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THE EFFECTS OF HYPOPHYSECTOMY AND ADMINISTRATION OF PILOCARPINE ON THE PAROTID GLAND OF THE RAT: A HISTOCHEMICAL AND BIOCHEMICAL STUDY

By

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ABSTRACT

The objectives of this study were (a) to elucidate the complex histochemical and biochemical changes which occur in the parotid gland after hypophysectomy, (b) to provide basic information for a re-evaluation of the possible endocrine role of the parotid gland and (c) to reconsider the effects of the administration of pilocarpine with the aid of newer histochemical techniques.

Histochemical procedures for ribonucleic acid, sulfhydryl and disulfide groups, carbohydrate-containing proteins, alkaline phosphatase, non-specific esterase, and succinic dehydrogenase were employed as well as procedures for the demonstration of zymogenic granules and mitochondria. A biochemical quantification and a separation by starch gel electrophoresis of the esterases of the parotid gland were performed.

A marked atrophy of the acinar cell occurred after hypophysectomy. This was evidenced by reduced cellular size and reduction in number, size, carbohydrate content and affinity for aniline blue of its zymogenic granules. No change was observed in the other parameters measured except a marked reduction in the alkaline phosphatase content of the myoepithelial cells and capillaries associated with the acinar cells.

A partial depletion of the cytoplasmic granules of the intercalated duct cells was also observed. No other defects were observed. There was no atrophy of the salivary ducts as shown by a lack of significant change in either cell height or duct width. Cytologic and chemical alterations were observed, however, in the salivary and interlobular ducts including a

decrease in affinity for leucofuchsin subsequent to periodic acid oxidation, slightly reduced ribonucleic acid, accumulation of glycogen and markedly depressed esterase. Similar effects were seen in the excretory ducts with the exception of the reduced esterase activity.

Two esterase-active proteins were demonstrated by starch gel electrophoresis. Hypophysectomy did not alter these in any way. Nor did it significantly alter the esterase content of the gland. A tendency toward a reduction was evident due to the reduced activity of the salivary and interlobular ducts. This was offset by the increased basal concentration of this enzyme in the acinar cells.

It was concluded that the degree of control by the hypophysis over the parotid gland, exerted either directly or indirectly through its dependent glands, is different from that over other enzyme-producing cells. The gastric chief cells, pancreatic acini, and serous tubules of the submandibular gland undergo a much greater morphologic change after hypophysectomy than does the parotid.

No evidence was obtained to support or reject the postulated endocrine role of the parotid. The lack of more marked changes in the salivary ducts is, however, evidence against such a concept, as they, under the tropic control of the anterior hypophysis, are reported to be the source of a salivary gland hormone (parotin).

The administration of pilocarpine affects both the acinar cells and the duct system. Degranulation was observed in both the acini and the intercalated duct cells. An increase in the ribonucleic acid content of both acinar cells and duct cells

occurred. Alteration in the position of the nucleus and mitochondria as well as an accumulation of glycogen was observed in the salivary, interlobular and excretory ducts.

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INTRODUCTION

Maintenance of the normal structure and function of the parotid gland is dependent on the action of hormones since Baker and Abrams ('55) demonstrated a marked reduction in weight of the parotid gland after hypophysectomy. Concurrently, acinar cells are reduced in size and depleted of zymogenic granules and the amylase content is reduced (Baker, Klegman, Logan and Garner, '56).

Additional interest in the relationship of the parotid gland to the endocrine system is created by the postulation of Japanese workers that the parotid gland is itself an endocrine organ. Ito ('54) and Ito and Okabe ('55) reported the chemical characteristics of a purified protein substance extracted from the parotid gland of cattle and human saliva, which they believed to be the salivary gland hormone described previously by Ogata (cited by Ito, '54). The failure of the tibial epiphysis to respond to pituitary growth hormone in hypophysectomized-sialoadenectomized rats (Shibata, Negishi, Saito and Todokoro, '54), and hypertrophy of the acidophils of the anterior hypophysis after sialoadenectomy (Akazaki, cited by Shibata, et al., '54), and conversely, a hypofunction of the thyroid and a reduction in the number of the acidophils of the anterior hypophysis after chronic administration of parotin (Tasaka, cited by Shibata, et al., '54) lead

these workers to postulate that the actions of growth hormone are mediated by the parotid gland.

The objectives of this study were (a) to elucidate the histochemical and biochemical changes which occur in the gland after hypophysectomy and (b) to provide basic information for a re-evaluation of the possible endocrine role of the parotid gland. It became apparent that the Hotchkiss periodic acid-Schiff procedure, which demonstrates cytoplasmic granules within the epithelial cells of the intercalated duct of the parotid gland, might aid in clarifying the possible secretory role of that portion of the duct system. Hence, these granules were studied after administration of pilocarpine.

MATERIALS AND METHODS

Young, adult male Sprague-Dawley rats were maintained in individual cages and fed a diet consisting of Purina Laboratory Chow. The animals were given water ab libitum but no food 24 hours prior to the termination of an experiment. The parotid glands were excised while the animals were under sodium amytal anesthesia.

Hypophysectomy. Hypophysectomy was performed by the parapharnygeal approach (Smith, '30). Operated animals were allowed to recover 10 to 15 days after surgery. Subsequently, a non-hypophysectomized control animal was pair-fed against each hypophysectomized rat. The completeness of hypophysectomy was ascertained in the

majority of cases by microscopic examination of serial sections of the pituitary region. In the remaining cases it was determined by the presence of reduced body weight and by gross examination of the pituitary region. Incompletely hypophysectomized rats were discarded.

Forty hypophysectomized and 40 non-hypophysectomized control rats were utilized. The mean body weight of the hypophysectomized animals was 181 ± standard deviation 4.8 gm before the operation and 142 ± s.d. 5.8 gm at the time of sacrifice; of the non-hypophysectomized animals it was 185 ± s.d. 1.2 gm at the beginning and 184 ± s.d. 4.1 gm at the termination. The post-operative period was 40-111 days; the post-operative periods for the animals used in any one procedure did not vary by more than 20 days. The parotid glands from each animal were used for several techniques (table 1).

Histological and histochemical techniques. Small pieces of parotid gland were fixed in Zenker-formol, Regaud's fluid, formalin-alcohol-acetic acid (FAA) and 1% trichloracetic acid in 80% ethanol. Specimens fixed in Zenker-formol and Regaud's fluid were treated subsequently with 3% potassium dichromate and double-embedded in celloidin and Tissuemat. They were sectioned at 3 or 4 μ. Fixation in FAA and subsequent dehydration in acetone were carried out at 2° C since portions of these specimens were used for the demonstration of an enzyme. They were then embedded in paraffin (mp 50° - 52° C),

TABLE 1

Number of Rats Used for Each Technique

Treatment	Techniques						
	Altmann-Masson	PAS and M.B.[1]	Alkal. phos.[2]	Succinic dehydrog.	Esterase		
					Histochem.	Quant. determ.	Electro-phoresis
Hypophysectomy	10	11	12	8	11	20	8
Non-hypophysectomy	10	11	12	8	11	20	8

1. Periodic acid-Schiff and methylene blue. 2. Alkaline phosphatase

sectioned at 4μ and mounted without the use of albumin.

For the demonstration of mitochondria and zymogenic granules, sections of the glands fixed in Zenker-formol and Regaud's fluid were stained with Regaud's hematoxylin and the Severinghaus ('39) Altmann-Masson procedure. For glycogen, carbohydrate-containing proteins, and nucleic acids, sections of specimens fixed in FAA were stained with the periodic acid-Schiff technique (PAS) of Hotchkiss ('48) and counterstained with a 0.05% solution of methylene blue containing 13.5% acetone and a citric acid-phosphate buffer at pH 5.6. To verify which basophilic substances were composed of ribonucleic acid, slides were digested in a 1% unbuffered solution of ribonuclease at 37° C for one hour before staining. Control slides were incubated in distilled water under the same conditions. In order to show which PAS-staining su stances were glycogen, some slides were digested in a 1% solution of α -amylase in a 0.01 M Sorensen phosphate buffer (pH 7.0) for 10 minutes at room temperature. Control slides were incubated in the buffer solution without amylase. Specimens fixed in FAA were also used to demonstrate alkaline phosphatase by the Gomori technique ('39), sodium glycerophosphate, fructose-diphosphate, glucose-6-phosphate, yeast nucleic acid and adenylic acid being used as substrates. Sections were incubated at a pH of 9.2 to 9.4 for one and one-half hours. For controls, other sections were heated to boiling, prior to incubation, in order to destroy alkaline phosphatase

activity.

