THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

SUBMAXILLARY AND PAROTID GLAND AMYLASE PRODUCTION AND HISTOLOGY IN THE LABORATORY RAT

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

BY

BARBARA ANNE SHIRLEY

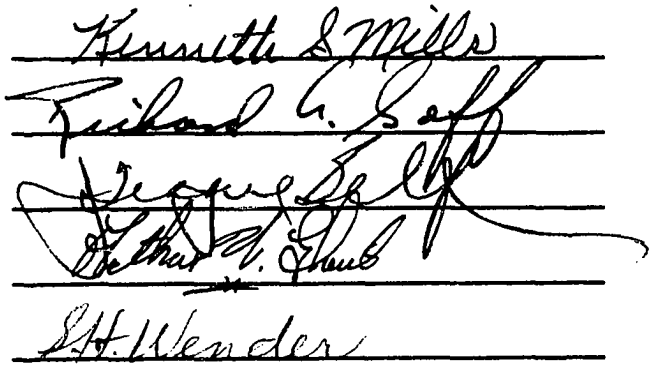
Norman, Oklahoma

1964

SUBMAXILLARY AND PAROTID GLAND AMYLASE PRODUCTION

AND HISTOLOGY IN THE LABORATORY RAT

APPROVED BY



DISSERTATION COMMITTEE

ACKNOWLEDGMENTS

Grateful recognition is due Dr. Harriet Harvey who directed this problem in its early stages, and to Dr. Kenneth Mills who continued the direction of the research and read the manuscript after Dr. Harvey's death.

I wish also to thank the Department of Zoology for providing the space and equipment needed; the VioBin Corporation for providing the Viokase; the National Science Foundation for a summer fellowship; and the National Institutes of Health for a graduate fellowship during the last two years of the research.

Recognition is due countless others who provided assistance and encouragement during the course of this research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

Figure Page

SUBMAXILLARY AND PAROTID GLAND AMYLASE PRODUCTION AND HISTOLOGY IN THE LABORATORY RAT

CHAPTER I

INTRODUCTION

The pancreas, submaxillary, and parotid glands produce amylolytic enzymes. In the pancreatic secretion the amount of amylase activity is mediated by a number of stimuli. The pancreatic stimuli studied most extensively would fall in three categories: neurohumors, hormones, and diet. Neurohumoral stimulation of the salivary glands has been studied in relation to quantity of saliva produced without analyzing the saliva for enzyme content. The effect of hormones and of diet on the salivary amylase content is not known.

A diet high in carbohydrate increases the amylolytic activity of pancreatic secretion or of a homogenate of pancreas tissue with a concomitant decrease in the trypsin

(Grossman, et al., 1942-43). A diet high in protein reverses this effect reducing the amylase and increasing the trypsin content of the pancreas.

Pancreozymin from the mucosa of the small intestine mediates an increase in the enzyme content of the pancreatic juice (Harper and Raper, 1943) while secretin from the intestinal mucosa stimulates the pancreas to secrete bicarbonate and water. Secretin causes an increase in the volume of pancreatic juice without increasing the output of amylase (Wang, et al., 1948).

Lin and Ivy (1957) noted that the administration of pancreozymin, methacholine, Urecholine (bethanacol), or acetylcholine would increase the pancreatic amylase activity without increasing the volume output of pancreatic juice.

The submaxillary and parotid glands also produce amylase with the parotid secretion being particularly rich in this enzyme. The stimuli which specifically affect the production of amylase by the salivary glands have not been clearly defined. The effect of nerve stimulation has not dealt with resulting changes in enzyme activity of the tissues.

A study of the effect of neurohumoral stimulation of the salivary glands has been made. Rawlinson (1933, 1935,

1936) and Babkin (1931, 1944) support the idea that the mucous cells of the submaxillary glands in the cat receive innervation from the chorda tympani while the demilune cells are innervated by the sympathetic nerve. Direct stimulation of the parasympathetic nerves of the parotid gland results in a copious secretion while adrenergic sympathetic stimulation produces a small amount of secretion (Richins and Kuntz, 1953). Adrenergic sympathetic stimulation of the submandibular gland is followed by a copious secretion. Cholinergic parasympathetic stimulation elicits an abundant secretion from the gland only after the adrenergic nerve components have been depressed. These studies were concerned only with volume of saliva produced and gave no information on amylolytic activity of the saliva secreted after stimulation of the glands.

