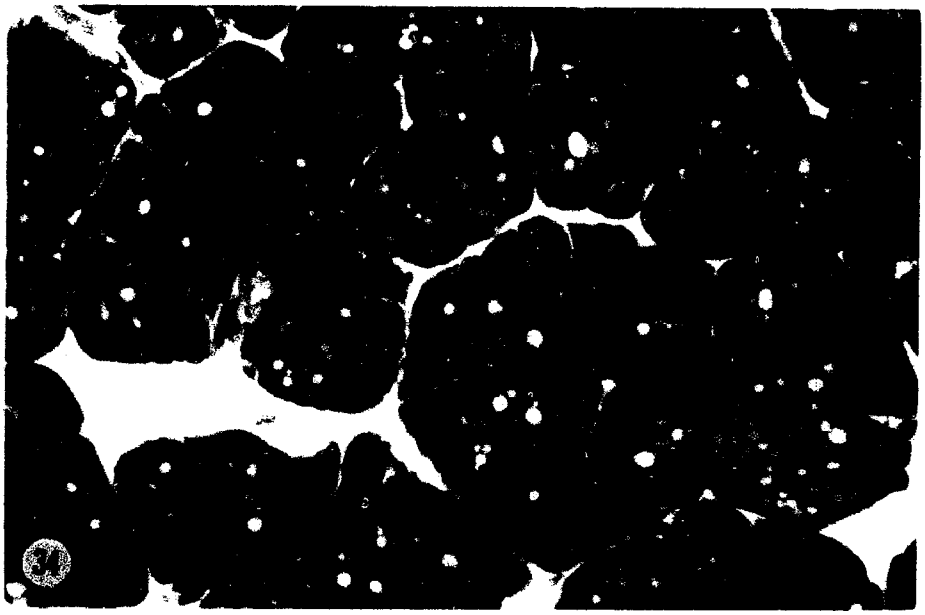
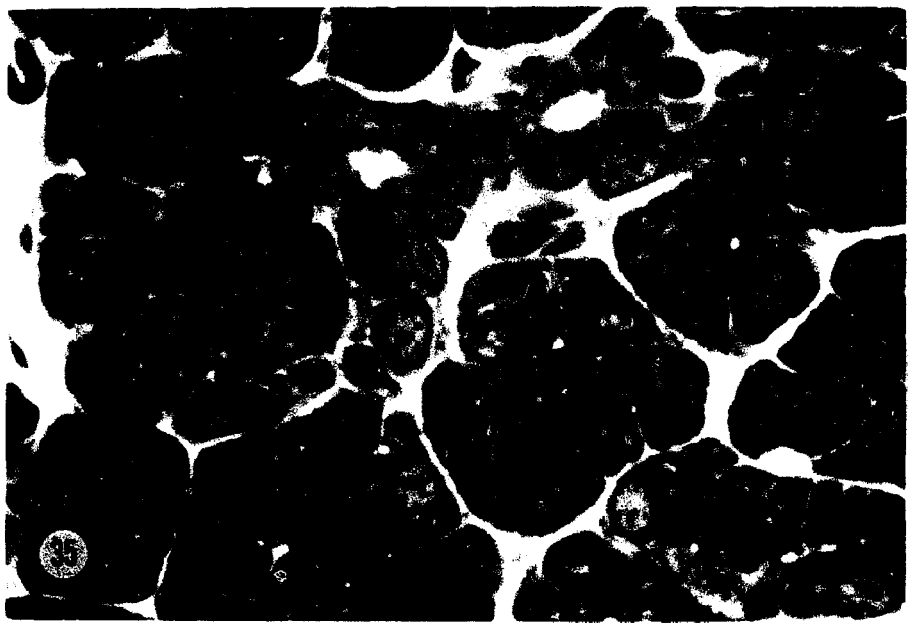

34

PLATE XIX

Figure 35. Light micrograph of parotid gland from 37-day old rat from which the superior cervical ganglia were removed at 22 days of age. Secretory granules completely fill the acinar cells, and most are stained by both dyes. Stained with TB-BF. X 1,180.