Sulfhydryl and disulfide groups were demonstrated on the trichloracetic acid-fixed glands by the method of Barrnett and Seligman ('52). For the demonstration of both groups, sections were incubated in 0.5 M thioglycolic acid adjusted to pH 8.0, at 50° C for two hours.

Succinic dehydrogenase and non-specific esterase were demonstrated in sections of non-fixed glands sectioned in the Cryostat. The technique of Farber and Louviere ('56) was used for the study of succinic dehydrogenase. After being mounted on slides within the Cryostat, the sections were air-dried at room temperature for 10 minutes for better adherence and preservation of the tissue. In contrast to the findings of Padykula ('52), sections air-dried for this period did not show loss of activity. Sections were incubated anaerobically for one and one-half hours. Slides incubated without sodium succinate in the incubation medium, but otherwise treated as described, served as controls.

Gomori's ('52) technique, with α-naphthol butyrate as the substrate, was used to localize non-specific esterase activity. Sections were treated prior to incubation in a manner identical to that utilized for the demonstration of succinic dehydrogenase. They were incubated in a substrate containing the azo-dye Garnet GBC, for one half hour at room temperature and subsequently fixed in formol-saline for 10 minutes. Control slides were incubated in a medium which did not contain α-naphthol butyrate.

Non-specific esterases also were studied by the quantitative procedure of Gomori ('53) and the starch gel electrophoretic method of Hunter and Markert ('57) for separation of the enzymes. For the quantitative procedure, the gland was homogenized, centrifuged, and diluted to a final concentration of 0.2 mg of tissue per ml. One ml of this dilution was incubated for one and one-half hours in 5 ml of buffered substrate containing 0.01 M Sorensen phosphate buffer at pH 7.0, 3×10^{-4} M α-naphthol butyrate, and the azo-dye, diazotized-4 diethylsulfonamide-2-aminoanisole, in 3.75% sodium lauryl sulfate. Solutions for determining the standard curve contained the same concentration of buffer, sodium lauryl sulfate and azo-dye.

For the separation of non-specific esterases by starch gel electrophoresis, small pieces of filter paper were saturated with the clear supernatant obtained from a centrifuged homogenate of the gland and inserted into starch strips buffered with a 0.03 M borate buffer at pH 8.5. A potential drop of 6 volts per cm was established along the starch strips. After $4\frac{1}{2}$ hours, for visualization of esterases, the strips were allowed to incubate in substrates containing α-naphthol butyrate, α-naphthol acetate, and the azo-dye Garnet GBC. This medium was buffered with a 0.08 M Sorensen phosphate buffer at pH 7.0.

Measurement of ducts and height of their epithelium. Sections stained with PAS and methylene blue were utilized in determining the effect of hypophysectomy on the diameter

of the salivary duct and the height of its epithelium. The smallest diameter was recorded only for those intralobular ducts which were cut transversely and showed some degree of basal striation. Measurements were made on 25 ducts in a single section from each animal. The data so obtained were treated statistically by a test of variance analysis (Snedecor, '56; Ostte, '54).

Administration of pilocarpine. Two groups of animals were used to study the effect of pilocarpine. The first consisted of non-treated rats which had been starved 48 hours to insure maximal storage of secretory granules within the epithelial cells of the acini and intercalated ducts. In an attempt to degranulate these cells, pilocarpine was injected in increasing doses for 6 hours. Five animals were given intraperitoneal injections of pilocarpine, expressed per 100 gm body weight, in the following doses: 0.1 mg at the beginning of the experiment, 0.5 mg at the second and third hours, and 0.8 mg at the 4th, 5th, and 6th hours. Five control animals were given injections of distilled water equaling 1.0 ml per 100 gm of body weight. The animals were sacrificed one-half hour after the last injection. Small portions of the parotid glands from each animal were fixed in FAA and Zenker-formol and stained as previously described.

The second group of animals consisted of hypophysectomized rats and their pair-fed controls. Three operated and three non-operated controls were given a single injection of pilocarpine hydrochloride at a dosage of one mg per 100 gm

body weight (one mg per ml of solution). Two hypophysectomized and two non-hypophysectomized animals, which served as controls, were given injections of water at a dosage of 1 ml per 100 gm of body weight. The animals were killed one hour after the injections and the tissues processed as described for the first group.

The animals which received pilocarpine injections all showed marked signs of parasympathetic stimulation, i.e., profuse salivation, diarrhea, shivering, and secretion by the Meibomian glands. None appeared to be near death.

OBSERVATIONS

The Normal Parotid Gland

Acinar cells. Acinar cells were typical serous cells and varied in size (fig. 1). After fixation in Zenker-formol or Regaud's fluid, the abundant zymogenic granules were of uniform size and morphology and stained intensely with aniline blue. After fixation in FAA, fewer granules were preserved so that the cytoplasm appeared vacuolated. Those granules which appeared within these vacuoles, stained by the PAS procedure with moderate intensity. With this procedure, the smaller acini contained more zymogenic granules in contrast to the larger ones, which showed only vacuolation.

The cytoplasm stained with methylene blue(fig. 3). Cells which were engorged with zymogenic granules rarely exhibited basal accumulation of the basophilic component. Digestion with ribonuclease removed completely the affinity

of cytoplasm for methylene blue, demonstrating that the presence of ribonucleic acid accounted for the basophilia. No glycogen was observed.

Mitochondria varied in shape from rods to filaments, were fairly abundant, and distributed uniformly throughout the cytoplasm. The nucleus, which usually contained a single prominent nucleolus, was somewhat compressed against the basal membrane of the cell by the abundant zymogenic granules.

Two types of cytoplasmic inclusion bodies were observed in all non-hypophysectomized animals. Their incidence in any one gland was low. One type, which appeared to be of cytoplasmic origin, was spherical, internally vacuolated, surrounded by a halo, and stained with PAS and aniline blue (fig. 6). It varied in size, sometimes filling the entire cytoplasmic space. The second type of inclusion (fig. 6) appeared to be of nuclear origin. It was spherical, homogeneous and intensely basophilic. Although variable in number and size, it often was larger than a typical nucleus. When present, no nucleus was observed, and the smaller ones appeared to have resulted from karyorrhexis. The two types of inclusions occurred together in the same cell or separately in different cells.

The technique for sulfhydryl groups stained cytoplasm, chromatin and the nuclear membrane. Incubation in thioglycolic acid, which reduces the disulfide groups to sulfhydryl groups, intensified the color in both locations (fig. 5). Since zymogenic granules were not preserved by

the fixation, their content of sulfhydryl and disulfide groups could not be determined.

No alkaline phosphatase was observed after fixation in FAA, acetone, 80% alcohol, or 10% buffered-formalin. A slight darkening of nuclei and cytoplasm was regarded as an artifact since it appeared in the control slides. However, alkaline phosphatase was present in the myoepithelial cells surrounding portions of the acini (fig. 10). These cells were more uniformly active about acini which were located close to intercalated ducts. The peri-acinar capillaries exhibited a high degree of activity.

The acinar cells contained a high concentration of esterase (fig. 15), a fine granular deposit of the coupled azo-dye being distributed evenly throughout the cytoplasm. No activity was observed when naphthol AS acetate was used as the substrate. Control slides revealed no granular deposit.

The succinic dehydrogenase system was demonstrated in the acinar cells (fig. 17), small crystals of the reduced neotetrazolium salt being distributed evenly throughout the cytoplasm. This distribution correlated well with that of mitochondria. In control slides incubated without sodium succinate, there was no deposition of the reduced neotetrazolium salt. Within two hours after preparation of these slides, rather large droplets appeared within the acinar cells which became pink in color. These droplets, of unknown nature, apparently dissolved the reduced neotetrazolium crystals.

Intercalated ducts. The intercalated ducts were long, branched, and narrow. Their transition into the salivary or striated ducts was abrupt. The ductular epithelium was squamous and extended into the acinus. After Zenker-formol fixation, the nucleus of the cell was elongate and contained coarse chromatin granules; the cytoplasm was homogeneous. The mitochondria, which were seen with difficulty, appeared as fine granules or short rods and were distributed in all parts of the cell.

Small granules, which varied in size and stained with aniline blue, were found within the cytoplasm of the epithelial cells throughout the length of the duct. With PAS, they stained more intensely and seemed more abundant (fig. 3). They were located, for the most part, in the apical portion of the cytoplasm and occurred most abundantly in the portion of the duct nearest the acinus. The epithelial cells proximal to the acinus were usually engorged while the more distal portions of the duct showed considerably fewer granules. They were resistant to digestion with α-amylase. No glycogen was observed. Distinct accumulations of basophilic material were located near the nucleus while a more diffuse basophilia commonly occurred throughout the cytoplasm. This property was not observed after digestion with ribonuclease.

