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THE EFFECTS OF HYPOPHYSECTOMY ON THE DIGESTIVE GLANDS OF THE MOUSE

by

Richard Mortson Koerker

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THE EFFECTS OF HYPOPHYSECTOMY ON THE DIGESTIVE GLANDS OF THE MOUSE

Richard Mortson Koerker

ABSTRACT

The objective of this study was to observe the influence of hypophysectomy on the secretory cells of the digestive glands in the mouse and to compare these findings with those reported for other mammalian species. Two experiments were carried out. In Experiment I, 11 hypophysectomized mice and 18 controls were allowed to eat ad libitum. In Experiment II, each of 12 non-hypophysectomized control mice was pair-fed against a hypophysectomized mouse. The experiments were terminated 8 to 10 weeks after the operation.

Widespread alterations occurred in the digestive glands of the mouse after hypophysectomy. Included were a reduction in weight of the stomach, pancreas and of the parotid, submandibular and sublingual glands. The diameters of the pancreatic, submandibular, sublingual and parotid acini were reduced. The gastric mucosa became thinner.

The important role played by the hypophysis in regulation of protein synthesis was reflected in the involutional changes observed in gastric chief cells, acinar cells of the pancreas and parotid gland, and in the submandibular serous tubules. These alterations included reduction in number of secretory granules, in cytoplasmic basophilia (RNA) and in size of cells, nuclei and nucleoli. The number of chief cells was reduced per gastric gland. The morphology of mitochondria was altered in gastric chief cells,

pancreatic and parotid acinar cells and serous tubular cells of the submandibular gland. The Golgi apparatus was contracted in pancreatic acinar cells.

Mucus-secreting cells were also affected by hypophysectomy but to a lesser degree. In normal mice, three varieties of mucous neck cell were differentiated histochemically, one variety containing neutral mucopolysaccharides (PAS-positive), another acid mucopolysaccharides (colloidal iron-positive) and a third variety possessing both neutral and acid mucopolysaccharides. After hypophysectomy the colloidal iron-positive variety was dominant. Mucous neck cells were increased in number per fundic gland but contained less secretory material. The mucous acini of the submandibular and sublingual glands also contained less secretory material and were smaller.

Cells concerned with ion transport or release were altered by hypophysectomy. Parietal cells became atrophic, contained fewer mitochondria and a greatly contracted intracellular canalicular system. Salivary ducts of the parotid and sublingual glands showed partial involution. Basal striations disappeared from the parotid salivary ducts.

In the parasympathetic ganglion of the submandibular gland, hypophysectomy caused a reduction in size of nerve cell bodies, in amount of Nissl substance and in size of nuclei and nucleoli.

It was concluded that, as in the rat, the protein-secreting cells of the digestive tract of the mouse depend on the support of pituitary hormones. The involutional changes observed in the submandibular ganglion probably represent the same type of

dependence. Likewise, those cells of the mouse digestive glands which secrete mucopolysaccharides or are involved in ion transport require the action of pituitary hormones for maintenance of their structure and function.

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INTRODUCTION

Schooley, Riddle and Bates ('41) first discovered that the pituitary gland influences the digestive system by demonstrating a loss in weight of the gastrointestinal tract in the pigeon after hypophysectomy. More recently others have shown that the hypophysis exerts significant control over the structure and function of digestive glands in the rat. Thus, all zymogenic cells which have been studied are dependent on the support of pituitary and other hormones for maintenance of normal structure and full secretory activity. These include the gastric chief cell (Baker and Abrams, '54), acinar cells of the pancreas (Haist, '55; Baker and Pliske, '57), parotid gland (Baker and Abrams, '55), zymogenic cells of the submandibular serous ducts (Lacassagne and Chamorro, '40) and cells of Paneth in the intestine (Baker et al., '58). In addition, mild structural involution (Baker and Abrams, '54; Baker and Clark, '61) and functional depression (Crafts and Walker, '47) take place in the gastric parietal cells after pituitary ablation.

In contrast to the rather detailed information available concerning the action of hormones on the digestive glands of the rat, relatively little is known about their effects in other mammals. After hypophysectomy, weight of the stomach decreases in the cat (Haeger et al., '53); gastric acid secretion falls in both the cat (Cutting et al., '37) and dog (Jacobson and Magnini, '64). Involution of the gastric mucosa and its chief cells occurs during

panhypopituitarism in man (Laumonier, '63). Similar mucosal atrophy results from hypophysectomy performed in the treatment of diabetes or cancer with hydrochloric acid secretion decreasing 50% as determined by the maximal histamine test (Dotevall and Westling, '63).

The purpose of this study was to examine the influence of hypophysectomy on the structure of the digestive glands of the mouse as a step toward determining whether the control exerted by hormones in the rat is ubiquitous among mammalian species. The mouse was chosen for this study because it is easily maintained in long-term experiments and little is known about the hormonal control of its digestive system. Detailed information is available only concerning the control exerted by hormones over the submandibular gland (Raynaud, '60). Although closely related to the rat, the mouse differs structurally and physiologically in many respects. Examples of such differences are the presence of an X-zone in the adrenal gland (Jones, '48), and in the submandibular gland of a high iodine-concentrating capacity (Taurog et al., '59), and high contents of nerve growth factor (NGF) (Levi-Montalcini and Angeletti, '64) and amylase (Raynaud and Rebeyrotte, '50; Junqueira et al., '64). The position of the mucous neck cells in the gastric mucosa (Fekete, '41) also differs from that in the rat.

In this report, the influence of hypophysectomy on the histology and histochemistry of the mouse gastric mucosa, pancreas and parotid, submandibular and sublingual glands

will be described. Not only will such information help to define the role of hormones in regulating mammalian digestive systems, but it will also serve as a basis for future experiments involving replacement therapy with hormones.

MATERIALS AND METHODS

The experiments were carried out on young adult male ICR Swiss mice. Hypophysectomy was performed by the transauricular method of Falconi and Rossi ('64) which was modified in the following ways. A polyethylene tube which produced minimal trauma was substituted for a metal tube as the sheath around the aspiration needle. Also, a Wintrobe pipette, with a small opening placed in its side, was inserted into the vacuum line to the aspiration needle. After the needle was inserted into the pituitary fossa, suction was created by covering the opening in the side of the pipette with the thumb. The Wintrobe pipette, with aspiration needle attached, served as a handle and permitted better control of the needle during the operation. It also facilitated visualization of the pituitary fragments as they were aspirated. The completeness of hypophysectomy was verified by histological observation of serial sections of the pituitary region. Incompletely hypophysectomized mice were discarded. A nonhypophysectomized mouse was retained as a control for each hypophysectomized animal.

The mice were fed a diet consisting of Purina mouse breeder chow supplemented with lettuce, bread and cod-liver oil. In order to evaluate the influence of food intake on the results obtained, the animals were arranged in two experiments. In Experiment I, 11 hypophysectomized mice and 18 controls were allowed to eat ad libitum. In Experiment II, each of 12 nonhypophysectomized control mice was pair-fed

against a hypophysectomized animal so that the control received the amount of food eaten by its operated mate on the previous day. During the first 7 days of this experiment the mice were not paired, but the operated animals were fed daily the average amount of food consumed by hypophysectomized mice as determined by previous observations. Each hypophysectomized mouse was kept in the same cage as its pair-fed control, the two mice being separated by a screen.

Since the ingestion of food elicits structural changes in the digestive glands, the animals were deprived of food for 8 to 12 hours before being sacrificed. This was shown to be the optimal starvation time by preliminary experiments in which it was observed that starvation for less than 8 or more than 12 hours caused great irregularity in the number of secretory granules present in the acinar cells of these glands. Indeed, practically complete degranulation occurred in both the parotid gland and pancreas of some control mice when more than a 12-hour starvation time was employed.

At the end of a postoperative period of 8 to 10 weeks, the stomach, pancreas and salivary glands were removed while the animals were under sodium amytal anesthesia. The organs were dissected free of contiguous tissue and weighed. Some difficulty was met in effecting a complete separation of the parotid gland from accompanying fat and connective tissue. Although dissection of the gland from fat and connective tissue after removal is successful in the rat (Baker et al.,

'64), better results were obtained in mice by dissecting the parotid gland from contiguous tissues in situ. After excising the sublingual and submandibular glands and the cervical lymph nodes, the cervical skin with parotid gland attached was stretched by grasping it between the thumb and index finger and then pulling it over the index finger. The parotid gland was then clearly visible, spread out in the connective tissue, and could be freed by separating it from the connective tissue with forceps. Histological sections showed that minimal fat and connective tissue were retained. Weights for the parotid gland which were reproducible from animal to animal were then obtainable.

The following fixatives were used for the purposes and times indicated: formalin-alcohol-acetic acid (FAA) (48 hours) for the demonstration of ribonucleoprotein, mucoprotein and glycogen; Champy's fluid (24 hours) for the Golgi apparatus; Regaud's fluid (96 hours) for mitochondria; Zenker-formol (5 hours) for ribonucleoprotein and mitochondria; and Bouin's fluid (24 hours) for general tissue structure. The extent to which each fixative was used is summarized in table 1.

After ligation at the pyloroduodenal junction FAA was injected through the esophagus into the stomach before excision in order to provide uniform distension of the organ and permit accurate observation of change in mucosal thickness. The esophagus was then ligated. After excision, the inflated stomach was submerged in fixative at 4° C for 48

hours. A strip of stomach was then removed from the greater curvature and prepared for embedding. The other stomachs were removed, cut along the greater curvature, rinsed with 0.9% saline and weighed. A strip of fundic mucosa on either side of the initial cut was removed and stretched out on a piece of stiff paper with the mucosa exposed and then fixed. A small piece was cut from one of the strips and fixed in Champy's fluid; the remainder of this strip was fixed in FAA. The second strip was placed in Regaud's fluid. The pancreas and salivary glands were placed directly in the fixatives after being weighed.

The specimens fixed in Regaud's fluid or Zenker-formol were post-chromated in 3% potassium dichromate for three days. Tissues fixed in Regaud's fluid were double-embedded in celloidin and Tissuemat. Tissues fixed in Zenker-formol, Bouin's fluid or FAA were embedded in Tissuemat. After fixation in Champy's fluid, tissues were immersed in 2% osmic acid for three days at 37° C and then double-embedded in celloidin and Tissuemat. Sections were cut at 3 to 5 μ.

A variety of staining procedures (table 1) were employed for the following purposes. Hematoxylin-Masson was used to reveal general tissue structure, azure II for cytoplasmic and nuclear basophilia, periodic acid-Schiff (PAS) (Hotchkiss, '48) for neutral mucopolysaccharides, colloidal iron (Rinehart and Abul-Haj, '51) for acid mucopolysaccharides, and PAS-colloidal iron (Mowry, '58) to differentiate acid from neutral mucopolysaccharides. Certain slides were

digested with α-amylase for 20 minutes to one hour in 0.1 M Sorensen's potassium phosphate buffer at a pH of 7.2 at 37° C as a control for the demonstration of glycogen. Other slides were incubated in the buffer solution without the enzyme. All of these slides were stained with PAS. For mitochondrial study, tissues fixed in Regaud's, Champy's or Zenker-formol fixatives were stained with the Altmann-Masson procedure (Severinghaus and Thompson, '39). For the demonstration of the Golgi apparatus, tissues fixed in Champy's fluid and post-osmicated were used with no stain.