Figure 36. Light micrograph of parotid gland from 37-day old rat that was partially desalivated and sympathectomized at 22 days of age. Acinar cells and secretory granules are similar in appearance to those in Fig. 35. Stained with TB-BF. X 1,180.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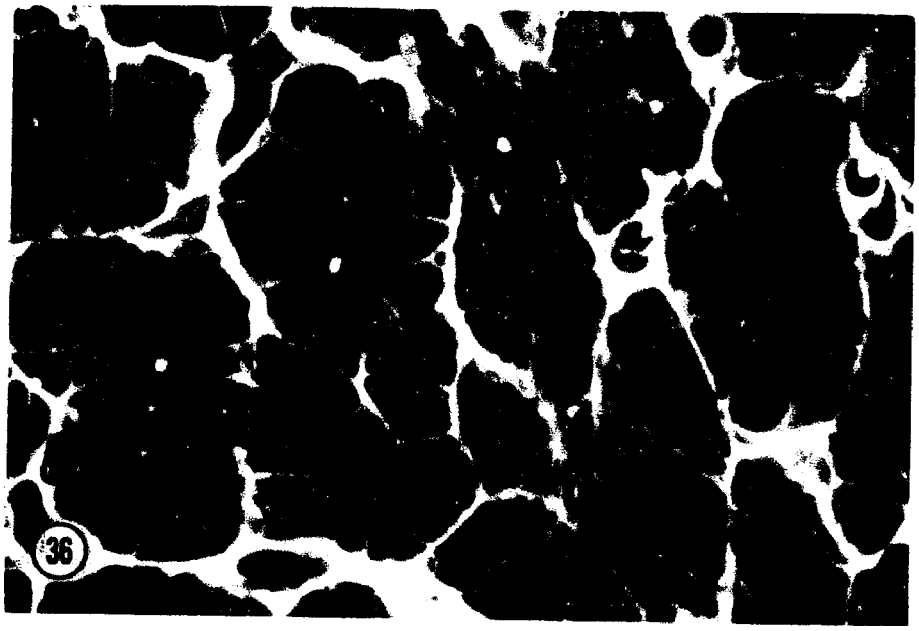
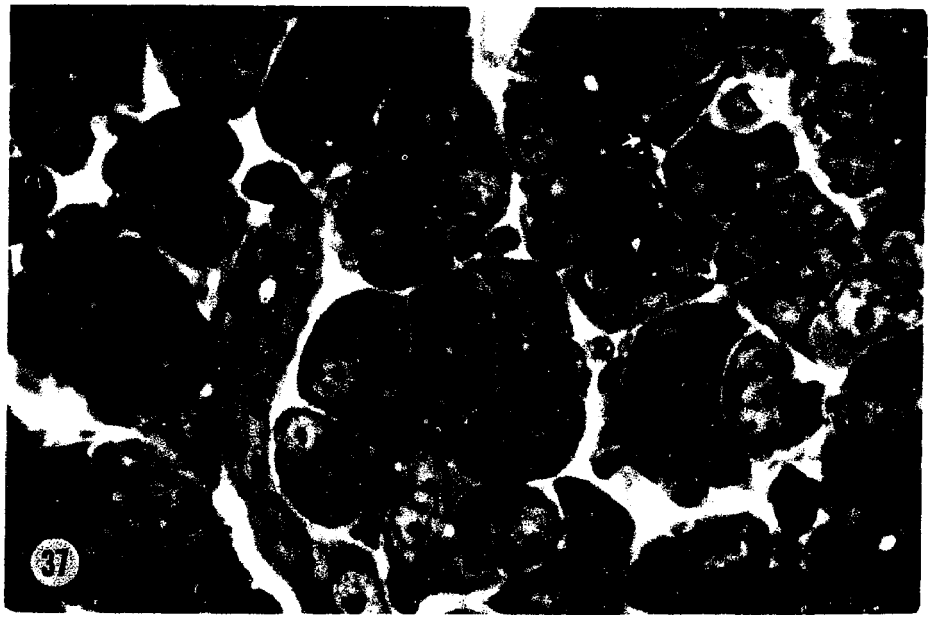

36

PLATE XX

Figure 37. Light micrograph of parotid gland from 37-day old animal that was parasympathectomized at 22 days of age. Numerous dark red staining secretory granules fill the secretory endpieces. Stained with TB-BF. X 1,180.