With the procedure for sulfhydryl groups alone, and for sulfhydryl and disulfide groups together, intercalated ducts stained more intensely than acinar cells (fig. 5). Alternate sections treated with the PAS technique demonstrated

the capacity of trichloracetic acid to preserve the cytoplasmic granules. Also, the portions of the duct containing the greatest number of these granules, was stained most intensely with the procedures for sulfhydryl and sulfhydryl and disulfide groups (fig. 5). From these observations it was inferred that the cytoplasmic granules of the intercalated duct contained a moderate concentration of sulfhydryl and disulfide groups.

The intercalated duct exhibited marked alkaline phosphatase activity (fig. 10). The myoepithelial cells and the basal cytoplasm of the duct cells contained a large concentration of this enzyme. Little activity was observed in the more apical cytoplasm of the duct cells except for the luminal border. The endothelium of the capillaries associated with these ducts exhibited a moderate amount of phosphatase.

No difference in localization of alkaline phosphatase was observed with the various substrates utilized for the demonstration of the enzyme. However, variation was noted in the degree of activity and sharpness of localization. Sections incubated with fructose-diphosphate as the substrate showed the greatest blackening of the active areas. Little difference was observed between sodium glycerophosphate and glucose-1-phosphate. Adenylic acid and yeast nucleic acid gave the least activity and sharpness of localization.

Fine granules of coupled azo-dye, indicative of the presence of esterase, were demonstrated throughout the

entire length of the intercalated ducts (fig. 15). The level of activity was comparable to that observed in acinar cells.

The succinic dehydrogenase system was operative in the epithelial cells lining the intercalated duct. The deposition of the reduced neotetrazolium salt appeared intermediate in amount between that observed in the acinar cells and in those lining the salivary and larger ducts.

Salivary (intralobular), interlobular, and excretory ducts.[1] The vesicular nuclei of columnar epithelial cells lining the salivary, interlobular and excretory ducts were located in approximately the middle portion of the cell. The characteristic basal striations of these cells were not apparent after the Altmann-Masson procedure due to the great number of granular to rod-shaped mitochondria which occurred in great numbers throughout the cell, excepting the apical portion (fig. 1).

The PAS procedure stained the epithelial cytoplasm lightly and the basal striations and luminal border, intensely (figs. 7 and 9). Sparsely distributed granules of variable size and regular occurrence were observed in the perinuclear and apical portions of the cell (fig. 9). They took the PAS stain weakly and were amylase-resistant. Glycogen was rarely observed. When present, it was usually

1. The term "excretory duct" encompasses the larger branches of the duct system, which were isolated by the presence of extensive connective tissue and possessed no intimate positional relationship to the paremchymal lobules.

restricted to the larger interlobular and excretory ducts, although small accumulations were observed occasionally in the cells of the salivary ducts.

Basophilic material was present in the apical portion of the cytoplasm and less often, in the middle and basal portions of the cell. It varied in form from a diffuse reticulum to clumps. This basophilia was ascribed to the presence of ribonucleic acid since it was not observed after digestion with ribonuclease. The salivary ducts contained a type of epithelial cell which resembled those described by Rawlinson ('35) in the cat. It was compressed laterally and stained intensely with methylene blue. A finely granular and strongly PAS-positive material was observed in the cytoplasm.

The cytoplasm of the salivary and interlobular duct cells was a deep rose color after application of the procedure for sulfhydryl groups, and prior reduction with thioglycolic acid intensified this reaction (fig. 5). Basal striations were delineated clearly. The more apical portion of these cells, which showed little specialization when prepared by other procedures, was deeply stained.

No alkaline phosphatase was observed (fig. 10). The capillaries and arterioles associated with the salivary ducts usually exhibited less activity than those surrounding the acini. Those adjacent to the interlobular and excretory ducts were always blackened intensely. Here, also, only a slight variation in the amount of phosphatase was revealed

by the use of different substrates.

The greatest esterase activity of the parotid gland was localized in the salivary, interlobular, and excretory ducts. However, this varied in degree between ducts and between cells within a single duct. Some salivary ducts showed a level of activity comparable with that of the acini while the majority exhibited much more (fig. 13). The interlobular and larger excretory ducts were always intensely active. The greatest concentration of esterase occurred in the basal portion of the epithelium and during incubation the azo-coupled dye appeared first in this region. Control sections incubated without α-naphthol butyrate exhibited only a background color. Little or no activity could be demonstrated with naphthol AS acetate as the substrate.

The salivary, interlobular and excretory ducts showed a high degree of activity by the succinic dehydrogenase system (figs. 17 and 18). Small granular crystals of reduced neotetrazolium were distributed throughout the cytoplasm of the epithelial cells except for their most apical portion. This distribution correlated well with that of mitochondria. This relationship was quite obvious in the excretory ducts (fig. 19) where the cells were taller and had, proportionately, more apical cytoplasm containing no mitochondria. As compared with the ducts, the lesser deposition of neotetrazolium salt in the acinar cells was proportional to the smaller concentration of

mitochondria found in them. No reduced neotetrazolium salt appeared in control sections which were incubated in a medium with no sodium succinate.

Separation of esterases by electrophoresis. Zymogram B (fig. 23) shows the two distinct esterase-reactive bands uniformly seen in all cases. No difference was observed with either ∝-naphthol butyrate or ∝-naphthol acetate employed as the substrate. Neither band was demonstrated after incubation with naphthol AS acetate. Zymogram C (fig. 23) reveals the esterase-reactive bands from a liver homogenate. This illustrates the constant relationship of migration of the reactive proteins of the parotid to those of the liver. Difficulty was encountered occasionally in digestion of the starch gel by the amylase present in the sample.

The Parotid Gland After Hypophysectomy

Acinar cells. A marked atrophy of the acinus occurred after hypophysectomy with reduction in the number and size of the intracellular zymogenic granules (fig. 2). After Zenker-formol fixation, most cells contained no zymogenic granules at all or only a few small ones in their most apical portion. A few cells, however, were filled with small zymogenic granules. These granules rarely stained as intensely with aniline blue as did those of the non-hypophysectomized animals. They were usually a brown to gray-blue color. After fixation with Regaud's fluid, small

zymogenic granules were preserved in all acinar cells. These granules also were brown after staining with aniline blue. Occasional acinar cells contained brown-staining granules which were located basally and centrally in addition to a few apical cyanophilic granules. With the PAS procedure following FAA fixation, the few granules present stained less intensely than in non-hypophysectomized rats (fig. 4).

The cytoplasm throughout the cells was strongly basophilic when stained with methylene blue. The basophilic component between the zymogenic granules or their vacuoles consisted of lightly stained granules which were more coarse than in non-hypophysectomized animals. In the basal portion of the cell, large intensely basophilic clumps appeared which were either homogeneous or lamellar (fig. 4). As described in non-hypophysectomized animals, a similar variation occurred in cellular size and preservation, and in staining affinity of zymogenic granules. The incidence of the two types of cytoplasmic inclusions was increased by hypophysectomy with probable involvement of more cells within an acinus. There was no alteration in the structure of the inclusions. No change was observed after hypophysectomy in either the distribution or degree of cytoplasmic reaction when stained for sulfhydryl groups alone or for sulfhydryl and disulfide groups together.

There was a marked reduction in the alkaline phosphatase of the myoepithelial cells associated with the acini (figs.

11 and 12), none being observed except on rare occasions. The activity of the peri-acinar capillaries was reduced. This change was not as uniform or as marked as that in the myoepithelial cells. Many capillaries were without demonstrable phosphatase while others were as active as those in non-hypophysectomized rats. No alteration appeared in succinic dehydrogenase activity (fig. 18). With respect to esterase, there was an increased basal concentration of the coupled azo-dye granules (fig. 16), suggesting an elevated concentration of the enzyme after hypophysectomy.

Little change was observed in the morphology of either the nucleus or mitochondria. The nucleus was spherical and usually contained a single large nucleolus. It was, however, less basally compressed. In those acini containing little granulation or vacuolation, it occupied a more central position. There was a decrease in number of mitochondria since they were not more concentrated in the smaller cells resulting from hypophysectomy. Mitochondria were restricted to perinuclear and basal positions of the cell.

Intercalated ducts. After hypophysectomy, the intercalated ducts appeared somewhat wider, shorter and with a slightly thicker epithelium (fig. 4). The cytoplasmic granules, which stained both with aniline blue and the PAS procedure, were of the same size and morphology as those seen in the glands from non-hypophysectomized animals. However, they were reduced in number (fig. 4). This was

seen most clearly in those cells nearest the salivary ducts, in which the granulation was either sparse or absent. Engorgement of epithelial cells near the acinus with granules, was not as common as in glands from non-hypophysectomized animals. There appeared to be no change in the basophilic material of the duct cells. It was, however, more readily apparent than in control glands because of the presence of fewer cytoplasmic granules. The concentration of sulfhydryl and disulfide groups was reduced commensurate with the reduction in cytoplasmic granules. Phosphatase (fig. 11), esterase (fig. 16) and succinic dehydrogenase (fig. 18) were not altered in distribution or degree of activity.