In order to secure an index of quantitative histological differences induced in the stomach by hypophysectomy, rough estimates of the number of various cell types in the fundic glands were made by counting the number of cells present in glands which were cut longitudinally throughout their entire length. Observations were made only in the fundic mucosa near the transverse ridge. Therefore, cell counts were made in only a few fundic glands per mouse.

The significance of the differences between quantitative data for control and hypophysectomized groups was determined by the Wilcoxon two sample test (Steel and Torrie, '60). Significance was attributed to differences with a probability of error of less than 5%.

TABLE 1

Summary of Distribution of Animals and Technical Methods Used

Number of Mice				Technical Methods	
Experiment I		Experiment II			
Hyp.	Control	Hyp.	Control	Fixation	Stain
Stomach					
11	18	12	12	FAA[1]	PAS[2]-azure II; colloidal iron-PAS
7	9	12	12	FAA	Colloidal iron
4	8	12	12	FAA	α-Amylase digestion; PAS
7	14	12	8	Regaud's fluid	Altmann-Masson
Pancreas					
10	18	12	12	Bouin's	Hematoxylin-Masson; azure II-eosin
7	14	10	12	Zenker-formol	Azure II-phloxine; Altmann-Masson
4	7	6	6	Champy's fluid	Altmann-Masson
3	4	5	3	Champy's fluid	Post-osmication
Salivary Glands					
11	17	12	12	FAA	PAS-azure II; colloidal iron; colloidal iron-PAS
2	6	5	3	FAA	α-Amylase digestion; PAS
7	14	10	11	Zenker-formol	Altmann-Masson

Hyp. = Hypophysectomized

[1]FAA = Formalin-alcohol-acetic acid

[2]PAS = Periodic acid-Schiff

OBSERVATIONS

Hypophysectomized mice lost weight after the operation (table 2). Reduction in food intake by pair-fed control mice (Experiment II, table 2) caused a loss of body weight, while the control mice which ate ad libitum continued to gain weight. However, the histology of digestive glands in the pair-fed control mice did not differ from that of the ad libitum-fed controls, except for the pancreas and submandibular gland. Pancreatic acini were smaller and the serous tubules of the submandibular gland were reduced in diameter as a result of pair-feeding. No other effects were noted which could be attributed to food restriction as determined by pair-feeding. Therefore, the effects of hypophysectomy as described in the following account apply to both experiments unless stated to the contrary.

With respect to the normal structure of the stomach, pancreas and salivary glands in the mouse, this presentation will be concerned only with those observations which have not been previously reported. The histology of these glands has been described by Fekete ('41). Additional information on the submandibular gland was presented by Lacassagne ('40a), Junqueira ('64), Junqueira et al. ('49 and '64), Raynaud ('60) and Schätzle ('62). The histochemistry of mucins in the submandibular and sublingual glands and stomach was analyzed by Spicer ('60, '63) and Spicer and Jarrels ('61).

The Stomach

Control mice. The differences between the mean stomach weights of the ad libitum- and pair-fed controls was not significant (table 2) but between the mean organ weight/body weight ratios the difference was significant ($P < .01$). Thus absolute weight of the stomach appears to be unaffected by reduced food intake.

Mucous neck cells were interposed among the parietal cells in the area of the mucosa just above the chief cells (fig. 1). In FAA-fixed material three histochemically different varieties of mucous neck cells were observed in the stomachs of control mice stained with colloidal iron-PAS (fig. 3). To one group of mucous neck cells colloidal iron imparted an aquamarine color. The stained material was in the form of tightly packed granules. The cytoplasm of a second group of mucous neck cells stained red with PAS. The third group contained both PAS-positive cytoplasm and colloidal iron-positive granules. In an individual stomach the colloidal iron-positive cells were usually smaller than the PAS-positive cells. Cells containing both PAS- and colloidal iron-positive material varied in size. All three varieties were present in a single animal, but usually one was numerically dominant. No single variety was consistently dominant in all control animals.

When PAS staining was used after FAA fixation, the cytoplasm of parietal cells stained pink and had a mottled appearance. Mucoprotein associated with the microvilli of

TABLE 2

The Effect of Hypophysectomy
on Weights of the Body and Stomach

Treatment	No. of Mice	Body Weight (g) Initial	Body Weight (g) Final	Stomach Weight (mg)	Stomach Organ Wt. (mg) / Body Wt. (g) x 10,000
Experiment I Ad libitum-fed					
Control	18	23.9±0.90[1]	33.1±0.46	230.2±17.74[2]	69.14±4.98
Hyp.	11	23.1±0.35	18.8±0.49	138.5± 9.92[3]	75.47±5.53
			P <.01	<.01	NS
Experiment II Pair-fed					
Control	12	25.9±1.37	24.7±1.21	275.7±14.96	112.63±5.06
Hyp.	12	23.7±1.13	19.4±0.58	154.9± 7.08	80.22±3.49
			P <.01	<.01	<.01

Hyp. = Hypophysectomized P = Probability of error NS = Not significantly different at P<.05

[1]Standard error of the mean
[2]10 mice
[3]6 mice

intracellular canaliculi stained more intensely, creating a perinuclear halo of PAS-positive material (fig. 5). The PAS-positive material was not glycogen, as indicated by its resistance to digestion with α-amylase, nor was it an acid mucopolysaccharide because it did not stain with colloidal iron. Large rounded mitochondria were found everywhere in the cytoplasm except in that area occupied by intracellular canaliculi (fig. 7). Thus, mitochondria were arranged in two rings, one surrounding the nuclear membrane and the other being situated inside the plasmalemma. The zone between these rings of mitochondria coincided with the position of intracellular canaliculi as indicated by the PAS-positive halo.

In addition to the basally located cytoplasmic basophilia of chief cells, a fine network of basophilic material outlined the secretory granules. The long, thread-like mitochondria of chief cells (fig. 9) were oriented randomly in the subnuclear region and vertically in the area lateral to the nucleus. Chief cell nuclei contained large nucleoli and fine chromatin material.

Hypophysectomized mice. The weight of the stomach was less in hypophysectomized than in control mice (table 2). These differences, when considered as absolute values or in relation to body weight (except in Experiment I), were significant at less than the 1% level of confidence, indicating that stomach may be more sensitive to hormonal withdrawal than the body as a whole. The results in Experiment II also

demonstrate that the reduction in stomach weight after pituitary excision is not due to reduced food intake.

The fundic mucosa in hypophysectomized mice was thinner than in the controls (fig. 2). The lumina of the fundic glands were often enlarged while in control mice they were seldom visible in microscopic sections. Dilation of the fundic lumina appeared more pronounced after fixation in Regaud's fluid than in FAA-preserved material. It was most prominent in the middle one-third of the mucosa (parietal cell area), less so in the base of the fundic gland, and was rarely evident in the neck region (figs. 2, 4, 6 and 8).

Although no demonstrable changes were observed in surface cells and nondifferentiated cells, some alteration was induced in mucous neck cells by pituitary excision. The number of mucous neck cells per fundic gland was increased in 18 of 21 hypophysectomized mice. The cells were usually smaller than those of controls (fig. 1) and in 17 of 22 cases contained less secretory material (fig. 4). In 21 of 22 animals, the colloidal iron-positive variety of mucous neck cell was dominant, and only rarely were other cell varieties seen. Transitional cells containing both PAS-positive and colloidal iron-positive material were dominant in the one exceptional case. The nucleus and mitochondria of mucous neck cells were unchanged.

Although the number of parietal cells per fundic gland appeared to be unchanged, they were strikingly atrophic after hypophysectomy when compared with controls (figs. 2,

4, 6 and 8). The cytoplasm maintained a pink mottled appearance with PAS staining. The perinuclear halo, which in controls marked the location of intracellular canaliculi, was no longer present. Rather this material was arranged as a diffuse clump of dense PAS-positive material on the luminal side of the nucleus indicating that a profound alteration had occurred in the structure of the intracellular canaliculi. This conclusion was confirmed by observation of stomachs fixed in Regaud's fluid and stained with the Altmann-Masson procedure (fig. 8) for mitochondria. In them the position of the canaliculi coincided with the clump of dense PAS-positive material seen on the luminal side of the nucleus. Thus, the intracellular canaliculi, which normally cupped around the nucleus from its luminal side and extended into the basal region of the parietal cell, were reduced to a small area between the nucleus and the glandular lumen. Consequently, instead of forming two distinctly separate perinuclear rings, mitochondria filled the entire cytoplasmic area basal and lateral to the nucleus (fig. 8) in parietal cells of hypophysectomized mice. Sometimes the luminal openings of the intracellular canaliculi appeared so extensively dilated that the nuclei of parietal cells seemed to be located adjacent to the glandular lumen. Atrophy of the parietal cells appeared to be partly responsible for the increased caliber of the glandular lumen. The total number of mitochondria was reduced. Nuclear size was slightly decreased and the chromatin appeared more compact

in 12 of 20 animals.

The number of chief cells per fundic gland was reduced and they were remarkably involuted (figs. 2 and 12). Chief cells were changed from the high columnar form characteristic of the controls to a low cuboidal shape (figs. 11 and 12). Fewer secretory granules were present per cell, with a concomitant disappearance of the expansive granule-filled apical area seen in control animals. In some hypophysectomized mice, chief cells had no visible granules at all. No change in the granular size was observed. The total amount of basophilic material and the intensity of staining with azure II were reduced. Mitochondria were shorter, wider and fewer in number (figs. 9 and 10). In contrast to their arrangement in controls, mitochondria appeared to follow the contour of the nucleus and were sometimes applied closely to the nuclear membrane. Chromatin was more compact. Nucleoli were smaller in 17 of 22 operated mice than in their controls.

The Pancreas

Control mice. The diameter of pancreatic acini of pair-fed controls was somewhat less than that of ad libitum-fed control mice, although glandular weights and gland weight/body weight ratios were not different in the two groups (table 3).

For study of the size, number and distribution of organelles, employment of several procedures was necessary.

TABLE 3

The Effect of Hypophysectomy
on Weights of the Pancreas and Parotid Gland

Treatment	No. of Mice	Pancreas		Parotid Gland	
		Weight (mg)	$\frac{\text{Gland Wt.(mg)}}{\text{Body Wt.(g)}} \times 10{,}000$	Weight (mg)	$\frac{\text{Gland Wt.(mg)}}{\text{Body Wt.(g)}} \times 10{,}000$
Experiment I Ad libitum-fed					
Control	18	291.31±30.20[1]	87.94±8.99	26.75±1.49[2]	8.06±0.58
Hyp.	11	115.04±13.72	60.48±6.68	16.07±1.43[3]	8.98±0.51
	P	<.01	<.05	<.01	NS
Experiment II Pair-fed					
Control	12	231.68±18.44	92.81±4.48	26.04±1.73[4]	10.91±1.11
Hyp.	12	167.32±19.60	85.31±9.04	16.94±0.91[4]	9.23±0.57
	P	<.01	NS	<.01	NS

Hyp. = Hypophysectomized P = Probability of error NS = Not significantly different at P<.05

[1]Standard error of the mean
[2]8 mice
[3]4 mice
[4]6 mice

Secretory granules were not preserved consistently after Bouin fixation but after preservation with Champy's fluid or Zenker-formol followed by Altmann-Masson staining, they stained intensely and uniformly (fig. 15). Small and large granules were seen in the same cell. Acini adjacent to pancreatic islets were usually filled with granules, while, in other portions of the gland, storage of granules varied from acinus to acinus. Evaluation of the amount of cytoplasmic basophilia in pancreatic acinar cells was difficult since the distribution of basophilic material was affected by the number of secretory granules. It was concentrated basally in granule-filled cells and more diffusely arranged in granule-poor cells. A fine network of basophilic material consistently surrounded the secretory granules. Mitochondria appeared as darkly stained threads of varied thickness and length (fig. 15). They were most numerous basal to the nucleus where they were arranged with their long axes parallel to the base of the cell. Those found along the lateral cell membrane were oriented perpendicularly to the base of the cell. The Golgi apparatus (fig.17) consisted of a reticular network located above the nucleus and dispersed among the supranuclear secretory granules. Large nuclei with chromatin clumped along the nuclear membrane and with large, irregularly shaped nucleoli were seen in ad libitum-fed controls. Nucleoli of pair-fed controls (fig. 13) were somewhat smaller and rounder in comparison with the irregularly shaped nucleoli of

ad libitum-fed controls.