Figure 38. Light micrograph of parotid gland from 37-day old animal that was partially desalivated and parasympathectomized at 22 days of age. Secretory granules are enlarged and stain with basic fuchsin. Toluidine blue-staining granules occur in intercalated duct cells (i). Stained with TB-BF. X 1,180.



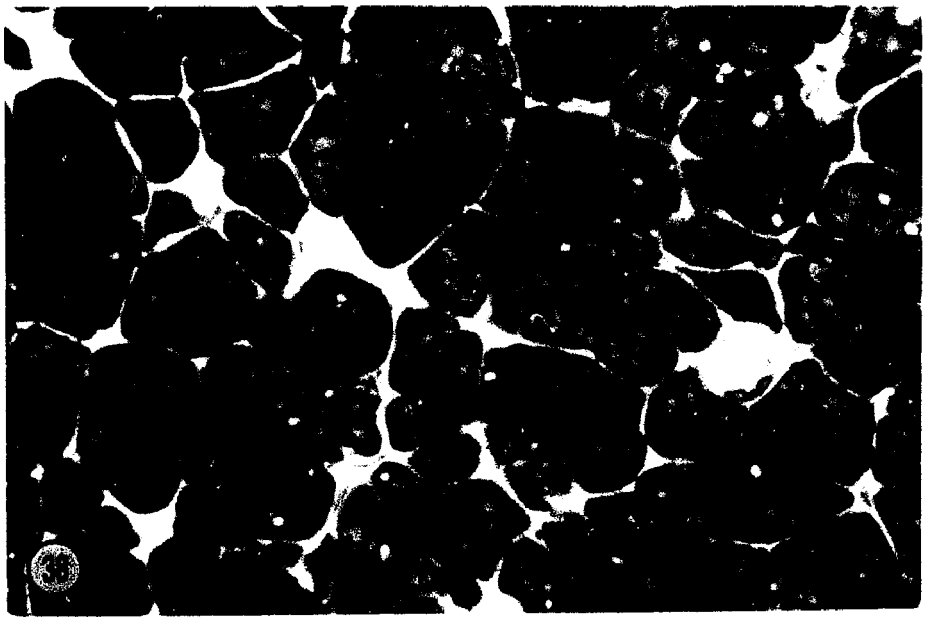
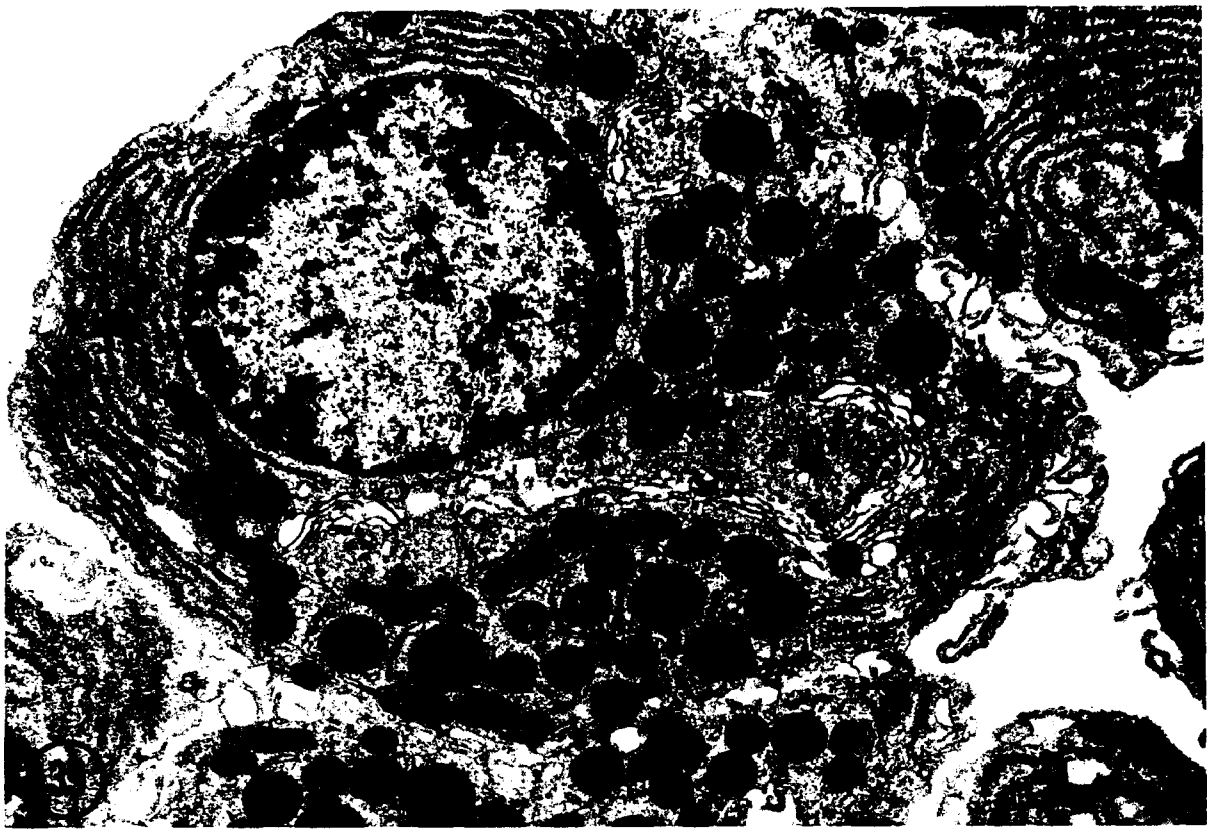