The stimuli which increase pancreatic amylase secretion with perhaps the exception of the hormonal stimuli produce many responses within an organism and the responding organs or glands could include the salivary glands and their enzyme production. For this reason it would seem feasible to explore the role of neurohumors, hormones, and diet in the secretion of salivary enzymes. The various studies concerning the stimulation of pancreatic enzyme production have

animals and enlargement of their salivary glands. The same result was obtained when the animals were fed a basal diet plus desiccated and defatted raw pancreas (Viokase powder). The submaxillary glands showed greater weight increases in animals fed greater amounts of dietary pancreas preparation.

The weight increase of the glands was similar to that produced by isoproterenol and the histological changes were similar. Again no enzyme study accompanied the extreme histological changes.

CHAPTER II

MATERIALS AND METHODS

The rats were from two sources: Colony 1 rats were from a colony maintained at the University of Oklahoma, and Colony 2 rats of the Sprague-Dawley strain were obtained from Sprague-Dawley, Inc. Rats from the two sources were never mixed within an experimental group. Since the sex hormones affect the salivary amylase activity (Harvey, 1957), mature male rats between the ages of 105 and 130 days were chosen so that the amounts of sex hormones being produced would be most comparable.

In each experiment the submaxillary gland from the right side was used for a determination of enzyme activity and the gland from the left side was used for histological preparation. The parotid gland from the right side was used for enzyme determination but histological preparation was not made.

All determinations of amylolytic activity of the salivary glands used the colorimetric method of Sobel and Meyers (1953) with slight modifications. This method requires a buffered glycogen substrate with which the gland homogenate preparation reacts for fifteen minutes at thirty-seven degrees centigrade. The buffered glycogen solution was prepared in half the concentration suggested in the original method and at the end of the reaction period undigested glycogen was precipitated with methyl alcohol, and removed by centrifugation for fifteen minutes and filtration through number one filter paper instead of utilizing a filtering agent such as Fuller's earth. After removal of the glycogen, 0.4 ml of the supernatant, 1.6 ml of distilled water, and 4.0 ml of Anthrone reagent were mixed in a colorimeter tube and after ten minutes the density of the color was determined with the Klett-Summerson colorimeter using the red filter. A standard was prepared in the same manner using 0.4 ml of a 10 mg per cent solution of glucose in place of the supernatant. The glycogen-anthrone units of amylase are expressed as follows: there is one unit of activity per ml of fluid when the quantity of liberated anthrone reacting substance present in 0.4 ml of supernatant equals that present in 0.4 ml of the glucose standard.

Immediately after the animals were killed the right submaxillary gland was removed whole, weighed, diluted 30 times with pH 7.1 phosphate buffer (assuming 1 gm of tissue equals 1 ml) and homogenized. The parotid is a diffuse gland and has a greater amylolytic activity so only a portion of the gland was removed, weighed, diluted 100 times with phosphate buffer and homogenized. An aliquot of the homogenate was diluted an additional 200 times just prior to the analysis for amylolytic activity. The total dilution of 20,000 times was necessary for the density of the color to be low enough for measurement with the colorimeter. The homogenates were frozen until activity was determined. This storage time was never more than one week.

Feeding of Special Diets

Forty rats were separated into four groups of equal number, individuals were weighed, and fasted for two days. One group was fed a high carbohydrate diet; another group was fed a high protein diet. The remaining two groups served as controls and were fed standard Rockland Rat Diet. All food and water were given ad libitum. The special diets were obtained from Nutritional Biochemicals Corporation. After four weeks the animals were weighed, sacrificed, and the salivary

glands excised for studies.

Pancreozymin and Secretin Administration

Six rats were given intracardial injections containing 20 Crick units of secretin in 1.0 ml saline (Crick, et al., 1949) and six rats were given 5 Crick units of pancreozymin in 1.0 ml of saline. Controls were given 1.0 ml of saline. The injection of secretin required one minute. Twenty minutes later the rats were killed and the salivary glands taken for study. Pancreozymin was injected over a two minute period and allowed to circulate for 10 minutes before the animals were killed and the glands removed.

Acetylcholine and Nor-adrenalin Administration

Acetylcholine was prepared in saline and administered intracardially in dosages of 1 mg in 0.1 ml to six rats. Controls were injected with the same volume of saline. The injection of acetylcholine required less than five seconds and the substance was allowed to circulate for twenty minutes before the animals were killed.