Hypophysectomized mice. Hypophysectomy caused pancreatic involution. This was shown by its lower absolute weight in hypophysectomized mice as compared with controls in both experiments (table 3). In Experiment I, the mean weight of the pancreas ($P < .01$) and the mean gland weight/body weight ratio were both reduced. In Experiment II, the absolute weights were reduced by pituitary ablation but the gland weight/body weight ratio was not.

Loss in weight of the pancreas was reflected in its histological alteration. Acinar diameter and cell size were less when compared with both ad libitum-fed and pair-fed controls (figs. 14 to 16). Although much less numerous, the quantity of secretory granules in acinar cells was more uniform among hypophysectomized mice than that in control animals. Nevertheless, peri-islet acinar cells showed less degranulation than other acinar cells. Cytoplasmic basophilic material basally and between granules was reduced in amount and less intensely stained. Mitochondria were thinner and shorter (fig. 16). The Golgi apparatus was condensed into a compact supranuclear body and no longer extended out among the secretory granules (fig. 18). Nuclei and nucleoli (fig. 14) were smaller; chromatin was more compactly arranged.

The Parotid Gland

Control mice. The absolute weight of the parotid gland

was not affected by pair-feeding but the difference between the mean gland weight/body weight ratios of pair-fed and ad libitum-fed controls was significant (table 3).

Acinar cells of the parotid gland were pyramidal and contained basal cytoplasmic basophilic material (fig. 22) which extended as a fine network among the apical secretory granules. Mitochondria were short rods and randomly oriented in the basal half of the acinar cell (fig. 19). Epithelial cells lining salivary ducts were columnar, lightly PAS-positive and possessed basal striations (fig. 22). Most mitochondria were located in the basal area of the cell parallel to the basal striations; a few were scattered in the apical cytoplasm. They were long and thin (fig. 20). The cells lining the intercalated ducts were low cuboidal and possessed a small area of cytoplasmic basophilia on either side of the nucleus. The background cytoplasm stained rose with PAS and sometimes contained PAS-positive granules (fig. 22). These granules were not glycogen, since digestion with α-amylase did not remove them.

Hypophysectomized mice. The mean weight of the parotid gland was reduced after hypophysectomy (table 3). However, the mean gland weight/body weight ratio remained unchanged.

Histological changes induced in the parotid gland by hypophysectomy were similar to, but not as great as, those elicited in the pancreas. Parotid acini were smaller, the epithelial cells being reduced in size and containing fewer secretory granules (figs. 21 and 23) than those of controls.

Cytoplasmic basophilia was clearly reduced in 13 of 22 hypophysectomized mice and questionably so in the other 9. Mitochondria were thinner and appeared to be more concentrated due to the loss of intervening secretory granules (fig. 21). Nuclei and nucleoli were unchanged.

Epithelial cells of the duct system in the parotid gland were also affected by hypophysectomy. The diameter of salivary ducts and size of their lining cells were reduced (figs. 21 and 23). Basal striations were no longer visible. Mitochondria were more evenly distributed throughout the cell and were not limited to the basal area. Those present in the basal area were shorter, randomly oriented and more densely packed. After hypophysectomy, cells lining the intercalated ducts were less basophilic. The number of PAS-positive granules was unaffected in these cells.

The Submandibular Gland

Control mice. The submandibular glands of pair-fed control mice weighed significantly less than those of ad libitum-fed controls ($P < .01$) but the means of the gland weight/body weight ratios for the two control groups did not differ significantly (table 4). Thus, weights of the submandibular gland and of the body responded to restricted food intake in parallel fashion.

The diameter of the submandibular serous tubules in pair-fed control mice was smaller than that of the ad libitum-fed controls. The large granules in the apical half

TABLE 4

The Effect of Hypophysectomy
on Weights of the Submandibular and Sublingual Glands

Treatment	No. of Mice	Submandibular Gland: Weight (mg)	Submandibular Gland: $\frac{\text{Gland Wt.(mg)}}{\text{Body Wt.(g)}} \times 10{,}000$	Sublingual Gland: Weight (mg)	Sublingual Gland: $\frac{\text{Gland Wt.(mg)}}{\text{Body Wt.(g)}} \times 10{,}000$
Experiment I Ad libitum-fed					
Control	18	83.52±3.11[1]	25.01±0.83	10.69±0.53	3.19±0.16
Hyp.	11	17.41±0.72	9.22±0.35	5.88±0.18	3.11±0.21
		P <.01	<.01	<.01	NS
Experiment II Pair-fed					
Control	12	57.21±4.72	22.78±1.14	12.08±0.73	4.85±0.21
Hyp.	12	19.60±1.05	10.09±0.50	8.36±0.49	4.28±0.26
		P <.01	<.01	<.01	NS

Hyp. = Hypophysectomized P = Probability of error NS = Not significantly different at P<.05

[1]Standard error of the mean

of the serous tubular cells stained with the Altmann-Masson procedure (fig. 28). A diffuse cytoplasmic basophilia was present in the basal area (fig. 24). Mitochondria were usually compressed into the basal region of the cell by the accumulated serous granules (fig. 28). In partly degranulated cells some mitochondria were scattered in the supranuclear zone. Mitochondria were randomly oriented and, when cut longitudinally, appeared as short rods.

The mucous acinar cells of the submandibular gland were filled with secretory material. Cytoplasmic basophilia was most dense in the basal area (fig. 24, inset) but occurred elsewhere also. When stained with colloidal iron-PAS, the mucous secretion stained a dark blue (fig. 26).

The parasympathetic submandibular ganglion was identified in the hilus of the submandibular gland surrounded by connective tissue. The ganglion cells were large and contained much Nissl substance. They had large nuclei with prominent round nucleoli (fig. 30).

Hypophysectomized mice. The mean weight of the submandibular gland in hypophysectomized mice and the mean gland weight/body weight ratios were reduced ($P < .01$) (table 4). A chronic sialitis was often present after hypophysectomy. The effect of hypophysectomy on the histology of the submandibular gland of the mouse has been described by Lacassagne and Chamorro ('40), and Shafer and Muhler ('60). The following new observations were made.

Marked atrophy of the serous tubules, with a loss of

serous granules and cytoplasmic basophilia and a decrease in nuclear size (figs. 25, 27 and 29), as described after hypophysectomy by other workers, was confirmed. In addition, glycogen granules became more prevalent in the apical area of serous tubular cells. Mitochondria were arranged perpendicularly to the base of the serous tubular cells (fig. 29). This arrangement of mitochondria occurred more frequently than in controls and imparted to the serous tubules the cytological appearance of inter- and intralobular striated ducts. The smaller diameter and the more numerous intracellular PAS-positive granules (fig. 25) in the involuted serous ducts were the only features which permitted one to differentiate them from the non-serous portions.

In addition to the reported atrophy of serous tubules, mucous acini (figs. 25 and 27) were reduced in diameter and their epithelial cells were smaller. PAS-positive material was decreased in total amount, less intensely stained and had a foamy appearance in contrast to the granular form characteristic of the mucin in most acini of controls. The colloidal iron-PAS procedure revealed the secretory material to be aqua-green, in contrast to the blue color of mucous acinar cells in controls. Cytoplasmic basophilia was reduced in total amount and staining intensity (fig. 25). Nuclei of mucous cells appeared slightly smaller but nucleoli were unchanged. Intercalated ducts were unaltered but appeared relatively more prominent because of the acinar involution.

The parasympathetic ganglion of the submandibular gland and its constituent nerve cells were smaller after hypophysectomy. Nissl substance (fig. 31) was reduced and nuclei and nucleoli were smaller.

The Sublingual Gland

Control mice. As compared with ad libitum feeding, restriction of food intake by pair-feeding did not affect the weight of the sublingual glands but did reduce the mean gland weight/body weight ratio ($P < .01$) (table 4).

The secretory material of mucous acinar cells (fig. 32) had a foamy appearance when revealed with PAS. With colloidal iron-PAS, the material staining with PAS was found in the apical part of the cell while other areas of the cell reacted intensely to colloidal iron. The nucleus was displaced basally by the accumulated intracellular secretion and cytoplasmic basophilic material was scattered on either side of it.

The cells of the salivary ducts in control animals had prominent basal striations (fig. 34) which stained with colloidal iron. In 6 of 21 animals, scattered cells of the salivary ducts were filled with granules (Spicer and Jarrels, '61) which did not react with PAS or colloidal iron, but stained intensely with the Altmann-Masson procedure (fig. 34). The granules were of the same size and staining properties as those of the serous tubules in the submandibular gland. Mitochondria (fig. 34) were situated along the

basal striations and scattered throughout the cytoplasm of the ductular cells.

Hypophysectomized mice. The mean absolute weight of the sublingual gland was reduced in hypophysectomized mice ($P < .01$) (table 4) but the mean gland weight/body weight ratios were not significantly different. Thus, the sublingual gland was no more sensitive to withdrawal of pituitary hormones than was the body as a whole. Histologically, the acinar diameter (figs. 33 and 35) was reduced and acinar cells contained less secretory material. The staining properties of the intracellular mucin, cytoplasmic basophilia, nuclei, nucleoli and mitochondria were not changed. Salivary ducts were narrower (fig. 35) and granulated cells had disappeared. Epithelial cells of salivary ducts were lower and had less distinct basal striations. The basal area of these cells (fig. 37) was variably vacuolated. Mitochondria were shorter, smaller (fig. 35) and no longer arranged between basal striations as in the controls. Nuclei contained clumped chromatin, and nucleoli were less distinct. Intercalated ducts became relatively more prominent after hypophysectomy due to the involution of the acini.

DISCUSSION

The Influence of Food Intake

Pair-feeding was employed to insure that the histological effects noted after hypophysectomy were not due to the approximately 30% reduction in food intake which typifies hypophysectomized animals (Hahn et al., '65). In addition, food restriction influences several pertinent aspects of cellular chemistry. The amount and activity of RNA are less in animals fed protein-free diets (Hultin, '64) and in rats starved for long periods of time, RNA and certain enzymes are decreased in both the submandibular gland and pancreas (Tamarin et al., '63).

Reduction in food intake of control mice to that consumed by hypophysectomized mice by pair-feeding impaired the gain in weight of the body and submandibular glands. Weight of the submandibular gland remained proportional to body weight but the mean gland weight/body weight ratios of the sublingual gland, parotid gland and stomach were greater in pair-fed controls as compared with those of ad libitum-fed controls. Thus, the reduction in weight of the sublingual and parotid glands and of the stomach, which followed pituitary ablation, was not caused by reduction in the amount of food ingested.