PLATE XXI

Figure 39. Electron micrograph of parotid acinar cells from 37-day old control animal. The secretory granules are uniformly electron dense. The Golgi apparatus (G) and rough endoplasmic reticulum (RER) are prominent. X 12,200.

Figure 40. Electron micrograph of parotid acinar cells from 37-day old rat that had submandibular and sublingual glands removed at 22 days of age. All secretory granules are electron lucent. X 12,200.



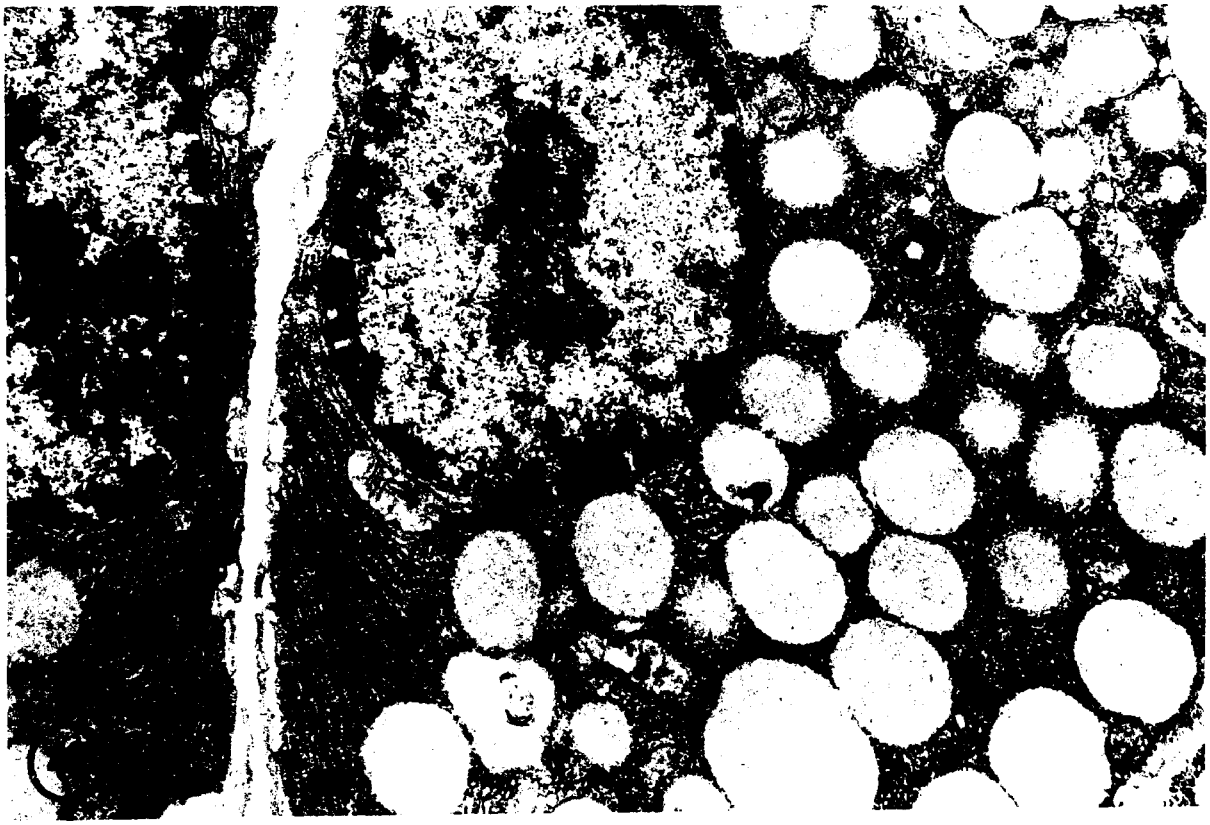
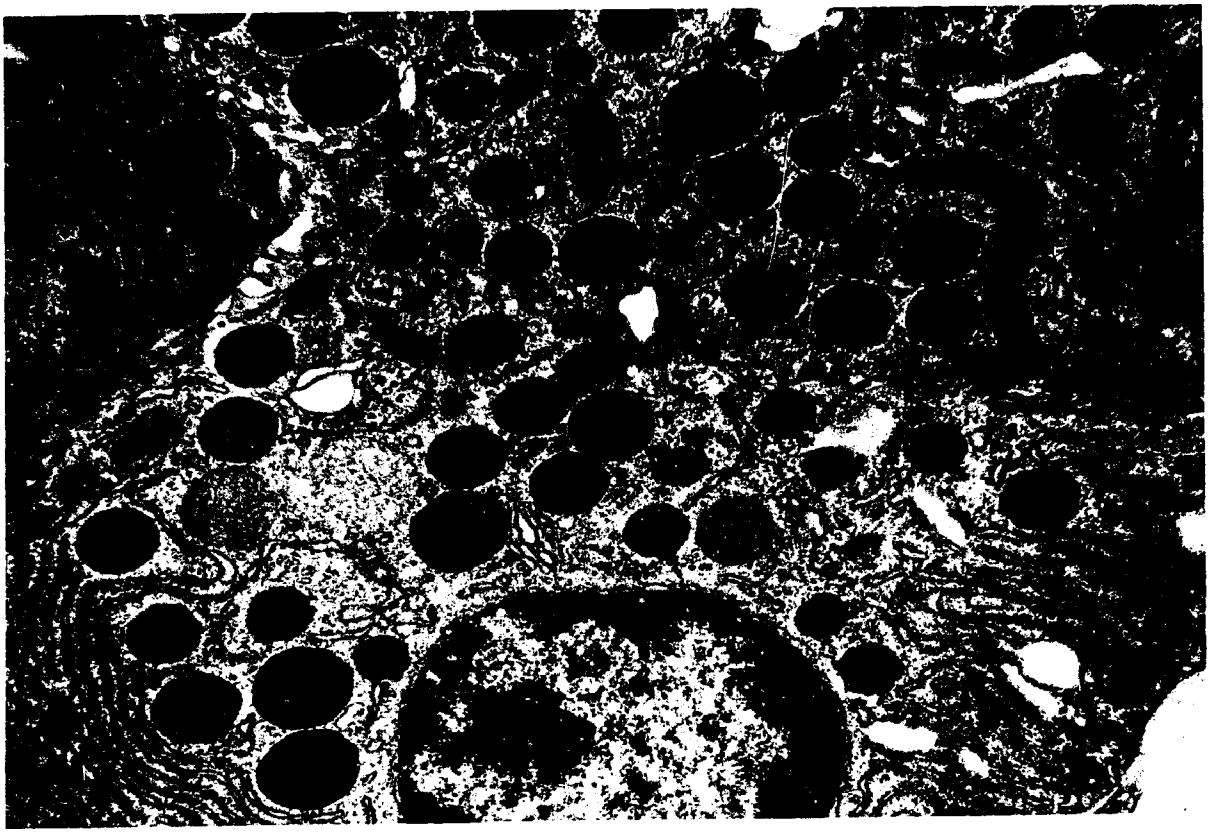


PLATE XXII

Figure 41. Electron micrograph of parotid acinar cells from 37-day old animal sympathectomized at 22 days of age. Secretory granules are unevenly electron dense. X 12,200.