Salivary (intralobular), interlobular and excretory ducts. Little change occurred in the cytology of the cells lining the salivary, interlobular and excretory ducts after hypophysectomy. These cells appeared somewhat narrower and the mitochondria more dense (fig. 2).

The mean diameter of the salivary ducts in non-hypophysectomized animals was 20.9 ± s.d. 0.17μ and in hypophysectomized, 21.1 ± s.d. 0.15μ. A similar increase was observed in the height of the epithelium, being 8.1 ± s.d. 0.08μ for the control glands and 8.3 ± s.d. 0.06μ after hypophysectomy. These differences were not significant. Variance analysis showed highly significant differences between animals in both groups. These differences were not due to variation in body weight or duration of post-operative period.

After hypophysectomy, the cytoplasm of epithelial cells lining these ducts stained less intensely with PAS than in non-hypophysectomized animals. The basal striations, luminal border and basement membrane reacted similarly. The most prominent alteration was a great increase in the amount of glycogen (fig. 8). in the epithelium of interlobular and excretory ducts, the glycogen occurring chiefly in the apical cytoplasm. The salivary ducts rarely showed such large accumulations and in them, the granules of glycogen were finer and more evenly distributed throughout the apical portions of the cell. Occasionally they occurred basally between the vertical striations. In all three types of ducts, great variation was observed in the amount of glycogen within different cells of one duct and in different ducts. Cytoplasmic basophilic material was reduced slightly by hypophysectomy. No change was observed in either the distribution or intensity of staining for sulfhydryl groups alone or sulfhydryl and disulfide groups together.

As in the controls, no alkaline phosphatase was observed in either the salivary (fig. 12), interlobular or excretory ducts after hypophysectomy nor did a reduction occur in those vessels associated with interlobular and excretory ducts. Hypophysectomy had no effect on the succinic dehydrogenase system (fig. 18). Of the 11 glands studied from hypophysectomized animals, 9 exhibited a marked fall in esterase activity in the salivary and inter-

lobular duct cells (fig. 14). The activity of these ducts after hypophysectomy was comparable to that observed in acinar cells. A similar change was not evident in the excretory ducts (fig. 14). In the two cases which did not follow this pattern, the level of activity in all three types of ducts equalled that in the control glands.

Biochemical determination of esterase. A tendency toward an increase in the concentration of esterase occurred after hypophysectomy. The difference between a mean of 23.6 ± s.d. 2.9 μg of α-naphthol/ 0.2 mg of tissue for hypophysectomized animals and 17.1 ± s.d. 1.3 μg for the non-hypophysectomized animals was not significant (table 2, .10>P>.05). However, when expressed in terms of total concentration, a reduction of from 14.92 ± s.d. 0.92 mg of α-naphthol for the non-hypophysectomized animals to 10.89 ± s.d. 1.2 mg of α-naphthol for the hypophysectomized rats was observed. This difference was significant at the 1 to 2 per cent level. A test of variance homogeneity of the various groups showed a significant lack of homogeneity. Since the Fisher t-test assumes variance homogeneity, a modified form of the test was used here. The length of the post-operative period had no linear effect upon the results.

Separation of esterases by electrophoresis. Hypophysectomy had no effect on the esterase-active bands of the parotid gland which were obtained with starch gel electrophoresis (fig. 23, zymogram A). This applies to intensity with which the bands stained and the distance which they

TABLE 2

The Effect of Hypophysectomy on the Non-Specific Esterase Activity of the Parotid Gland

Treatment	No. of Rats	Days Posthyp.	Body wt. (gm)		Mean par. wt. (mg)	Mean esterase activity (mg α-naphthol)[1]	
			Initial	Final		Per 0.2 mg Par. x10^{-2}	Total
Non-hypophysectomy	20	45-71	184 ± 2[2]	187 ± 7	179.8 ± 6.5	1.71 ± 0.13	14.92 ± 0.92
Hypophysectomy	20	45-71	185 ± 2	145 ± 2	92.4 ± 2.9	2.36 ± 0.29	10.89 ± 1.2
					P = .001[3] df[4] = 27	.10>P>.05 df = 25	.02>P>.01 df = 37

Par. = parotid. wt. = weight

1. Released during 1½ hrs. of incubation at 37° C.
2. Standard error of the mean.
3. Modified Student-Fisher t-test (Dixon and Massey, '51)
4. df = degrees of freedom

migrated. No difficulty with amylase digestion occurred in the preparation of zymograms from hypophysectomized animals which probably results from the reduced amylase content of the gland (Baker, Klegman, Logan and Garner, '56).

The Effect of Pilocarpine Administration

The profound stimulation produced by the graded regimen of pilocarpine injections lead to a total degranulation of the majority of acinar cells. Some acini were affected less (figs. 21 and 22, lower portion) than others (fig. 22, upper area). Mitochondria were reduced greatly in size and were more granular to rod-shaped, in contrast to their filamentous shape in control glands. They were restricted to the basal and perinuclear regions and were rarely observed apically. Basophilic material was increased greatly in concentration and appeared in clumps or lamellae in the perinuclear area of the cell. Nuclei were enlarged and hydropic.

A marked degranulation of the intercalated duct cells occurred also (figs. 21 and 22). The granules remaining in the cytoplasm stained with the same intensity but appeared smaller. The cells were hypertrophied and the nuclei, hydropic. There was a marked increase in cytoplasmic basophilia. The basophilic material was arranged in small clumps and lamellae located chiefly near the nucleus.

Little alteration was observed in the general morphology of the cells lining either the salivary, interlobular, or

excretory ducts after prolonged stimulation with pilocarpine. Accumulation of glycogen was especially common in the epithelial cells of the interlobular and excretory ducts (fig. 20). It was located both basally and apically. Cytoplasmic basophilia was increased after prolonged stimulation. The somewhat enlarged and slightly hydropic nucleus assumed a more apical position in the cell. Mitochondria, while apparently unchanged in either number or form, extended farther into the apical portion of the cell. In the excretory ducts, both after prolonged and short-term stimulation, blebs formed at the apical surface of the epithelium. These protrusions contained no stainable material but appeared to be extensions of the homogeneous, pale-staining areas as described for glands of control rats.

Similar but less profound changes were seen in parotid glands taken from non-hypophysectomized animals only one hour after they had received 1.0 mg of pilocarpine per 100 gm body weight. As exceptions, alteration in mitochondria and the appearance of glycogen were not seen. At this dosage, glands from hypophysectomized animals were affected more profoundly than those from non-hypophysectomized animals with acinar cells being totally degranulated and mitochondria more densely arranged. Intercalated duct cells were also more extensively degranulated. The larger ducts in hypophysectomized rats did not respond differently from those of non-hypophysectomized rats except that their

epithelium accumulated more glycogen.

DISCUSSION

The Normal Gland

Acinar cells. The observations on the acini of normal parotid glands are of interest from several standpoints, especially as they pertain to other published accounts. In contrast to the findings of Noback and Montagna ('47), who observed only a faint apical basophilia, techniques used in this study revealed a significant affinity of the entire cytoplasm for methylene blue. Since the zymogenic granules stained lightly with PAS, it may be inferred that they contain carbohydrate. Similar results were obtained by Lillie ('51) in man and some rodents. Aunap ('31) carefully described the effect of administration of pilocarpine on the acinar cells. Observations made on the present material are in agreemant with his findings.

Although Deane ('47) described the presence of demonstrable alkaline phosphatase in acinar cells, and Noback and Montagna ('47) reported activity in the basal portions of the cell, with only a faint apical reaction, no alkaline phosphatase was observed in the acinar cells in this material. The slight darkening which occurred, was observed also in the control slides inactivated by boiling. The myoepithelial or basket cells associated with

the acini were intensely reactive, in agreement with the findings of Silver ('54) in sheep. Similarly, the myoepithelial cells of the rat, guinea pig and human mammary gland (Dempsey, Bunting and Wislocki, '47) and of the eccrine and apocrine sweat glands of man (Kopf, '57) are exceedingly active. The function of this cell type in the parotid gland and the significance of its high concentration of alkaline phosphatase remain obscure. The intense alkaline phosphatase activity found in capillaries, arterioles and venules associated with the acini, agrees with the observations of Deane ('47) and Hill and Bourne ('54).