Since parasympathetic stimulation is tonic in action, it was decided that the best study would involve removal of the gland from the influence of the tonic stimulus.

Homotransplants of part of the parotid were made in 26 animals by inserting parotid tissue hypodermally in the abdominal region. A piece of the tissue was analyzed as control for enzyme activity at the time the transplantation was done. The transplants were allowed to grow for seven to fourteen days then removed and weighed. Since the quantity of transplanted tissue was usually insufficient for both enzyme studies and sectioning, transplants in six of the animals were used for histological studies and the rest for the study of enzyme activity.

After seven days the animals were sacrificed and the transplanted tissue removed. Twenty minutes before they were killed five of the rats in this group were given intracardially 1 mg of acetylcholine dissolved in 0.1 ml of saline. The enzyme content of the transplants was determined and a comparison made between non-injected and acetylcholine injected animals.

To study the effect of sympathetic stimulation on salivary enzyme production, six rats were injected intracardially with 10 µg of nor-adrenalin dissolved in 1 ml of saline. Control animals were given 1 ml of saline. The animals were killed and the salivary glands removed twenty minutes after the injections.

Isoproterenol Administration

Six rats were injected intraperitoneally with 25 mg (0.25 ml) of an aqueous solution of isoproterenol twice daily. A second group of six animals was injected once each day with 5 mg (0.25 ml) of isoproterenol. Eleven control animals were injected with like volumes of distilled water. The animals were weighed on the first day and weighed and killed on the tenth day of the experiment.

Feeding of Pancreas Tissue

Thirty-two rats were divided into four groups of unequal number. The individual animals were weighed at the beginning and the end of the experiment. Six animals were fed fresh raw rat pancreas tissue which made up 5% of their diet. The diet was restricted to that amount which was completely consumed within one day. This daily diet was weighed and the control group of six rats was fed the same quantity of Rockland Rat Diet. These two groups were fed for ten days and at the end of this time the animals were killed and the salivary glands analyzed. A group of ten rats was fed desiccated and defatted raw hog pancreas[1] (Viokase powder)

---

[1]Provided by the VioBin Corporation, Monticello, Illinois.

in the amount of 4% of the total diet by weight. Ten control animals were fed a like quantity of Rockland Rat Diet. Five of these experimental animals and five of these controls were fed for fourteen days and the remaining five experimental and five control animals were fed their respective diets for twenty-eight days before being killed. The rats fed pancreas powder were fed for a longer period of time than those fed pancreas tissue because they ate very little for the first few days.

Histological Methods

The submaxillary glands from the left side were fixed in Zenker-formol solution (Bensley and Bensley, 1945) immediately after being excised. They were passed through graded alcohols, cleared in oil of wintergreen and embedded in paraffin. Sections were cut at 6-10 micra and stained with Mallory's Triple Connective Tissue stain (Bensley and Bensley).

The isoproterenol injected animals and the animals fed desiccated pancreas tissue had the greatest changes in salivary amylase content after treatment. The area occupied by submaxillary terminal tubules containing red-staining granules was determined in order to give a more quantitative

analysis of the histological changes which occurred simultaneously with enzyme changes.

For the quantitative analysis an outline of the granule-containing terminal tubules was sketched with a camera lucida at 440 x. The area of the tubules was determined by tracing the perimeter of the drawing with a planimeter. The actual area of each field counted was 52.8 mm^2 but tables show areas of 100 mm^2 calculated from these data. Salivary gland slides from two animals in each group were used for the terminal tubule counts and three areas on each slide were counted. In each case the terminal tubule area was determined on the periphery of the section where the tubules stained more deeply.