Figure 42. Electron micrograph of parotid acinar cells from 37-day old animal sympathectomized and partially desalivated at 22 days of age. The secretory granules have the same mottled appearance as those in Fig. 41. X 12,200.



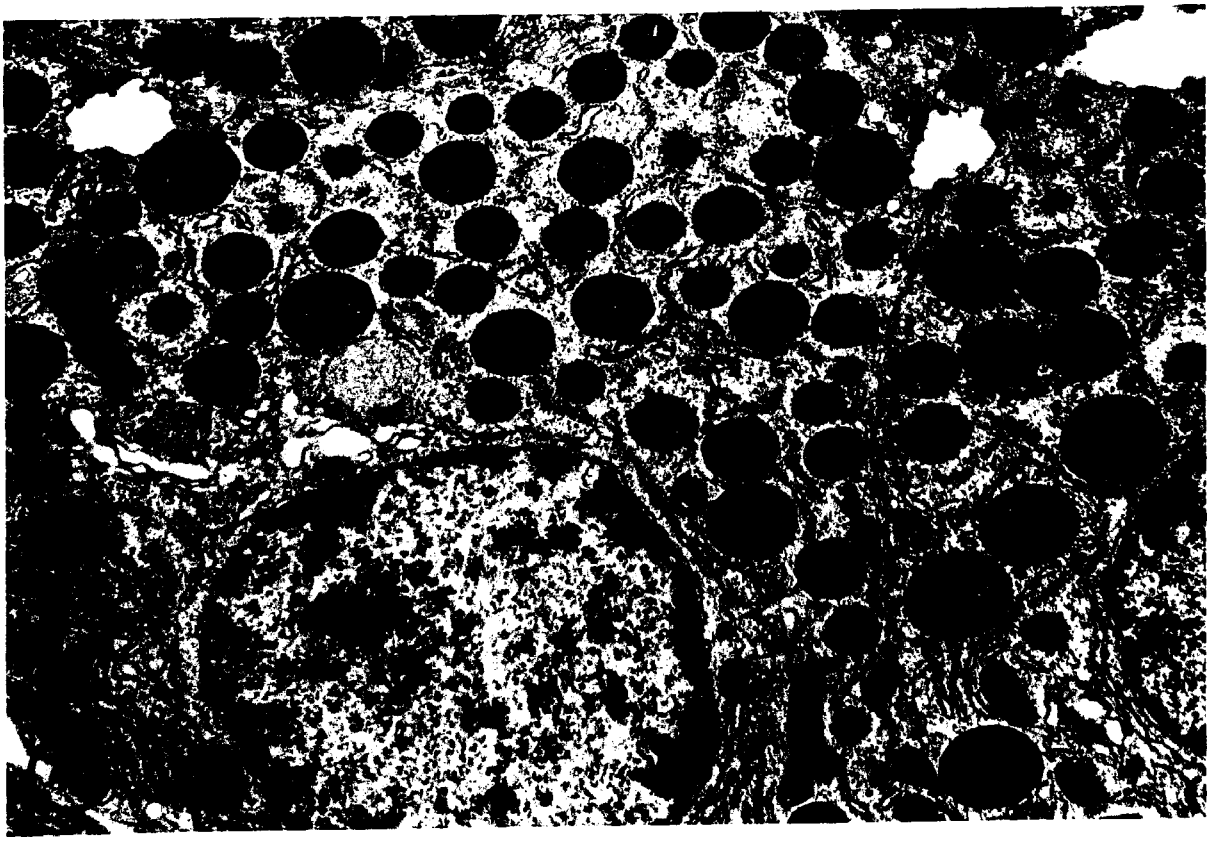


PLATE XXIII

Figure 43. Electron micrograph of parotid acinar cells from 37-day old rat parasympathectomized at 22 days of age. Secretory granules are moderately electron dense, and some have greater density at the periphery. An acinar lumen (L) is located at the top of the figure. X 12,200.