There is disagreement concerning the presence of nonspecific esterase in acinar cells. Chessick ('53) found no activity; Hill and Bourne ('54) observed slight activity throughout the cytoplasm and near the Golgi area; and Burstone ('56) found a "slight overall background reaction." The pronounced activity observed in the acini in the present investigation is undoubtedly due to better preservation of the enzyme by avoidance of fixation. While the metabolic role of esterase has not been clarified, its significant concentration within acinar cells indicates an important physiological role.

The distribution of succinic dehydrogenase reported here agrees with that described by Padykula ('52) even though the improved method of Farber and Louviere ('56) was employed. This procedure involves the use of methylene blue as an intermediate electron transporter which increases the reduction of neotetrazolium salts and shortens the

incubation time. It is believed that the technique "actually localizes a carrier which reacts with succinic dehydrogenase" rather than the enzyme itself (Farber, Sternberg and Dunlap, '56).

A search of the literature revealed only one description of the occurrence of cytoplasmic inclusions in the parotid acinar cells similar to those described here. Duff and Corbett ('45) found such inclusions in the pancreas of mice, rats, and rabbits and in the salivary glands of the rat. They suggested a viral etiology rather than a disturbance of secretory function as the inclusions were not affected by the administration of pilocarpine or atropine. In the present investigation, pilocarpine likewise was observed to have no effect.

Intercalated ducts. The cytological and histochemical characteristics of the epithelium lining intercalated ducts strongly suggests that they perform a secretory function. The presence of cytoplasmic granules and the postulation that they represent the stored secretion of these cells was reported by Ito and Narita (cited by Yoshimura, '56). Hill and Bourne ('54) described their marked affinity for leucofuchsin subsequent to oxidation with periodic acid. The present work is in agreement with these findings. Since fewer of these granules were visible after fixation with Zenker-formol or Regaud's fluid and staining by the Altmann-Masson procedure, than after FAA fixation and staining with the PAS technique, one may infer

that the former fixatives did not preserve the granules well or that some had less affinity for aniline blue. They probably contain a mucoprotein since they are resistant to digestion with saliva (Hill and Bourne, '54).

Alkaline phosphatase, esterase and succinic dehydrogenase were exceedingly active in the epithelium of the intercalated duct. With respect to alkaline phosphatase, this was in sharp contrast to the remaining portions of the duct system. The intense activity of the underlying myoepithelial cells raises the possibility that the blackening of intercalated ducts may have been an artifact due to diffusion of the reaction products or the enzyme itself (Gomori, '52). The intensity and consistency of the reaction makes this seem improbable. This evidence, in addition to the depletion of granules and increase in cytoplasmic ribonucleic acid which followed pilocarpine stimulation, indicates that the intercalated ducts perform a secretory function.

Salivary (intralobular), interlobular and excretory ducts. The salivary, interlobular and excretory ducts of the rat parotid were similar cytologically, except that the epithelium became higher and the basal striations, higher and more prominent in progression towards the excretory duct. In the region of transition from intralobular to intercalated duct, few basal striations are present.

Much effort has been expended in attempts to demonstrate

a secretory function by these ducts (Babkin, '50; Hill and Bourne, '54). As yet one can only point to the occurrence of various enzymes as indicative of a high rate of metabolic activity, i.e., non-specific esterase (Chessick, '53; Hill and Bourne, '54; Burstone, '56), succinic dehydrogenase (Padykula, '52) and alkaline phosphatase (Deane, '47; Noback and Montagna, '47; Hill and Bourne, '54). With a few exceptions, this investigation agrees with these findings. In contrast to Deane ('47) and Noback and Montagna ('47), who described alkaline phosphatase in the epithelium of the salivary duct, and Hill and Bourne ('54) who reported it to be present in the distal edges of some cells lining the larger intralobular and interlobular ducts, no activity was observed in either type of duct. Deane's microphotographs suggest that she was dealing with intercalated ducts rather than salivary ducts. An important physiological function by these cells is suggested also by the presence of a large number of mitochondria, cytoplasmic PAS-stained granules (Hill and Bourne, '54) and ribonucleic acid. Bixler, Muhler, Webster, and Shafer ('57) reported the absence of stainable ribonucleic acid in these cells; the observations of Noback and Montagna ('47) and this investigation disagree with their conclusion.

The striking responsiveness of the epithelium to stimulation by pilocarpine constitutes additional evidence for a secretory function. The increased ribo-

nucleic acid content, the slight apical shift in position of nucleus and mitochondria, and the accumulation of glycogen after prolonged stimulation are suggestive of altered physiology. Obviously such profound stimulation is beyond the physiological range, yet these changes are indicative of a responsiveness to an accepted mode of parasympathetic stimulation. The occurrence of blebs at the apices of the cells lining the interlobular and excretory ducts may be an artifact. However, they were not observed in the non-stimulated glands. One is tempted to conclude from the presence of glycogen that the ducts may serve as a source of salivary glucose.

The responsiveness to pilocarpine of the epithelium in both acini and ducts indicates that both may respond to parasympathetic stimulation. If this conclusion is valid, it stands in contradiction to the postulation that different cell types are innervated either by sympathetic or parasympathetic fibers exclusively (Babkin, '50).

The Effects of Hypophysectomy

In addition to the partial atrophy and degranulation of acinar cells after hypophysectomy (Baker and Abrams, '55; Yoshimura, '56), the granules were also smaller and stained less intensely with aniline blue and PAS. The latter observation indicates that the granules were altered qualitatively as well as quantitatively. In contradiction to Bixler, Muhler, Webster, and Shafer ('57),

no decrease in the content of ribonucleic acid in acinar cells was observed. This is of particular interest in view of the important role played by ribonucleic acid in protein synthesis (Brachet, '55). The reduced capacity of the acinar cells to synthesize protein as evidenced by its reduced content of zymogenic granules and amylase cannot be ascribed to a reduced ribonucleic acid concentration. It is possible, however, that hypophysectomy induces a chemical change in this component. The increased number of cytoplasmic inclusions in the acinar cells after hypophysectomy is indicative of an increased susceptibility to the viral agent, if these inclusions are the result of such an infection.

Study of various enzymes in the acinar cell did not reveal a fundamental defect. The lack of change in the succinic dehydrogenase, which is concentrated in mitochondria, indicates that at least with respect to this enzyme, the mitochondria are probably unaffected by hypophysectomy. However, alkaline phosphatase was clearly reduced in the peri-acinar capillaries. If this enzyme plays a role in transport of metabolites as postulated for the renal tubular epithelium and the absorptive epithelium of the small intestine (Knox, Auerbach and Lin, '56), a reduction in phosphatase might lead to impaired transport of these metabolites. This condition could be involved in the atrophy of acinar cells after hypophysectomy. Similar reductions in the concentration of alkaline phosphatase in

capillaries of the thyroid, testis, and skeletal muscle after hypophysectomy and in the seminal vesicle (with sodium glycerophosphate but not fructose diphosphate as the substrate), and uterus after gonadectomy, were described by Dempsey, Greep, and Deane ('49).

In contrast to the observations of Yoshimura ('56), hypophysectomy caused a reduction in the number of cytoplasmic granules in the epithelium of intercalated ducts. Also, in disagreement with Yoshimura ('56), no atrophy was observed in the epithelium of salivary ducts. This is evident not only in the lack of significant change in the cytology or sulfhydryl and disulfide content as described here, but also in the lack of significant alteration in either cellular height or diameter of the duct. In these respects, the interlobular and excretory ducts also appeared unchanged. The slight increase in the diameter of salivary ducts and height of epithelial cells and the similar changes observed in the epithelium of the intercalated ducts were accredited to a reduced tension on these ducts as a result of the acinar atrophy after hypophysectomy. It is certain, however, that some alteration of the physiology of these cells occurred. The accumulation of glycogen, as described by Yoshimura ('56) and confirmed here, slight reduction in PAS-staining, somewhat diminished basophilia and the marked drop in non-specific esterase activity of the salivary and interlobular ducts were probably indicative of a depressed metabolism. The absence of change in the activity of the

succinic dehydrogenase system correlated well with the lack of modification in mitochondria.

It was hoped that the use of several techniques would help to elucidate more clearly the effect of hypophysectomy on esterases in the parotid gland. It is becoming increasingly apparent that more than a single enzyme is responsible for the hydrolysis of the esters used in demonstrating esterase activity. With the development of a method for electrophoretic separation with subsequent histochemical demonstration of these enzymes (Hunter and Markert, '57), an extremely useful tool became available for the study of their individual reactions to physiological change. In this investigation, however, the two esterase-active bands demonstrated in homogenates of the parotid gland were not effected by hypophysectomy.

Hypophysectomy did not alter significantly the concentration of the two esterases when determined biochemically. However, there was a tendency toward reduction. When examined histochemically, significant changes were observed in the localization of esterases. The marked reduction in esterase activity in the salivary and interlobular ducts was partially offset by the increased concentration which occurred in the basal portion of the acinar cells. As would be anticipated on the basis of the reduced glandular weight, the total esterase content of the parotid was reduced significantly.