Starvation of animals before sacrifice was necessary because histological variation induced by food ingestion at this time would have interfered with evaluation of the

effects of pituitary ablation. Certain periods of starvation altered the histological picture of some of the digestive glands in the mouse, particularly of the pancreas and parotid. The ingestion of hair may have been a factor in eliciting this irregularity since control mice starved for more than 12 hours were active and displayed cleaning behavior. As a result, hair was found in their stomachs. More than 12 hours of starvation did not cause degranulation of these glands in hypophysectomized mice. In contrast, hypophysectomized mice were quiet, did not display cleaning behavior and their stomachs were empty. With the starvation time of 9 to 12 hours which was used routinely in these experiments, pancreases and parotid glands of control mice remained granulated and their histology could be compared with that of hypophysectomized mice. In rats, starvation for 18 to 24 hours did not elicit degranulation of the pancreas or parotid gland (Baker, '58).

Normal Histology of the Mouse Digestive System

The histology of the digestive glands of the normal mouse differs from that of other mammals in several unique ways. First, with respect to the stomach, the presence of mucous neck cells in the mouse and some other mammals is still debated. Using the electron microscope, they were observed by Corpron ('66) in the rat, by Sedar ('64) in the dog and by Ito and Winchester ('63) in the bat. However, Lillibridge ('64) was unable to distinguish mucous neck

cells in man. Helander ('62 and '64) claimed that they could not be differentiated from surface cells of the mouse gastric mucosa by means of electron microscopy.

My observations show clearly that mucous neck cells are present in the mouse. However, their location in the fundic gland differs from that in most other mammals. Rather than being situated in the neck as is true of the rat (Baker and Abrams, '54) and most other mammals, they are interposed among parietal cells just above the lower one-third of the mucosa, as described by Fekete ('41). Since Helander ('64) failed to differentiate mucous neck cells from surface cells in the fundic mucosa of the mouse with the electron microscope, it is significant that the blue color imparted to mucous neck cells by colloidal iron differs from the purple color of the surface mucous cells. This staining difference indicates that surface and mucous neck cells are different in type. The mucoid cells described by Helander ('64) appear not to include the mucous neck cells described for other species, but his "atypical zymogen cells," except for the character of their cytoplasmic granules, resemble closely the mucous neck cells of rats as described by Corpron ('66).

The three histochemically different varieties of normal mucous neck cells, as described in this study, have not been previously reported for any mammalian species. Whether they represent different functional types, different stages in a secretory process, or indicate transformation of one distinct

type into a second is not now clear. Spicer ('60) was unable to demonstrate acid radicals in the mucopolysaccharide in mucous neck cells of the mouse without prior chemical treatment. Since Townsend ('61), using Bowie's stain on rat stomach, detected pepsinogen granules in mucous neck cells which were seemingly undergoing transition to chief cells, it is necessary to consider the possibility that the granules which stained with colloidal iron represent pepsinogen. This does not seem possible since the chief cell granules did not stain with this procedure.

The halos of PAS-positive material in the parietal cells of the normal mouse stomach, which make visualization of the position of intracellular canaliculi possible, have not been reported by others. Ito ('65) described an "enteric" coat of PAS- or colloidal iron-positive material on the luminal surface of the intestinal epithelial cells of the mouse and other mammals. This coat is filamentous in appearance as seen in electron micrographs and was interpreted to be an extension of the outer leaflet of the cell membrane. However, Ito ('61) reported that intracanalicular microvilli of the mouse are not similarly covered. Nevertheless, the halo of PAS-positive material may represent a mucopolysaccharide coat over the intracanalicular microvilli which has not yet been observed.

The location of mitochondria in parietal cells of the normal mouse appears to be a secondary reflection of the distribution of the intracellular canaliculi. The position

of the PAS-positive halo in parietal cells corresponds with the unstained area between the two rings of mitochondria as revealed in Altmann-Masson preparations. Electron micrographs of mouse parietal cells (Ito, '61; Helander, '62) show that the intracellular canaliculi are located in this region. The arrangement of the intracellular canaliculi appears similar in the parietal cells of the bat and mouse when observed with both light and electron microscopes (Ito, '61; Ito and Winchester, '63).

The presence of the granule-containing cells of the salivary ducts in the mouse sublingual gland was reported by Spicer and Jarrels ('61). Since these cells are found predominantly in male mice and resemble the granulated cells of the serous tubules in the submandibular gland, they probably are of like functional significance.

Comparison of the Effects of Hypophysectomy in the Mouse with Other Mammals

In most respects the digestive glands of the mouse responded to hypophysectomy in a manner similar to those of the rat and other animals.

The stomach. Involution of the gastric mucosa appears to be characteristic of pituitary deficiency. Reduction in volume (Crean, '63a) and thinning of the gastric mucosa after hypophysectomy have been observed in the cat (Haeger, Jacobsohn and Kahlson, '53), rat (Abrams and Baker, '54) and man and also in association with panhypopituitarism

(Laumonier et al., '63). Jacobson and Magnini ('64) reported no change in thickness or histology of the gastric mucosa of two hypophysectomized dogs, but at least one of their animals received cortisone, corticotropin and growth hormone during the experiment. Since the period of time between termination of the hormonal replacement therapy and sacrifice is not clear, the validity of their conclusion is doubtful. The absence of histological change in the surface mucous cells and nondifferentiated cells after hypophysectomy of the mouse agrees with findings in the rat (Baker and Abrams, '54; Corpron, '66).

Mucous neck cells of the mouse were altered significantly by hypophysectomy, in contrast to the minor changes reported in other animals. The irregular reduction in the amount of mucoid material in individual mucous neck cells of the mouse agrees with that seen in the rat (Baker and Abrams, '54) with the light microscope although no alteration in ultrastructure was observed by Corpron ('66). Unique to the mouse is the increase in the number of mucous neck cells and the predominance of the colloidal iron-positive histochemical variety of mucous neck cells which resulted from pituitary ablation.

In contrast to the minor changes reported in parietal cells of the rat after hypophysectomy, their structure is greatly altered in the mouse. In agreement with Crean's ('63a and b) observations in the rat, the number of parietal cells per fundic gland did not seem to be reduced by

hypophysectomy in the mouse. The decrease in parietal cell size was much greater in the mouse than in the rat. Mitochondria were depleted from parietal cells in both species when observed with the light microscope although Corpron ('66) did not confirm this reduction in the rat with the electron microscope. Of utmost significance is the great alteration in canalicular structure as revealed by two staining procedures. This modification was not apparent in light microscopic studies of rat gastric mucosa and only slight alteration in size of microvilli of intracellular canaliculi was seen by Corpron ('66) with the electron microscope in hypophysectomized rats. This may be the first time that such great histological alteration of parietal cells has been induced in any animal by experimental manipulation. Although some workers (Vial and Orrego, '60; Sedar and Friedman, '61) have reported ultrastructural alteration of the parietal cells after stimulation, Ito ('61) claimed that form of intracellular canaliculi cannot be correlated with any physiological state because of their great normal variation.

The decrease in size of chief cells and their nuclei, in the number of pepsinogen granules and mitochondria, and the loss of cytoplasmic basophilia induced in the mouse by pituitary ablation are identical with the changes observed in the hypophysectomized rat (Baker and Abrams, '54) and in general similar to those occurring in human panhypopituitarism (Laumonier et al., '63). Corpron ('66) verified

these changes in the rat with the electron microscope.

The pancreas. The pancreas of the mouse undergoes the same changes as seen in other animals after hypophysectomy. These include a loss in weight as observed in the dog (Koster, '30), pigeon (Schooley et al., '41) and rat (Haist, '55), and decrease in acinar diameter, acinar cell size, number of secretory granules, amount of cytoplasmic RNA, number and size of mitochondria, and nuclear size, as observed in the rat (Baker and Abrams, '55; Sesso et al., '55; Baker and Pliske, '57; Sesso and Valeri, '58). Condensation of the Golgi apparatus in pancreatic acinar cells after hypophysectomy has been demonstrated also in the rat with both light and electron microscopes (Baker, personal communication). A decrease in nucleolar size has not been previously reported although an increase was observed in hypophysectomized rats after hormonal replacement (Baker, '58).

The parotid gland. Changes in the parotid gland of the mouse after hypophysectomy were generally similar to those occurring in the rat although the loss in weight appears to be somewhat greater in the rat (Baker and Abrams, '55), and alteration in morphology of acinar mitochondria in the mouse contrasts with Dewey's ('58) negative findings in the rat. In both mouse and rat (Baker and Abrams, '55) decrease in cytoplasmic basophilia is not as great as in the gastric chief cells and pancreas.

The atrophy of the salivary ducts in the hypophysectomized

mouse appears more pronounced than that seen by Baker and Abrams ('55) and Dewey ('58) in the rat, but is of approximately the same degree as that reported by Yoshimura ('56). The change in morphology of mitochondria and disappearance of basal striations in salivary duct cells was observed by Yoshimura ('56) in the rat. However, no increase in glycogen content of duct cells was detected in the mouse in contrast to the reports of Yoshimura ('56) and Dewey ('58) for the rat.

The submandibular gland. The loss in weight of the submandibular gland after hypophysectomy agrees with reports for the mouse (Lacassagne and Chamorro, '40), dog (Argonz, '61), cat (Kahlson and Renvall, '56), and rat (Baker and Abrams, '55; Sreebny et al., '57). Atrophy of the serous tubules with loss of their zymogenic granules also seems to be a general phenomenon since it has now been observed in the mouse (Lacassagne and Chamorro, '40), rat (Sreebny, '53; Baker and Abrams, '55; Yoshimura, '56), cat (Kahlson and Renvall, '56), and dog (Argonz, '61). Bixler et al. ('57) saw no cytoplasmic basophilia in serous tubular cells of the normal rat submandibular gland. However, I observed a diffuse cytoplasmic basophilia in these cells in the normal mouse and disappearance of it after hypophysectomy.

Earlier workers (Lacassagne and Chamorro, '40; Gabe, '50; Taurog et al., '59) reported no acinar changes after hypophysectomy. However, in this study, a decrease in submandibular acinar size with a loss of cytoplasmic

basophilia and secretory material were observed in the mouse, and somewhat similar changes were found in the rat by Baker and Abrams ('55), Yoshimura ('56) and Argonz and de Corral Saleta ('61) and in the dog by Argonz ('61). Bixler et al. ('57) reported a fall in RNA content of the acinar cells in the rat and Kronman ('63) and Baker et al. ('64) described a decrease in the PAS-staining intensity of the mucous acini of the rat after hypophysectomy. Sreebny et al. ('57), however, saw no changes in the rat submandibular acinar cells. Therefore, complete agreement does not exist but most evidence indicates that the hypophysis exerts a regulatory influence over the production of submandibular mucus.

The sublingual gland. Of all digestive glands, the sublingual glands are affected the least by removal of the pituitary gland. However, as in the rat (Baker and Abrams, '55), the mean absolute weight, the mean gland weight/body weight ratio and the amount of mucin in acinar cells were decreased. In contrast, Kronman ('63) saw no histological changes in the rat. The involution of striated ducts with accompanying increased mitochondrial concentration and the loss of scattered granule-containing cells (serous cells) which occurred in the mouse are new findings.