Figure 44. Electron micrograph of parotid acinar cells from 37-day old animal parasympathectomized and partially desalivated at 22 days of age. The density of secretory granules is greatly reduced so that they are almost electron lucent. X 12,200.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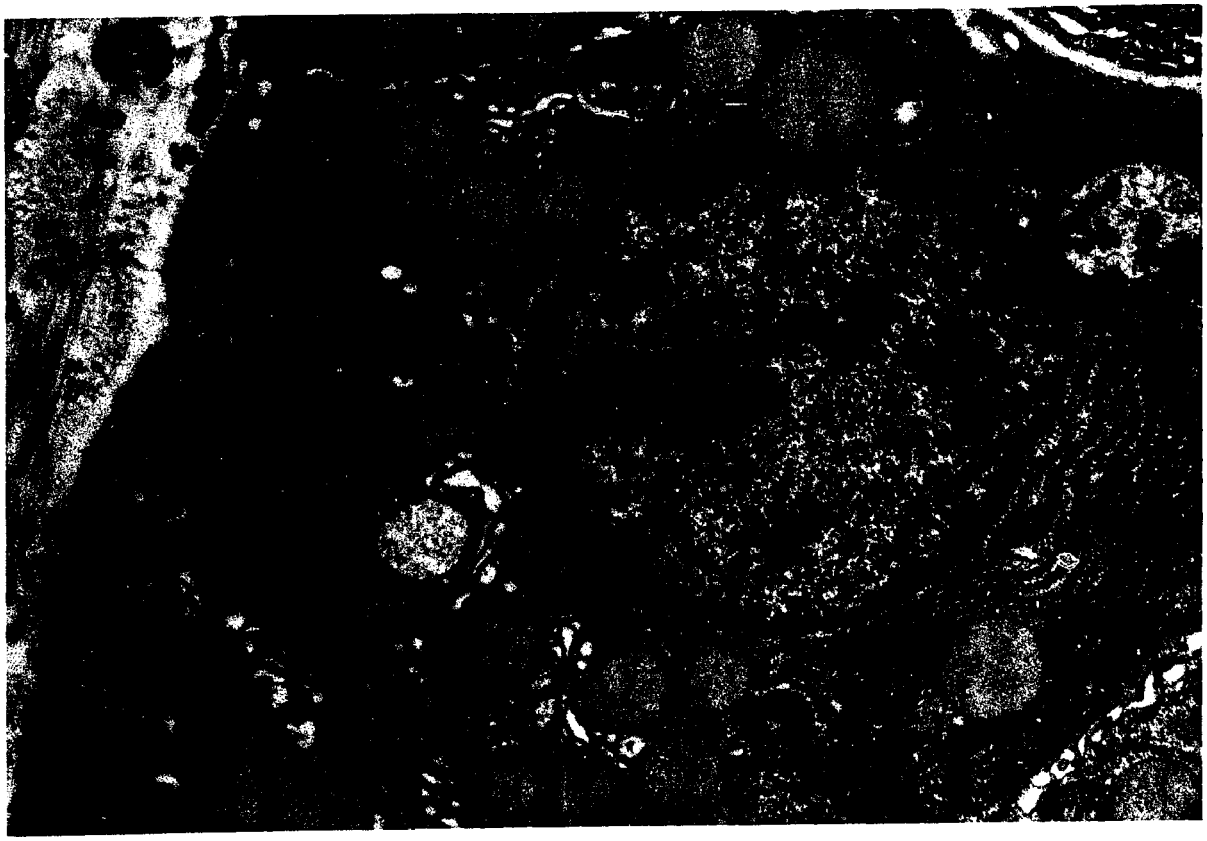

GRADUATE SCHOOL
UNIVERSITY OF ALABAMA IN BIRMINGHAM
DISSERTATION APPROVAL FORM

Name of Candidate Alice Hardin Morgan

Major Subject Anatomy

Title of Dissertation Compensatory Changes in the Parotid Gland of the Immature Rat Induced by Partial Desalivation and Denervation

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Date 2 June 1978