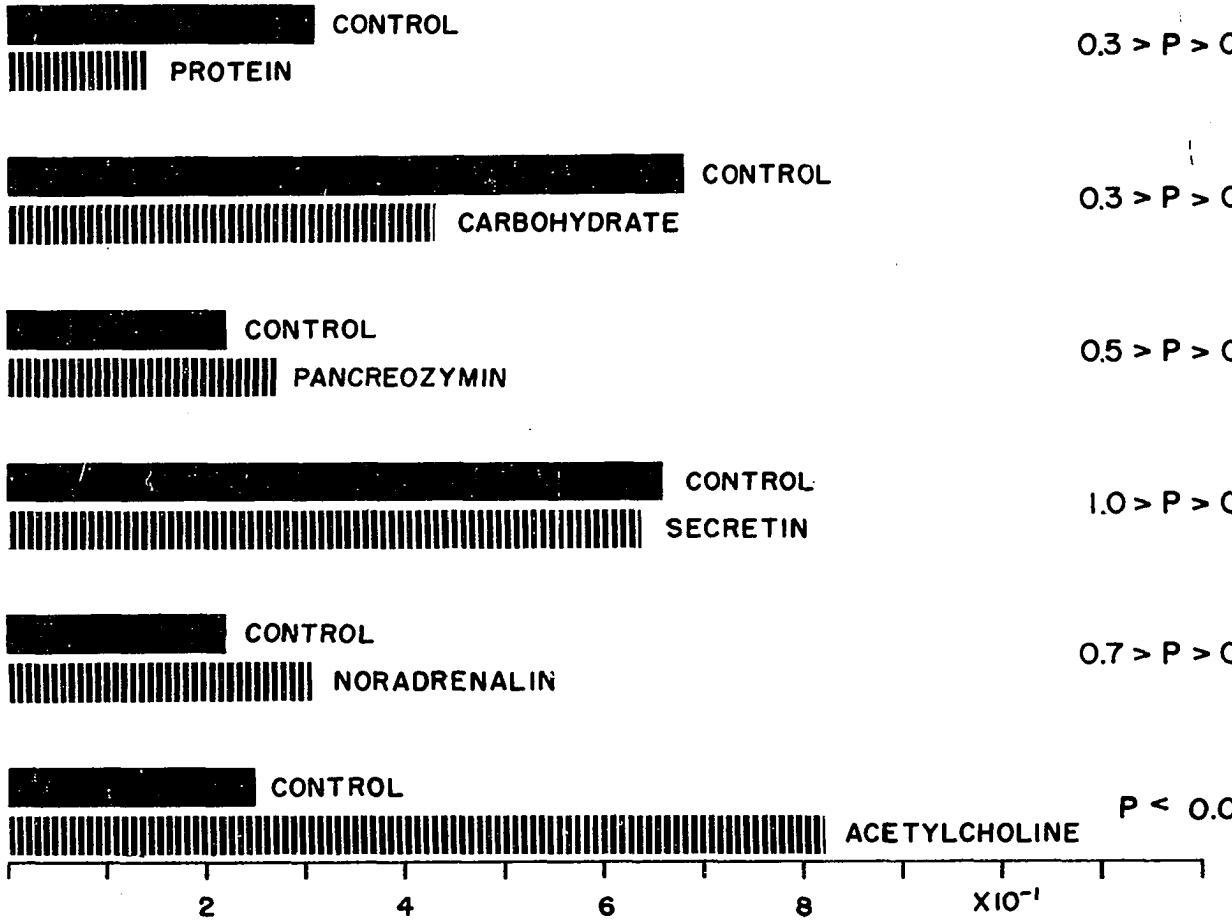
CHAPTER III

RESULTS

Feeding of Special Diets

No significant change in the amylolytic activity per mg of submaxillary gland resulted from feeding a diet high in carbohydrate ($0.3>P\geq0.2$) or high in protein ($0.3>P>0.2$). Although there was a slight lowering of enzymatic activity in both cases, this change in mean activity was not significant (Table 1, Figure 1). The difference in activity of the submaxillary glands of the two control groups is likely because the animals were of different strains. The animals fed the high carbohydrate diet and the corresponding control group were from colony 1. In contrast the animals fed the high protein diet and the animals of their control group were Sprague-Dawley rats from colony 2. This difference in enzyme activity between the two strains was observed in most experiments. The variation between control groups permits comparison of an experimental group only with its controls.

FIG. 1 MEAN SUBMAXILLARY AMYLASE LEVELS



UNITS ACTIVITY PER 100 MG TISSUE

	PROBABILITY (t-TEST)	NUMBER OF ANIMALS	RAT COLONY
CONTROL	0.3 > P > 0.2	10	2
PROTEIN		10	
CONTROL	0.3 > P > 0.2	11	1
CARBOHYDRATE		9	
CONTROL	0.5 > P > 0.4	6	2
PANCREOZYMIN		6	
CONTROL	1.0 > P > 0.9	4	1
SECRETIN		6	
CONTROL	0.7 > P > 0.6	6	2
NORADRENALIN		6	
CONTROL	P < 0.01	6	1
ACETYLCHOLINE		6	

The colony of animals used in each experiment is indicated on each graph and table.