The Effect of Hypophysectomy on Protein Synthesis

The considerable evidence now available for the rat and mouse indicates that, in general, protein-producing cells of the digestive system undergo greater atrophy after

hypophysectomy than do other cell types studied. For the mouse this conclusion was made evident by the structural responses to pituitary ablation of gastric chief cells, acinar cells of the pancreas and parotid glands, serous cells in the submandibular and sublingual ducts, and parasympathetic ganglion cells associated with the submandibular gland. Biochemical data are available from study of other species to prove that this histological involution represents a true functional depression. Thus, in the rat, storage or secretion of the following enzymes is decreased after hypophysectomy: pepsin (Baker and Abrams, '54), pancreatic protease (Haist, '55; Baker, Reid and Thoms, '56; Sesso and Valeri, '62; Sesso et al., '63), pancreatic amylase (Barrett et al., '55; Sesso and Valeri, '62), pancreatic lipase (Bicknell and Baker, '62), amylase and protease in the submandibular gland (Sreebny, '53; Sreebny et al., '57), and amylase in the parotid gland (Baker, Klegman, Logan and Garner, '56).

The structural and histochemical alterations in zymogenic cells interpreted in relation to biochemical data give some indication of where in the pathway of protein synthesis a deficiency may exist in hypophysectomized animals. The decreases in nucleolar size, amount of cytoplasmic basophilia, size of the Golgi apparatus and the number of secretory granules all may be taken as indicative of interference at various points in this pathway. Since RNA of the nucleolus is representative of rRNA (De Robertis et al., '65), reduction in size of the nucleolus after hypophysectomy

suggests that a block occurs in the formation of rRNA. Retarded incorporation of amino acids into protein after hypophysectomy has been demonstrated in the microsomal fraction of hepatic cells (Korner, '59). The reduction in cytoplasmic basophilia after pituitary ablation reflects a reduction in RNA although which RNA fraction accounts for the basophilia has not yet been revealed. Nevertheless, the profound loss of basophilia in chief and pancreatic acinar cells clearly indicates a depletion of endoplasmic reticulum and ribosomes from the cytoplasm, such a loss having been demonstrated by Corpron ('66) in the rat chief cells. The involvement of rough-surfaced endoplasmic reticulum and ribosomes in protein synthesis has been demonstrated by both biochemical (Korner, '59 and '60; Hultin, '64; and many others) and electron microscopic studies (Kurosumi, '61; Caro and Palade, '64). A deficiency in the capacity of ribosomes to form proteins after hypophysectomy has been demonstrated by Korner ('59 and '60) and Richardson et al. ('66). The latter workers concluded that the polysomes of hepatic cells from hypophysectomized rats could not translate mRNA. Similarly, Liao and Williams-Ashman ('62) observed a decrease in amino acid incorporation into protein by ribosomes of the prostate gland after castration. If cytoplasmic basophilia is a reflection of the quantity and condition of rough-surfaced endoplasmic reticulum in the cell and is directly related to its capacity to produce protein, the decreases in amount of basophilic material in

zymogen-producing cells of the mouse and rat after hypophysectomy constitute histological evidence of a deficiency in protein synthesis at the point of polypeptide formation.

Currently accepted concepts hold that polypeptides formed at the ribosomes pass into the cisternae, through which they move to the Golgi apparatus. Here, the secretory product becomes visible microscopically as it is enclosed by a smooth-surfaced membrane. A direct relationship between size of the Golgi apparatus and secretory activity has long been recognized in many types of secretory cells (Kirkman and Severinghaus, '38; Dalton, '61; Zeigel and Dalton, '62). Hence, the contraction of this organelle in pancreatic acinar cells of both mouse and rat after hypophysectomy clearly indicates a depression in the production of protein. The depletion of secretory granules from zymogenic cells also reflects decreased production of secretion, since there is no evidence that the release of secretion from the cell is accentuated by hypophysectomy.

The Effect of Hypophysectomy on Mucopolysaccharide Production

The production of mucin appears to be less dependent on hormonal control than is the synthesis of protein. Surface cells of the gastric mucosa showed no change after hypophysectomy in the mouse or rat (Baker and Abrams, '54; Corpron, '66). However, Robert et al. ('66) reported that the rat stomach contains less mucus after hypophysectomy. Following

adrenalectomy of the rat, a decreased amount of mucin was found in the mucous surface cells of the stomach (Baker and Bridgman, '54); whether this condition resulted from decreased production or augmented release was not ascertained. Although the sialomucin of the sublingual acinar cells differs in its histochemical properties from that in the submandibular gland (Spicer and Jarrels, '61), mucin in both glands was reduced in amount after hypophysectomy, indicating some interference in its synthesis. A similar inference may be drawn from the lesser amount of secretory material present in the mucous neck cells of the mouse and rat (Baker and Abrams, '55) after hypophysectomy. The dominance of the colloidal iron variety (acid mucopolysaccharide) of mucous neck cell in the mouse after pituitary ablation may indicate a suppression of neutral mucopolysaccharide production or alteration in a secretory cycle of these cells.

The Effect of Hypophysectomy on Acid Production and Ion Transport

Gastric acid production is influenced by pituitary hormones. A reduction in acid production and/or an elevation of pH occurred in the stomach of the rat (Crafts and Walker, '47; Baker and Abrams, '55), cat (Cutting et al., '37), dog (Jacobson and Magnini, '64) and man (Doteval and Westling, '63) after hypophysectomy. This effect correlates well with the decrease in size (Baker and Abrams, '54) and

in total number of parietal cells (Friedman, '53; Crean, '63a and b) present after pituitary ablation of the rat and with the profound involution of parietal cells observed in the mouse.

Much disagreement exists among investigators regarding the functional significance of parietal cell structure. Surface area and length of the canalicular microvilli were used as an index of cellular activity under other experimental conditions by Vial and Orrego ('60) and Sedar and Friedman ('61) who reported an increase in surface area of microvilli following stimulation of the stomach. However, Ito ('61) refuted these claims. It is conceivable that if maintenance of the morphology of microvilli is dependent on an "enteric" coat (Ito, '65), an alteration in its composition may be partly responsible for the collapse of intracellular canaliculi after hypophysectomy of the mouse. The greatly reduced secretory surface area which I observed in the canaliculi of the mouse, coupled with the decrease in number of mitochondria and cellular size, provide histological evidence for a reduction in acid production by these cells.

Ion transport also occurs in the ducts of salivary glands, where cell morphology may be correlated with function. Junqueira ('64) demonstrated a relationship between the histology of the salivary ducts and ion composition of the saliva. The involution of salivary ducts which occurred in the mouse parotid and sublingual glands indicates that

ion transport across their epithelium was altered. Taurog et al. ('59) were not able to detect any change in the iodine-concentrating capacity of the submandibular gland after hypophysectomy in the mouse. In accord with this observation, I found that hypophysectomy did not cause basal striations to disappear from salivary duct cells in the mouse submandibular gland as it did from the parotid and sublingual glands.

The Pathways of Hormonal Control over the Digestive System

The pathways of hormonal control over secretory cells of the digestive glands have not been fully delineated. Little is known about the humoral factors which regulate acid production by the stomach or the hormonal pattern required for full support of parietal cells. Crean ('63a and b) was able to restore partially the total number of parietal cells in the hypophysectomized rat by treatment with growth hormone. The fall in acid production resulting from hypophysectomy was only incompletely corrected by cortisone acetate administration to the dog (Jacobson and Magnini, '64), and growth hormone therapy of the rat (Crean, '63b). More normal response was reported in the rat to result from treatment with growth hormone, corticotropin or adrenocorticosteroids begun 7 to 10 days after the operation (Kyle and Welbourn, '56).

Pathways of hormonal control over enzyme production are

more clearly defined. Enzyme production in the rat pancreas and parotid gland depends to a variable degree on thyroxin (Kinash and Haist, '55; Townsend and Baker, '59; Sesso et al., '63; Nishikawara et al., '64), growth hormone (Kinash et al., '53; Sesso and Valeri, '58; Baker et al., '61; Light and Baker, '62) and adrenocorticoids (Baker, '58; Gülzow et al., '60; Light and Baker, '62; Nishikawara et al., '64). The hormones responsible for protein production in the serous tubules of the submandibular gland are primarily testosterone and thyroxin in the mouse (Lacassagne, '40b; Lacassagne and Chamorro, '40; Raynaud, '60) and rat (Grad and Leblond, '49; Arvy and Gabe, '50; Clark et al., '57; Sreebny et al., '57; Argonz and de Corral Saleta, '61).

More work is needed to ascertain fully the hormones which regulate gastric chief cells. Combined thyroidectomy, adrenalectomy and gonadectomy elicited structural and functional changes in chief cells (Abrams and Baker, '54) comparable with those resulting from hypophysectomy. However, therapy with a combination of somatotropin, thyroxin and corticosterone (STC) failed to effect full structural repair in the hypophysectomized rat (Baker, '58). Also, the hormones required for support of zymogenic cells in the mouse are not known although Kojima ('17) reported an increase in acinar and nuclear size in the pancreas by feeding thyroid substance to the mouse. It may be inferred from this evidence that zymogenic cells are maintained by a pattern of hormones which varies somewhat with respect to

the cell type in question. Generally, adrenocorticosteroids and thyroid hormones are involved, usually as a supplement to the action of growth hormone.

The Effect of Hypophysectomy on Nerve Cells

Little information is available regarding the influence of pituitary hormones on nerve cells located outside the central nervous system. In the only other previous report Atech and Turchini ('65) observed that after pituitary ablation of the rat, neurons of the sympathetic chain ganglia were reduced in number, nerve fibers were smaller, and perikarya contained less Nissl material. The structure of these cells was partially restored by thyroxin therapy. My observations showed that similar regressive changes occur in the parasympathetic submandibular ganglion after hypophysectomy. The involutional changes observed in the submandibular ganglion after hypophysectomy raise additional questions regarding the mode of action of pituitary hormones on the submandibular gland and its associated ganglion. There are several possibilities. First, the withdrawal of pituitary hormones may cause involution of the submandibular ganglia, which then would lead secondarily to involution of the submandibular gland. The size and weight of the submandibular gland are under neural regulation in rats since superior cervical ganglionectomy reduces its weight and prevents its normal growth as well as the enlargement which follows incisor amputation (Wells, '63). Second,

hypophysectomy may elicit involution of the submandibular gland by withdrawal of the supporting hormones which act directly on it, with regression of the ganglion following as a secondary response. This view would also have experimental support. Nerve growth factor (Levi-Montalcini and Angeletti, '64) produced by the serous tubules of the submandibular gland is necessary for embryonic growth of peripheral sympathetic and sensory ganglia. Castration decreases the content of NGF in the submandibular gland concomitant with atrophy of its serous tubules (Levi-Montalcini and Angeletti, '64). Although Levi-Montalcini and Angeletti ('64) claimed that NGF is specific for sympathetic nerve cells in adult mammals, the rate of protein synthesis is increased by NGF in nerve cells of the dorsal root ganglia (Scott et al., '66). The effect of NGF on parasympathetic ganglia has not been studied but it might be expected that a decrease in the factor would cause a reduced rate of protein synthesis in the submandibular parasympathetic ganglion which would be accompanied by depletion of Nissl substance, and reduced nucleolar and cellular size. Finally, the submandibular gland and its ganglion may both be regulated directly by pituitary hormones and the changes observed after hypophysectomy would then arise independently of each other.

The Effect of Hypophysectomy on Replacement of Gastric Epithelial Cells

Much uncertainty exists concerning the lineages of epithelial cell types in the stomach of the rat. Hunt ('58) and Hunt and Hunt ('62), using radioautography, and Townsend ('61), using histochemical techniques, demonstrated apparent transformation of mucus-containing cells into chief and parietal cells. Corpron ('66) supported this view with electron microscopic observations. If chief cells in the mouse arise from mucous neck cells, the decrease in number of chief cells and increase in number of mucous neck cells point toward an arrest or decrease in the rate of transformation of mucous neck cells into chief cells, or possibly to an increase in the mitotic rate of mucous neck cells after hypophysectomy. However, Clark and Baker ('63) showed that the mitotic rate of mucous neck cells is decreased by hypophysectomy of the rat, concomitant with an increase in the mitotic rate of surface mucous cells.