The parotid gland is affected the least by hypophysectomy

of the enzyme-producing structures associated with the digestive system (Baker and Pliske, '57). The gastric chief cells undergo marked atrophy and loss of ribonucleic acid with a concomitant reduction in pepsin production (Baker and Abrams, '54). The serous tubules of the submandibular gland in the rat (Gabe, '50) and in the mouse (Lacassagne and Chamorro, '40) become atrophic and their granular content is depressed. Concurrently, the proteolytic (Sreebny, '54; Shafer and Muhler, '55) and amylolytic (Shafer and Muhler, '55; Sreebny, Meyer and Bachem, '56) activity of this gland is reduced by hypophysectomy. The pancreatic acini show focal degranulation with acinar atrophy. There is a reduction in the basal chromophilia, in number and size of mitochondria and in the non-specific esterase (Baker and Pliske, '57). The severe changes observed in the ribonucleic acid content of these structures and the changes observed in the mitochondria of the pancreatic acinar cell find no counterpart in the parotid. In fact, the acinar cell of the parotid after hypophysectomy presents little evidence of impaired secretory function except for the reduction in zymogenic granules. The same is true for the duct system. It appears that the degree of control exercised by the pituitary hormone(s), directly or indirectly through some target organ or other metabolic process, is different for the parotid than it is for the other digestive organs. Clarification of the mode of pituitary control over the parotid must await additional

studies involving ablation of other endocrine glands and replacement therapy with hormones of the hypophysis and of other dependent glands. The present status of our knowledge on this has been reviewed (Baker and Abrams, '55; Baker, '57; Baker and Pliske, '57).

While no definitive conclusions may be reached on the basis of this work concerning the postulated endocrine role of the parotid, certain pertinent inferences may be drawn. First, if the salivary ducts are the source of the salivary gland hormone (parotin) (Ogata, cited by Yoshimura, '56), and if its secretion is regulated by somatotropin (Shibata, Nigishi, Saito and Todokoro, '54), a marked atrophy of the cells lining these ducts would be expected after hypophysectomy. This is not the case. Second, no cell type can be demonstrated in the parotid gland which shows a polarization toward blood vessels which resembles that of endocrine cells in general.

SUMMARY

Histochemical and biochemical techniques were employed in an attempt to clarify the complex changes which occur in the parotid gland of the rat after hypophysectomy. Acinar cells were smaller and zymogenic granules, reduced in size, number and staining affinity for aniline blue and the PAS stain. No alteration was observed in the activity of the succinic dehydrogenase system, in the morphology of mitochondria, or in the content of ribo-

nucleic acid or of sulfhydryl and disulfide groups in the acini. However, alkaline phosphatase was reduced in the myoepithelial cells and capillaries associated with the acinar cells. If this enzyme(s) is involved in transport of metabolites, such a reduction might account, at least in part, for the acinar atrophy.

Cytoplasmic granules in the lining cells of intercalated ducts were partially depleted by hypophysectomy. Ribonucleic acid, alkaline phosphatase, succinic dehydrogenase and non-specific esterase were unchanged in these cells.

The height of the epithelium in and the width of salivary ducts were not changed. However, ribonucleic acid content, PAS staining and esterase activity were decreased and glycogen, increased in the epithelium of salivary and interlobular ducts. Similar changes were observed in the excretory ducts with the exception of the reduced esterase activity.

Two esterase-active proteins were demonstrated in homogenates of the gland by starch gel electrophoresis but were not altered by hypophysectomy. Although the localization of esterase, as determined histochemically, was changed by pituitary ablation, biochemical analysis revealed no alteration in the quantity of enzyme present.

It was concluded that the magnitude of hormonal control exerted by the hypophysis over the parotid gland is less than that directed toward gastric chief cells, pancreatic acini, or serous tubules of the submandibular gland. The

weight of the evidence obtained is against the concept that the salivary ducts are regulated by pituitary growth hormone.

Following administration of pilocarpine, degranulation and increased ribonucleic acid content occurred in cells of both acini and intercalated ducts. In the salivary, interlobular and excretory ducts, there was alteration in the position of the nucleus and mitochondria, and accumulation of glycogen.

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PLATE 1

Explanation of Figures

Figures 1 and 2: fixation in Zenker-formol, stained with Altmann-Masson, 4 μ . x1325. Figures 3, 4 and 6: fixation in FAA, stained with PAS and methylene blue, 4 μ . x600. Figure 5: fixation in trichloracetic acid, stained with sulfhydryl and disulfide procedure. x450.

1 Non-hypophysectomized control. Acinar cells (above) are engorged with large zymogenic granules. Nuclei are hyperchromatic. In the salivary duct epithelium (bottom) mitochondria are most concentrated basally.

2 Hypophysectomized 57 days previously. Acinar cells are reduced in size and contain fewer zymogenic granules which are less cyanophilic. Nuclei are less compressed. The salivary duct epithelium (bottom) is not atrophic but the cells are narrower. Their mitochondria are stained more intensely.

3 Non-hypophysectomized control. Acinar cells are vacuolated; the cytoplasm throughout the cell is intensely stained with methylene blue. Epithelial cells lining the intercalated duct are engorged with PAS-positive granules.

4 Hypophysectomized 111 days previously. Cytoplasm of the acinar cell is vacuolated. No loss in intensity of cytoplasmic basophilia is apparent; basal masses of basophilic material are present (arrow). The PAS-positive granules of the intercalated duct are reduced in number but not in size.

5 Non-hypophysectomized control. Acinar cytoplasm is lightly stained. No zymogenic granules are seen. Intercalated ducts (I) are more intensely stained than acini. The cytoplasm of the salivary duct (S) cells is stained intensely.

6 Hypophysectomized 60 days previously. A large homogeneous basophilic (B) and a PAS-positive inclusion (arrow) are seen.