Table 1

Effect of Diet on Mean Submaxillary Gland Weight and Enzyme Activity+

Diet (Colony)	Body Wt. (gm) Initial	Final	Submax. Wt. (mg)	Mg. Gland/ 100 gm Body Wt.	Activity/ 100 mg Tissue*
Control (1)	338	323.3	231.2	71.4	0.68
CHO (1)	342	357.8	291.2	81.3	0.43
Control (2)	414	397.8	267.9	66.9	0.31
Protein (2)	366	365.5	255.7	70.5	0.14

+All values are means.
*Activity expressed in anthrone units.

The sizes of the submaxillary glands of the control group and the group fed the high protein diet were not significantly different. The high carbohydrate diet caused a significant increase ($0.02 > P > 0.01$) in relative submaxillary gland weight calculated as mg per 100 gm body weight. Since there was no significant difference in activity per unit weight of tissue between the control and experimental animals, the increase in gland size would cause an increase in total gland activity.

Neither test diet caused histological changes in the submaxillary glands.

The parotid gland amylase levels of the controls and those of the group fed a diet high in protein were not significantly different, but in the group fed a high carbohydrate diet there was a significant ($P<0.01$) decrease in amylase activity per unit weight of parotid tissue (Figure 2, Table 2) to 62.7% of that of the controls.

Table 2

Effect of Diet on Parotid Enzyme Activity+

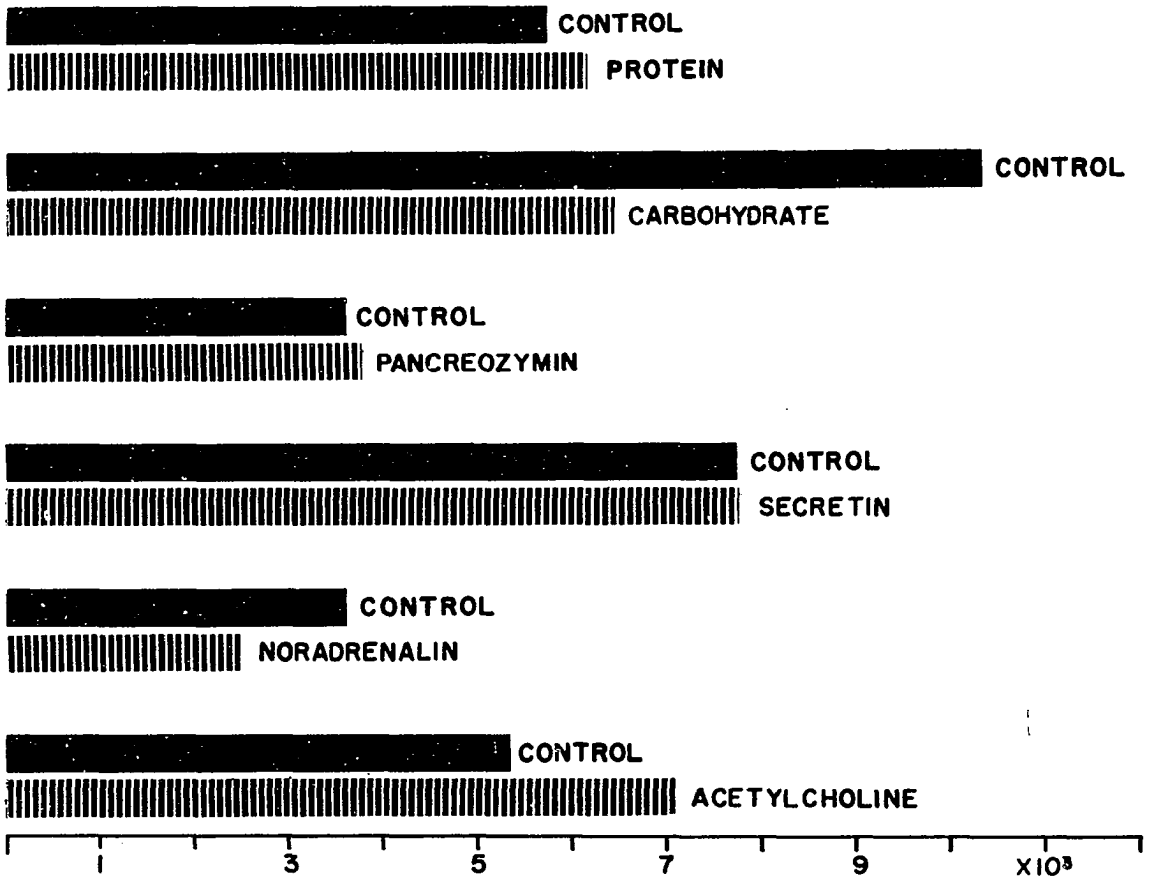
Diet (Colony)	Body Wt. (gm) Initial	Final	Activity/100 mg Tissue*
Control (1)	338	323.3	10,322
CHO (1)	342	357.8	6,471
Control (2)	414	397.8	5,708
Protein (2)	366	365.5	6,158