SUMMARY

Widespread alterations occurred in the digestive glands of the mouse after hypophysectomy. Included were a reduction in weight of the stomach, pancreas and of the parotid, submandibular and sublingual glands. The diameters of the pancreatic, submandibular, sublingual and parotid acini were reduced. The gastric mucosa became thinner.

The important role played by the hypophysis in regulation of protein synthesis was reflected in the involutional changes observed in gastric chief cells, acinar cells of the pancreas and parotid gland, and in the submandibular serous tubules. These alterations included reduction in number of secretory granules, in cytoplasmic basophilia (RNA) and in size of cells, nuclei and nucleoli. The number of chief cells was reduced per gastric gland. The morphology of mitochondria was altered in gastric chief cells, pancreatic and parotid acinar cells and serous tubular cells of the submandibular gland. The Golgi apparatus was contracted in pancreatic acinar cells.

Mucus-secreting cells were also affected by hypophysectomy but to a lesser degree. In normal mice, three varieties of mucous neck cell were differentiated histochemically, one variety containing neutral mucopolysaccharides (PAS-positive), another acid mucopolysaccharides (colloidal iron-positive) and a third variety possessing both neutral and acid mucopolysaccharides. After hypophysectomy the colloidal iron-positive variety was dominant. Mucous neck

cells were increased in number per fundic gland but contained less secretory material. The mucous acini of the submandibular and sublingual glands also contained less secretory material and were smaller.

Cells concerned with ion transport or release were altered by hypophysectomy. Parietal cells became atrophic, contained fewer mitochondria and a greatly contracted intracellular canalicular system. Salivary ducts of the parotid and sublingual glands showed partial involution. Basal striations disappeared from the parotid salivary ducts.

In the parasympathetic ganglion of the submandibular gland, hypophysectomy caused a reduction in size of nerve cell bodies, in amount of Nissl substance and in size of nuclei and nucleoli.

It was concluded that, as in the rat, the protein-secreting cells of the digestive tract of the mouse depend on the support of pituitary hormones. The involutional changes observed in the submandibular ganglion probably represent the same type of dependence. Likewise, those cells of the mouse digestive glands which secrete mucopolysaccharides or are involved in ion transport require the action of pituitary hormones for maintenance of their structure and function.

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ILLUSTRATIONS

PLATE 1

Explanation of Figures

Figures 1 and 2: fixed in FAA, stained with PAS-azure II, 4 μ, x100. Figures 3 and 4: fixed in FAA, stained with colloidal iron-PAS, 4 μ, x1000.

1 Gastric mucosa of a pair-fed control mouse. Chief cells (C) are columnar and have basally located cytoplasmic basophilia and a granule-filled apex. They are found in the lower one-third of the mucosa. Parietal cells (P) are primarily located in the middle one-third of the mucosa, as well as being scattered in the neck and base of the fundic glands. Mucous neck cells (arrow) are interposed among parietal cells in the area just superficial to the chief cells. Mucous surface cells extend from the gastric pits (GP) onto the surface.

2 Gastric mucosa of a mouse 70 days after hypophysectomy. As compared with figure 1, the mucosa is thinner. Chief cells (C) appear to be decreased in number and size and have fewer granules and less cytoplasmic basophilia. Parietal cells (P) are smaller. Mucous neck cells (arrow) appear more numerous. Mucous surface cells are unchanged. The lumina of the fundic glands are dilated.

3 Gastric mucosa of an ad libitum-fed control mouse. Three histochemically different varieties of mucous neck cells are shown. Colloidal iron-positive mucous neck cells (a) stain darkly with colloidal iron (black). PAS-positive mucous neck cells (b) are lightly stained with PAS (grey). A transition phase is shown in which PAS-positive mucous neck cells contain colloidal iron-positive granules (arrow). Parietal cells (P) are large.

4 Gastric mucosa of a mouse 70 days after hypophysectomy. As compared with figure 3, the total number of mucous neck cells appears to be increased per fundic gland. Only the colloidal iron-positive mucous neck cells (black) are present. The lumina of the fundic glands are dilated (arrow). Parietal cells (P) are reduced in size.

PLATE 1



PLATE 2

Explanation of Figures

Figures 5 and 6: fixed in FAA, stained with PAS-azure II, 4 μ, x1000. Figures 7, 8, 9 and 10: fixed in Regaud's fluid, stained with Altmann-Masson, 3 μ, x1550.

5 Gastric mucosa of an ad libitum-fed control mouse. The parietal cells are large and have large nuclei with prominent nucleoli and sparse chromatin. A perinuclear halo of PAS-positive material (arrow) indicates the position of the intracellular canaliculi which cup around the nucleus from the luminal side.

6 Gastric mucosa of a mouse 68 days after hypophysectomy. The parietal cells (P) are smaller and have smaller nuclei and nucleoli and coarsely clumped chromatin. No halos of PAS-positive material are present but clumps of PAS-positive material are found on the luminal side of the nucleus (arrow). The fundic lumina are dilated as compared with the control (fig. 5).

7 Gastric mucosa of an ad libitum-fed control mouse. The parietal cells (P) are large and have large rounded mitochondria (M) located along the cell and nuclear membranes. The area (arrow) between the two rows of mitochondria indicates the location of the cup-shaped intracellular canaliculi and corresponds with the PAS-positive perinuclear halo (fig. 5).

8 Gastric mucosa of a mouse 86 days after hypophysectomy. The parietal cells (P) are smaller. The intracellular canaliculi do not cup around the nucleus, being limited to the luminal area of the cell. The luminal openings of the canaliculi are dilated (arrow). Mitochondria (M) are reduced in number and tend to be arranged around the basal aspect of the nucleus in a cup-like formation. The glandular lumen (L) is dilated as compared with that of the control (fig. 7).

9 Gastric mucosa of a pair-fed control mouse. Chief cells are illustrated. Their apical portions are filled with large secretory granules (G). The mitochondria (arrow) are long and thread-like and are located predominantly basal and lateral to the nucleus (N).

10 Gastric mucosa of a mouse 68 days after hypophysectomy. Chief cells are illustrated. Their secretory granules are sparse. Mitochondria (arrow) are shorter and wider than in the control (fig. 9). Their arrangement follows the contour of the nucleus (N).

PLATE 2



PLATE 3

Explanation of Figures

Figures 11 and 12: fixed in FAA, stained with PAS-azure II, 4 μ, x1000. Figures 13 and 14: fixed in Zenker-formol, stained with azure II-phloxine, 3 μ, x600.

11 Gastric mucosa of a pair-fed control mouse. The chief cells (C) are columnar. The cytoplasmic basophilia (E) is dense basally and basophilic material is sparsely distributed among the secretory granules. Secretory granules (G) are not fixed but the distribution of vacuoles shows that they filled the apex of the cell. The nuclei have prominent nucleoli (arrow) and fine chromatin.

12 Gastric mucosa of a mouse 68 days after hypophysectomy. The chief cells are low cuboidal. The cytoplasmic basophilia (E) is reduced to smaller areas around the nucleus. Almost no secretory granules are present. The nuclei are smaller, the chromatin coarser and the nucleoli (arrow) smaller as compared with the control (fig. 11).

13 Pancreatic acinar cells from a pair-fed control mouse. The pancreatic cells are large and filled with granules (G). The cytoplasmic basophilia (E) is abundant basally and distributed as a fine network among the apical secretory granules. The nuclei have prominent nucleoli (arrow).

14 Pancreatic acinar cells from a mouse 57 days after hypophysectomy. The acini and acinar cells are reduced in size and have fewer secretory granules (G) as compared with the control (fig. 13). The cytoplasmic basophilia (E) is reduced in total amount and staining intensity. The nuclei have more compact chromatin and smaller nucleoli (arrow).

PLATE 3

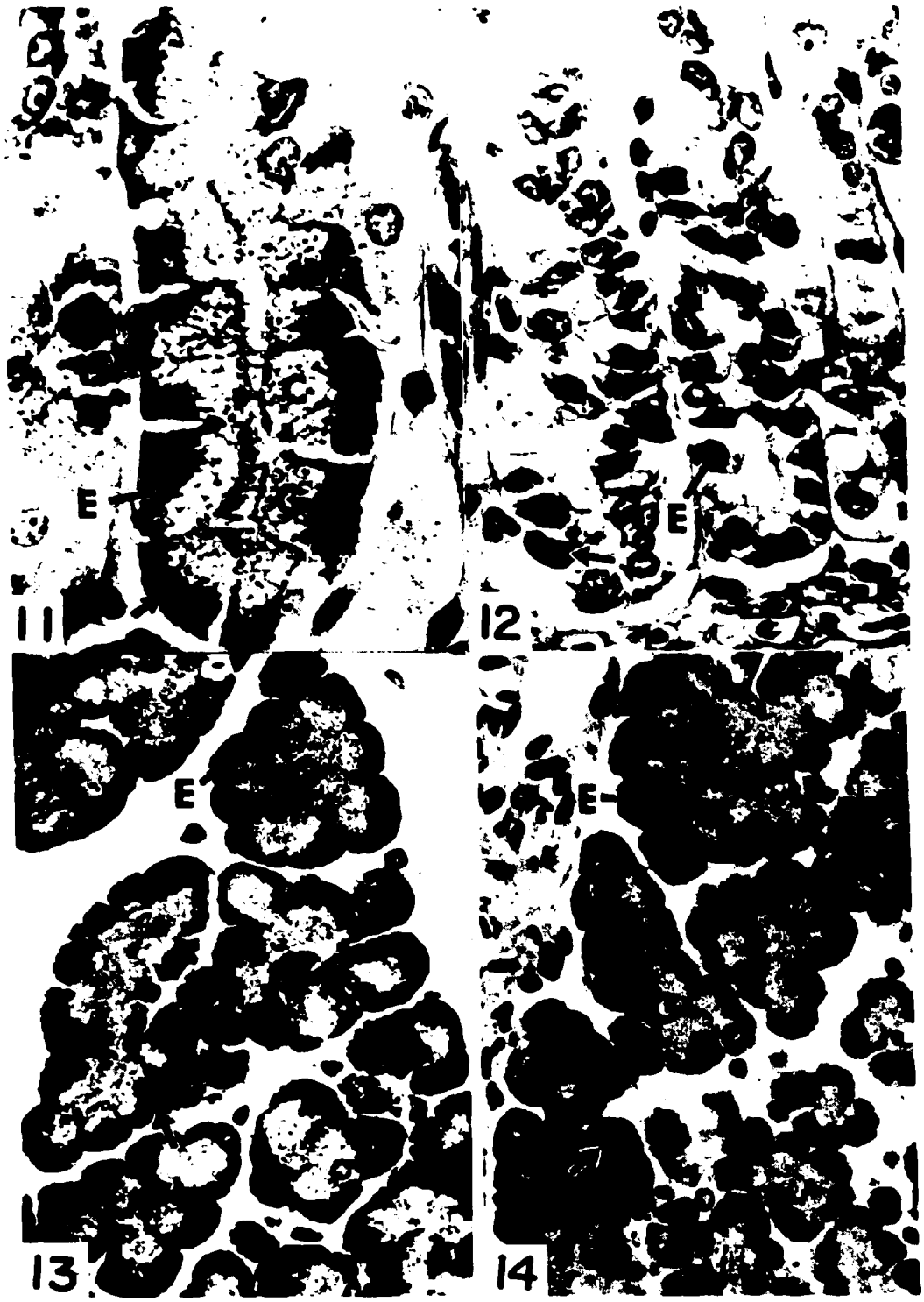


PLATE 4

Explanation of Figures

Figures 15 and 16: fixed with Zenker-formol, stained with Altmann-Masson, 3 μ, x1700. Figures 17 and 18: fixed with Champy's fluid, post-osmicated, 3 μ, x1000. Figures 19, 20 and 21: fixed with Zenker-formol, stained with Altmann-Masson, 3 μ, x1000.