PLATE 1

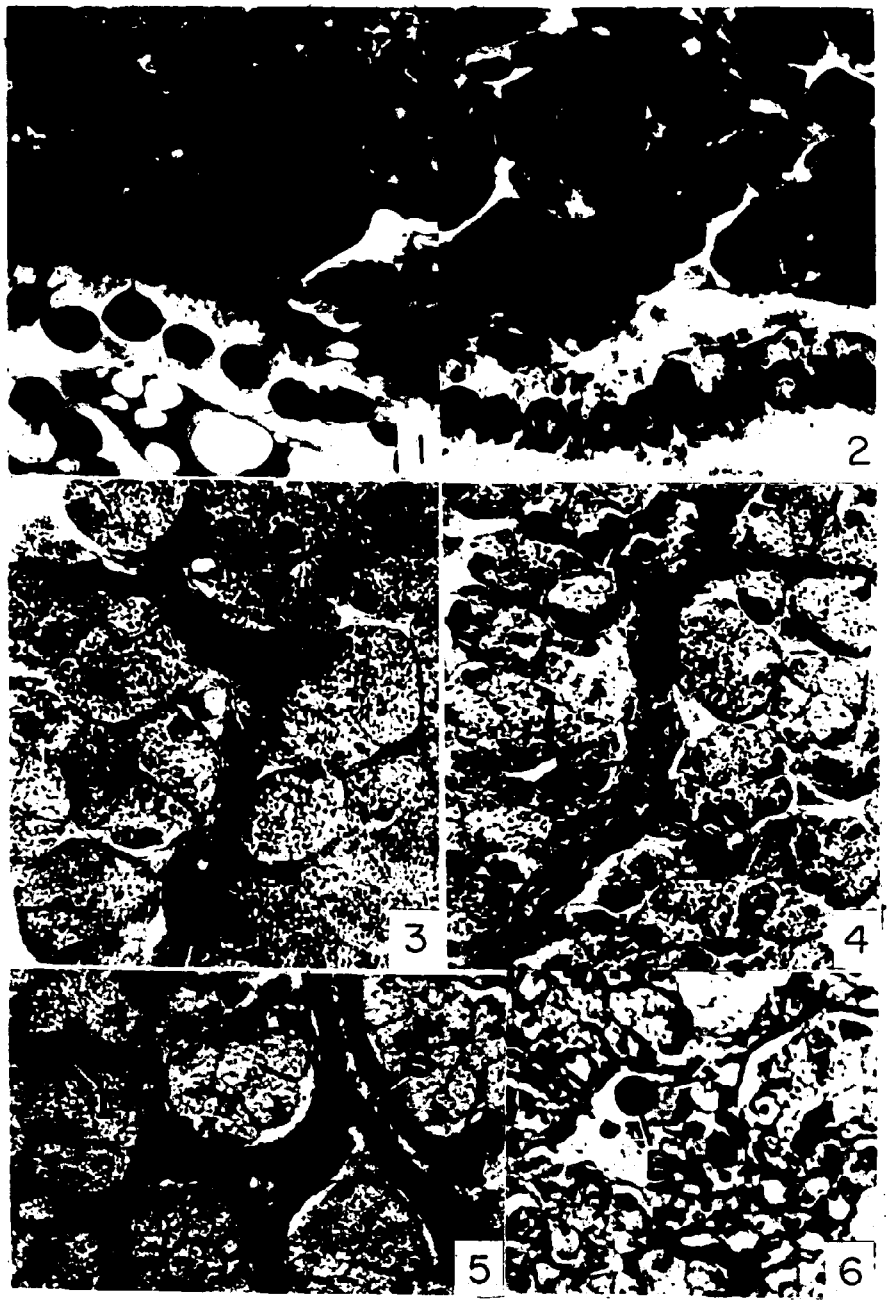


PLATE 2

Explanation of Figures

Figures 7 and 8: fixation in FAA, stained with PAS and methylene blue, 4μ . x600. Figure 9: fixation in FAA, α-amylase digested, stained with PAS and methylene blue. x638. Figures 10, 11 and 12: fixation in cold FAA, alkaline phosphatase with sodium glycerophosphate as substrate, 4μ . x338.

7 Non-hypophysectomized control. The cytoplasm of the lining epithelium of the interlobular ducts is stained lightly by PAS. A fine, sparse granulation is seen throughout the cytoplasm. The basal striations (arrow) and the basement membrane are stained with PAS.

8 Hypophysectomized 11 days previously. Large accumulations of glycogen are present in the cells lining the salivary (S) and interlobular ducts (R).

9 Hypophysectomized 46 days previously. Amylase digestion removed all of the intensely PAS-staining material from the ducts (compare with fig. 8). The staining of basal striations and fine cytoplasmic granulation is unchanged.

10 Non-hypophysectomized control. Alkaline phosphatase is possibly present in the intercalated ducts (I) and definitely present in the myoepithelial cells associated with the acini (arrow). Arterioles (A) and peri-acinar capillaries (C) show marked activity while the venules (V) are less active. The salivary duct (S) is negative.

11 Hypophysectomized 40 days previously. Phosphatase activity is unchanged in the intercalated duct (I) but reduced in peri-acinar capillaries (arrow).

12 Hypophysectomized 40 days previously. No alkaline phosphatase is observed in the salivary duct (S). The activity of myoepithelial cells is reduced.

PLATE 2

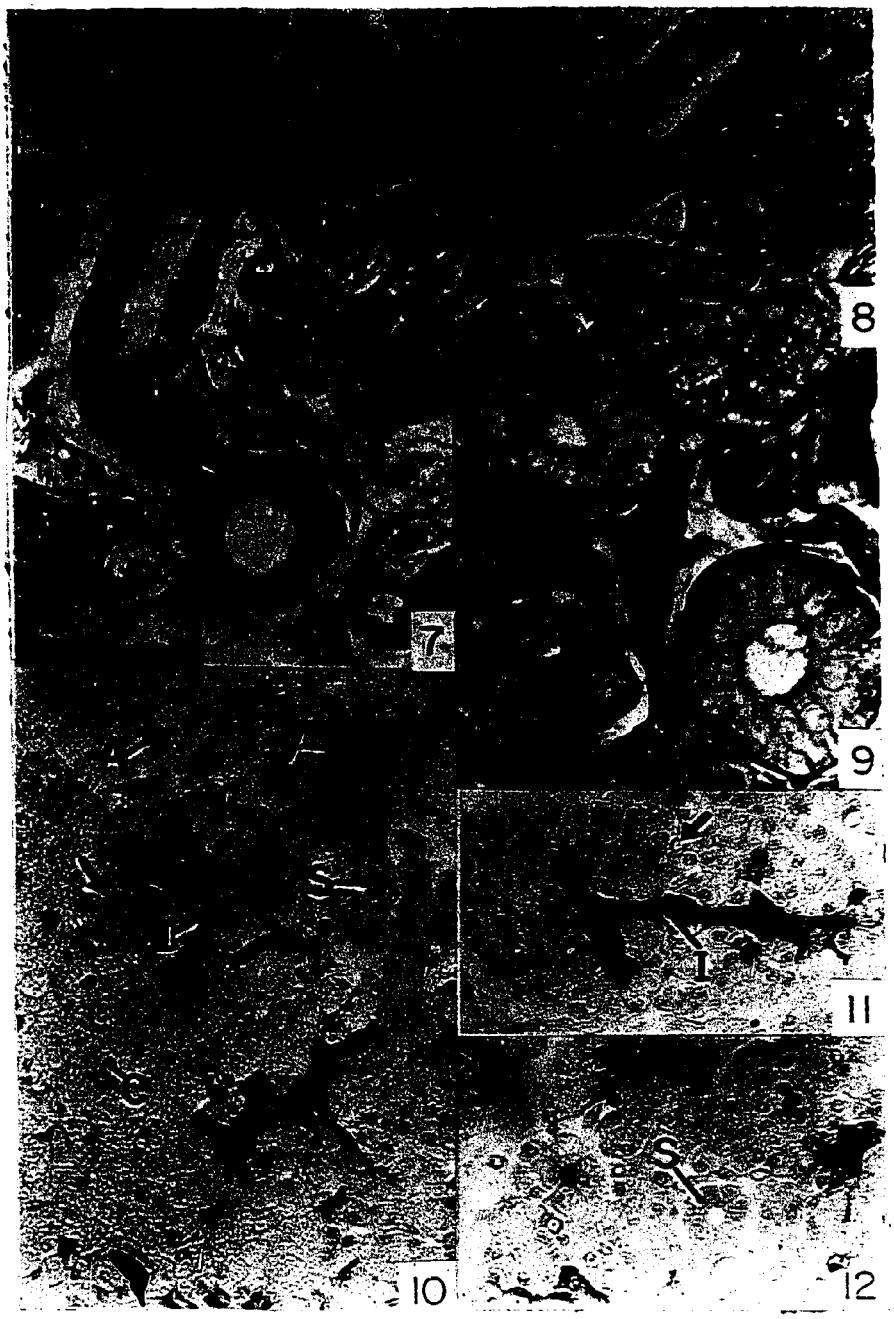


PLATE 3

Explanation of Figures

The parotid glands for figures 13 through 16 were sectioned in a Cryostat at 10μ . Incubated with α - naphthol butyrate and Garnet GBC for esterase. Figures 13 and 14: x64. Figures 15 and 16: x338.

13 Non-hypophysectomized control. The majority of the salivary (S) and interlobular (R) ducts and the large excretory duct (bottom) show intense esterase activity. Variation in activity among cells within all ducts is apparent.

14 Hypophysectomized 46 days previously. Esterase in acini and the large excretory duct is unchanged. The activity in salivary (S) and interlobular ducts (R) is reduced.

15 Non-hypophysectomized control. The apparent reduction in acinar size is an artifact. Considerable esterase is distributed uniformly throughout the cytoplasm of the acinar cells. A comparable degree of activity is seen in the intercalated duct (I), while the abrupt transition of this duct into the salivary duct is clearly marked by the greater activity of the latter (S).

16 Hypophysectomized 44 days previously. Esterase is not reduced in the acini or intercalated duct (I). Comparison with figure 15 reveals a basal concentration (arrow) of activity in the acinar cells. The activity of the salivary duct (S) is reduced.