15 Pancreatic acinar cells from a pair-fed control mouse. The mitochondria (arrow) are long string-like structures in the basal area and extend to the secretory granules (G) in the apex of the cells.

16 Pancreatic acinar cells from a mouse 73 days after hypophysectomy. The mitochondria (arrow) are shorter and finer than those of the control (fig. 15). They often follow the curvature of the nucleus and are located basal, lateral and apical to it. Fewer secretory granules (G) are present.

17 Pancreatic acinar cells from an ad libitum-fed control mouse. The Golgi apparatus (arrow) is distributed throughout most of the supranuclear region of the cell among the secretory granules.

18 Pancreatic acinar cells from a mouse 86 days after hypophysectomy. The Golgi apparatus (arrow) is smaller and more compact than that in the control (fig. 17).

19 Parotid acinar cells from a pair-fed control mouse. The acinar cells are filled with granules (G). Mitochondria (arrow) are rod-like and are randomly oriented.

20 Parotid salivary ducts from a pair-fed control mouse. Mitochondria (arrow) are primarily located basal to the nucleus paralleling the basal striations (B). A few mitochondria are present apical to the nucleus.

21 Parotid gland from a mouse 71 days after hypophysectomy. The acini are smaller with fewer granules (G). The acinar mitochondria (arrow) appear more numerous than those in the control (fig. 19) due to the loss of granules. They are located around the nucleus and are thinner. The salivary ducts (SD) are smaller in diameter and their cells are lower and narrower as compared with those of the control (fig. 20). Basal striations are

PLATE 4

(continued)

Explanation of Figures

absent. Nuclei are smaller. Mitochondria of the salivary duct (SD) are more concentrated, smaller and randomly oriented. They are found around the nucleus as well as basal to it.

PLATE 4

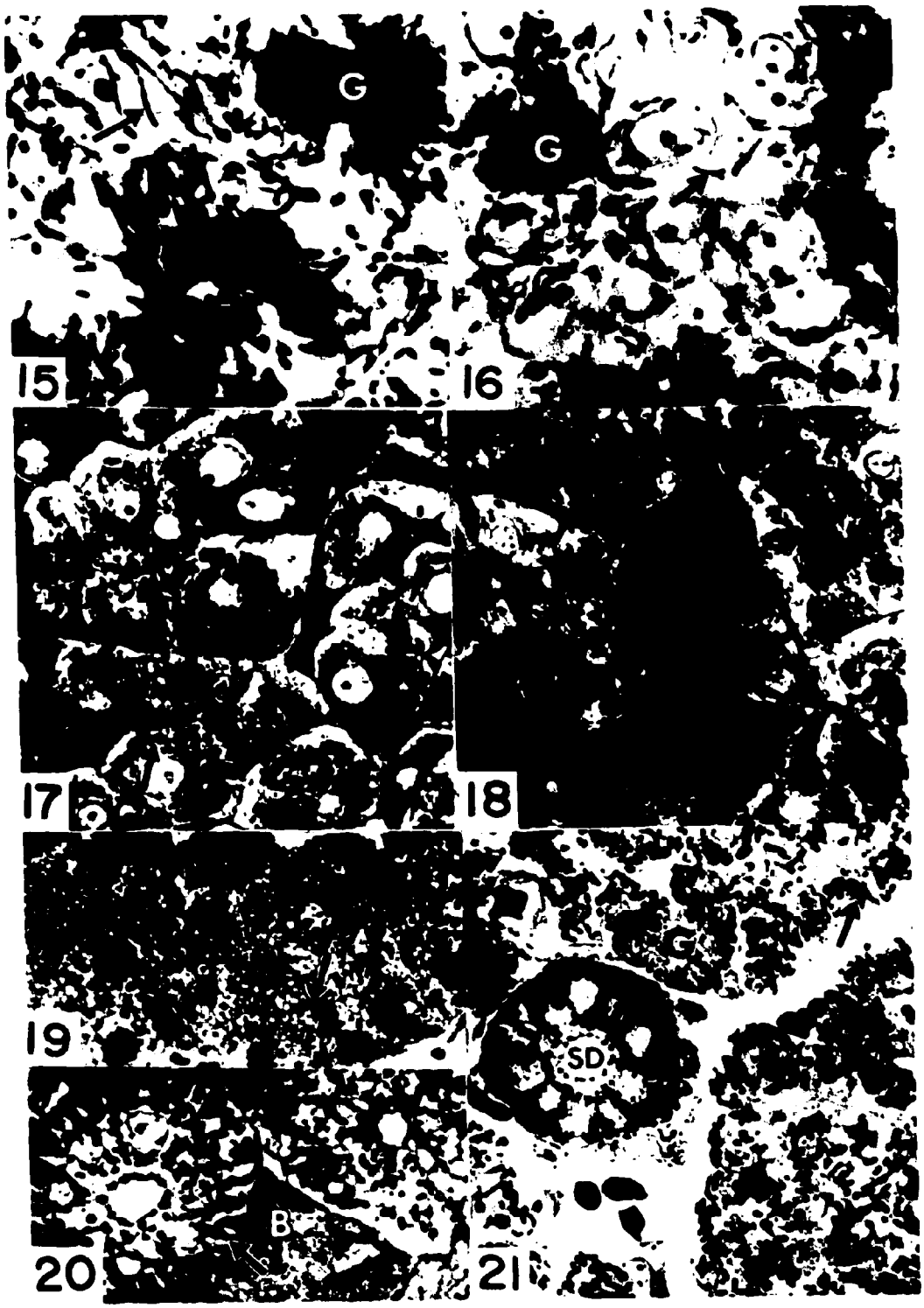


PLATE 5

Explanation of Figures

Figures 22 and 23: Fixed in FAA, stained with PAS-azure II, 5 μ, x400. Figures 24 and 25: fixed in FAA, stained with PAS-azure II, 5 μ, x400; insets 24 and 25: x924.

22 Parotid gland of a pair-fed control mouse. The acinar cells have dense basal cytoplasmic basophilia (E) and are filled with secretory granules (G). Intercalated ducts (I) are seen. The salivary ducts (SD) are lined by columnar cells with prominent basal striations (B).

23 Parotid gland of a mouse 71 days after hypophysectomy. The acinar diameters are smaller than those of the control (fig. 22). The acinar cells have fewer secretory granules (G) and less cytoplasmic basophilia (E). The intercalated ducts (I) are not greatly changed. They appear prominent because of the acinar involution. The salivary ducts (SD) are smaller and lined by low cuboidal cells. Cells of the salivary ducts have lost their basal striations and have smaller nuclei.

24 Submandibular gland from a pair-fed control mouse. The serous tubules are filled with granules (G) and possess minimal basal cytoplasmic basophilia (E) (inset). Mucous acini are filled with secretory material (S) (inset) and have dense basal cytoplasmic basophilia (E') (inset).

25 Submandibular gland of a mouse 57 days after hypophysectomy. The serous tubules are atrophied with loss of granules and cytoplasmic basophilia. The mucous acini are smaller and possess less secretory material (S) (inset) and somewhat less cytoplasmic basophilia (E) (inset) as compared with the control (fig. 24, inset). Cells of the serous tubules appear to contain more PAS-positive granules (arrow) than those found in the control (fig. 24).

PLATE 5

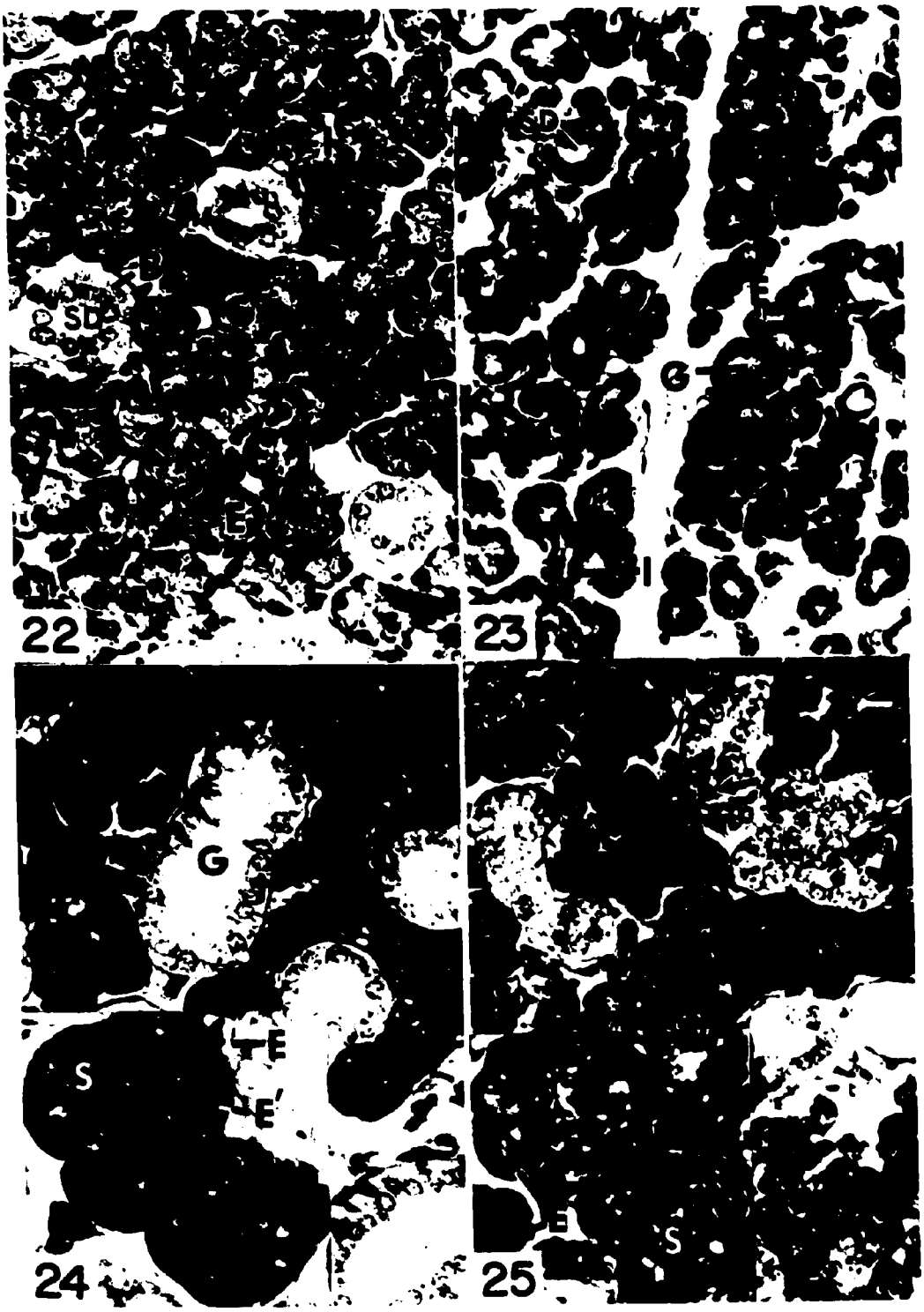


PLATE 6

Explanation of Figures

Figures 26 and 27: fixed in FAA, stained with colloidal iron-PAS, 5 μ, x400. Figures 28 and 29: fixed in Zenker-formol, stained with Altmann-Masson, 3 μ, x1000.

26 Submandibular gland of a pair-fed control mouse. Mucous acini are filled with secretory material (S) which stains intensely with colloidal iron. Epithelial cells of serous tubules are charged with secretory granules (G).

27 Submandibular gland of a mouse 71 days after hypophysectomy. The mucous acini are smaller and contain less secretory material. The secretory material (S) stains less intensely with colloidal iron as compared with that of the control (fig. 26). Serous tubules are profoundly atrophied (arrow).

28 Submandibular gland from an ad libitum-fed control mouse. Mitochondria of the serous tubular cells (arrow) are small, randomly oriented and located chiefly in the basal half of the cells. Serous tubular cells are filled with granules (G).

29 Submandibular gland from a mouse 68 days after hypophysectomy. The serous tubules (ST) are atrophied and depleted of granules. Nuclei of the serous tubular cells are smaller as compared with those of the control (fig. 28). Mitochondria (arrow) tend to be wider as compared with those of the control (fig. 28). Some mitochondria are oriented perpendicularly to the base of the cell.

PLATE 6

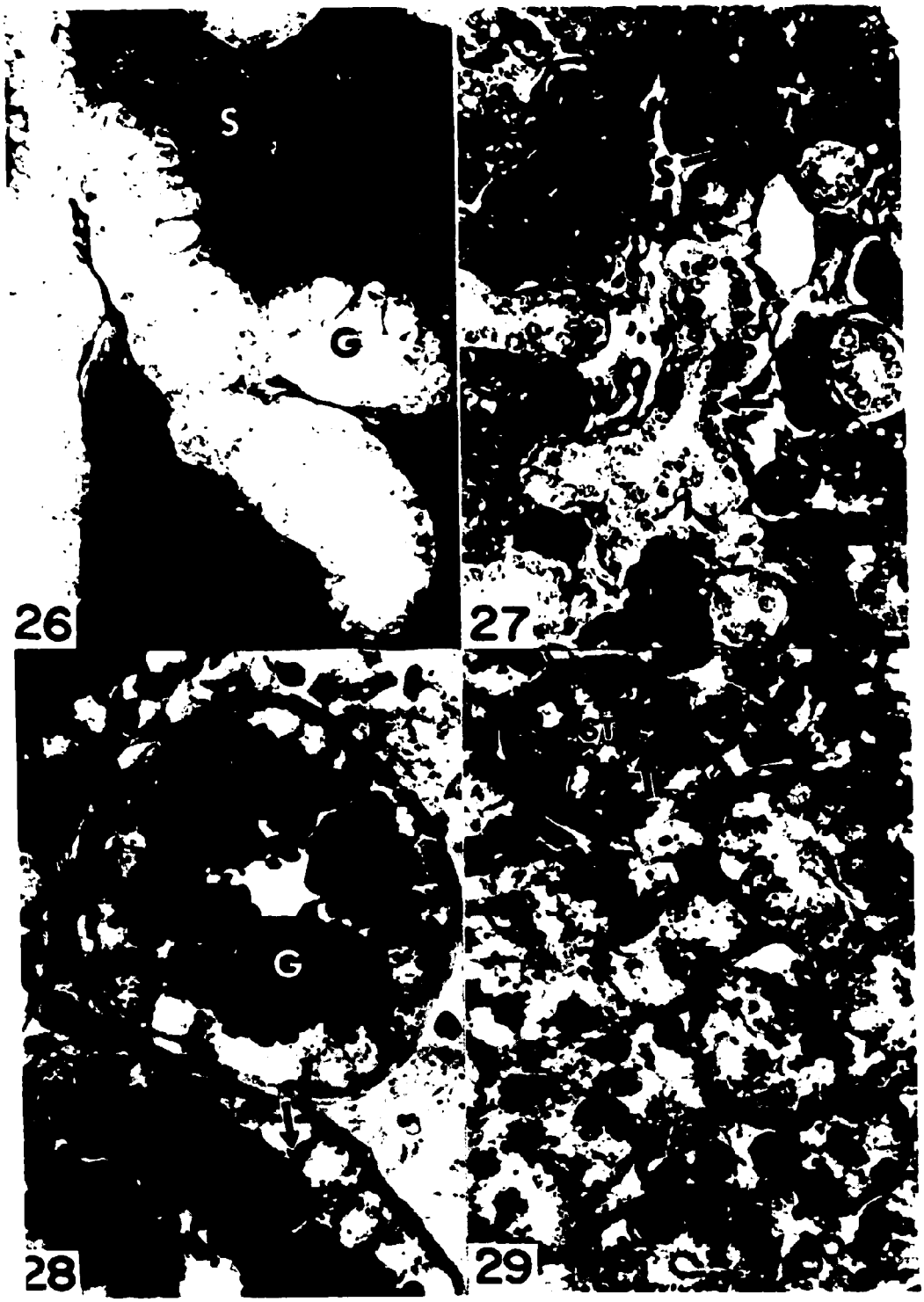


PLATE 7

Explanation of Figures

Figures 30 and 31: fixed in FAA, stained with PAS-azure II, 5 μ, x400. Figures 32 and 33: fixed in FAA, stained with colloidal iron-PAS, 5 μ, x400.

30 Submandibular ganglion from an ad libitum-fed control mouse. The ganglion cells contain much Nissl substance (NS) and have large nuclei with prominent nucleoli (arrow).

31 Submandibular ganglion of a mouse 86 days after hypophysectomy. The ganglion cells are smaller, with less Nissl substance (NS) and smaller nuclei and nucleoli (arrow) than found in the control (fig. 30).

32 Sublingual gland from a pair-fed control mouse. The mucous acini are filled with secretory material (S). The salivary duct (SD) is lined with high columnar epithelium. An intercalated duct (arrow) is shown.

33 Sublingual gland from a mouse 68 days after hypophysectomy. The mucous acini are smaller and have less secretory material (S). Cells of the salivary duct (SD) are involuted as compared with the control (fig. 32). Intercalated ducts are not involuted (arrow).

PLATE 7

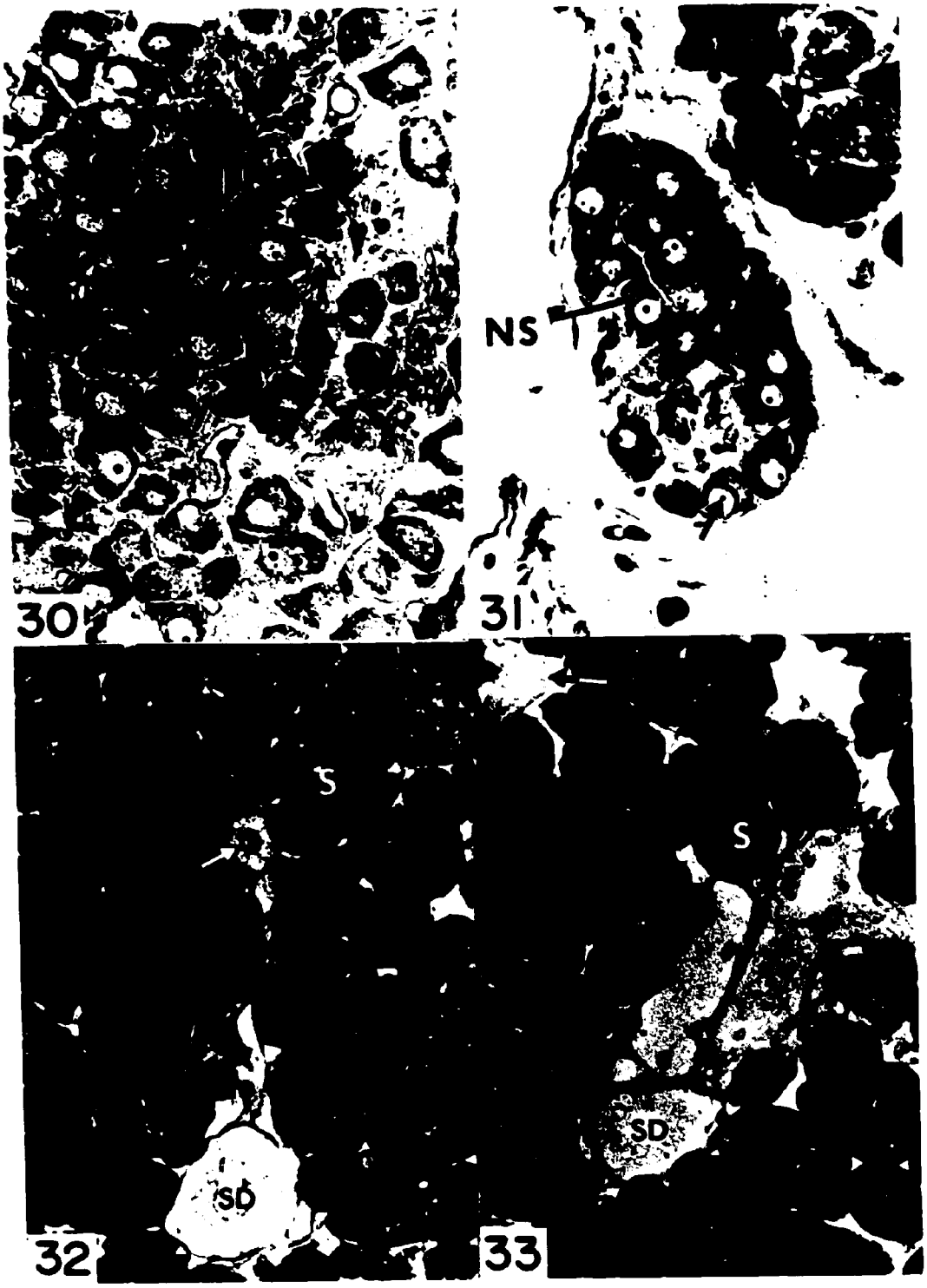


PLATE 8

Explanation of Figures

Figures 34 and 35: fixed in Zenker-formol, stained with colloidal iron-PAS, 3 μ, x1000. Figures 36 and 37: fixed in FAA, stained with colloidal iron-PAS, 5 μ, x2062.

34 Sublingual gland of an ad libitum-fed control mouse. Mitochondria (arrow) of salivary duct (SD) cells are concentrated basally, paralleling the basal striations. Scattered granulated cells (G) are present in the ducts. Mucous acini (A) are illustrated.

35 Sublingual gland of a mouse 87 days after hypophysectomy. Cells of salivary ducts (SD) are smaller, with less prominent basal striations and smaller nuclei. The mitochondria (arrow) are more concentrated, randomly arranged in the basal area and smaller than the control (fig. 34). No granulated cells are present. Mucous acini (A) are smaller.

36 Salivary duct in the sublingual gland of a pair-fed control mouse. The duct cells have large nuclei and considerable material in the infranuclear region which stained with colloidal iron (arrow).

37 Salivary duct of a sublingual gland of a mouse 68 days after hypophysectomy. The duct cells are lower, have smaller nuclei and less infranuclear cytoplasmic material which stained with colloidal iron. Some vacuoles (V) are present in the basal area.

PLATE 8