PLATE 3

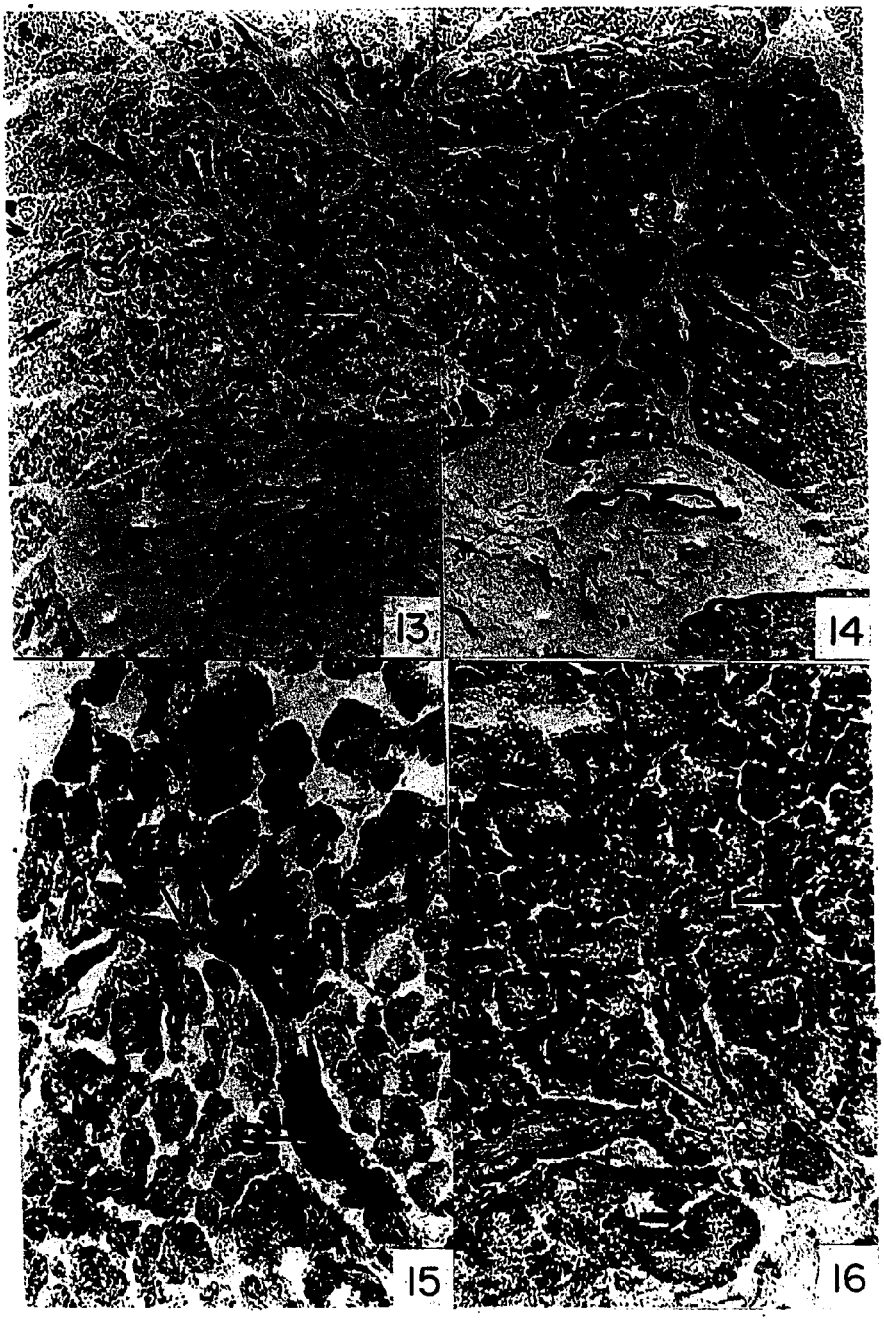


PLATE 4

Explanation of Figures

Figures 17, 18 and 19: unfixed and sectioned in a Cryostat at 10 μ ; succinic dehydrogenase, x158. Figures 20, 21, and 22: fixation in FAA, stained with PAS and methylene blue, 4 μ.

17 Non-hypophysectomized control. Acinar cells possess succinic dehydrogenase activity. Droplets within the acinar cells have dissolved neotetrazolium crystals and appear as dark rings. Marked activity is seen in the intercalated, salivary (S) and interlobular (R) ducts.

18 Hypophysectomized 52 days previously. No change is observed in the succinic dehydrogenase activity of either the acini, intercalated duct (I), salivary duct (S) or interlobular duct (R).

19 Non-hypophysectomized control. Reduced neotetrazolium is concentrated basally in a large excretory duct.

20 Non-hypophysectomized, treated with pilocarpine. Glycogen is observed in the salivary duct (S). Basophilia is increased in the intercalated duct (I). x675.

21 Water-injected control. Acinar cells are large and filled with zymogenic granules. The intercalated duct cells, especially those nearest the acini, are engorged with PAS-positive granules. x638.

22 Non-hypophysectomized, treated with pilocarpine. Acinar cells are reduced in size, depleted of zymogenic granules, and contain large clumps and lamellae of basophilic material in the basal portion of the cell. The nuclei appear hydropic. Intercalated duct cells are enlarged, depleted of granules, and reveal markedly increased basophilia (arrow). x638.

PLATE 4

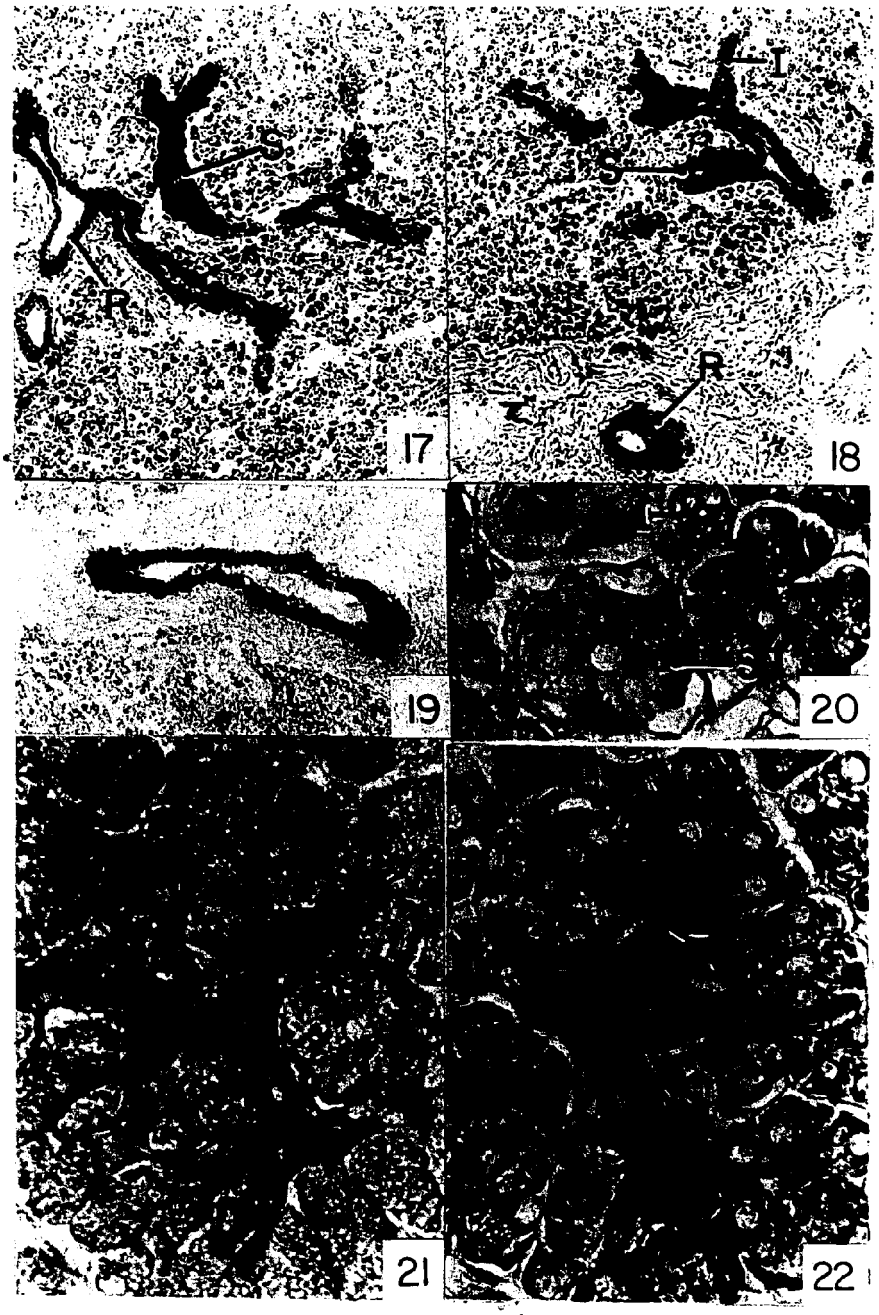


PLATE 5

Explanation of Figures

Zymograms prepared according to the method of Hunter and Markert ('57), α-naphthol butyrate as substrate. The dark spots visible on the zymograms are artifacts and due to secondary absorption of the azo-coupled complex.

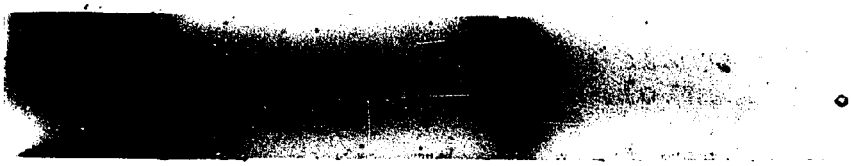
23 A. Parotid gland 44 days after hypophysectomy.

B. Parotid gland from a non-hypophysectomized control.

C. Liver from non-hypophysectomized control.

PLATE 5

A.



B.



C.