+All values are means.
*Activity expressed in anthrone units.

Hormone Administration

There was no noticeable difference in granulation of terminal tubules and no significant difference in submaxillary

FIG. 2 MEAN PAROTID AMYLASE LEVELS



	PROBABILITY (t-TEST)	NUMBER OF ANIMALS	RAT COLONY
CONTROL	1.0 > P > 0.9	10	2
PROTEIN		10	
CONTROL	P < 0.01	11	1
CARBOHYDRATE		9	
CONTROL	0.9 > P > 0.8	6	2
PANCREOZYMIN		6	
CONTROL	1.0 > P > 0.9	4	1
SECRETIN		6	
CONTROL	0.3 > P > 0.2	6	2
NORADRENALIN		6	
CONTROL	0.2 > P > 0.1	6	1
ACETYLCHOLINE		5	

enzyme activity after the injection of either pancreozymin or secretin (Figure 1).

The relative gland weights of the submaxillary glands are expressed as mg tissue per 100 gm body weight and are very nearly the same for experimental and control animals (Table 3). However, there was a difference between the relative submaxillary gland weights of animals in the pancreozymin experiment and those in the secretin experiment. Again the animals in the two experiments were of different strains and this difference in weights perhaps represents one variation between the two strains of animals.

Table 3

Effects of Pancreozymin and Secretin on Mean Submaxillary Gland Weight and Enzyme Activity+

Group (Colony)	Body Wt. (gm)	Submax. Wt.(mg)	Mg. Gland/ 100 gm Body Wt.	Activity/ 100 mg Tissue*
Control (2)	371.9	215.6	57.8	0.22
Pancreozymin (2)	344.2	204.8	59.5	0.27
Control (1)	342.8	244.7	71.5	0.66
Secretin (1)	328.1	230.8	70.5	0.64

+All values are means.

*Activity expressed in anthrone units.

The response in the parotid glands to the administration of either pancreozymin or secretin was the same as in the submaxillary glands. The mean amylase activity of the experimental groups was not significantly different from that in the control groups (Figure 2, Table 4).

Table 4

Effects of Pancreozymin and Secretin on Parotid Enzyme Activity+

Group (Colony)	Body Wt. (gm)	Activity/100 mg Tissue*
Control (2)	371.9	3,600
Pancreozymin (2)	344.2	3,760
Control (1)	342.8	7,725
Secretin (1)	328.1	7,770

+All values are means.
*Activity expressed in anthrone units.

Neurohumor Administration

The intracardial injection of acetylcholine caused a significant increase ($P<0.01$) in the amylolytic activity of the submaxillary glands (Figure 1).

The increase in activity of the parotid gland (Figure 2) after acetylcholine injection was not significant

(0.2>P>0.1).

There was no significant change in the amylolytic activity per mg of either submaxillary or parotid tissue after the injection of nor-adrenalin (Tables 5 and 6). The submaxillary gland, however, showed a significant increase in weight when compared to controls (0.05>P>0.02) and consequently increased total gland amylase activity.

Table 5

Effects of Neurohumors on Mean Submaxillary Gland Weight and Enzyme Activity+

Group (Colony)	Body Wt. (gm)	Submax. Wt.(mg)	Mg. Gland/ 100 gm Body Wt.	Activity/ 100 mg Tissue*
Control (1)	322.5	219.4	68.3	0.25
Acetylcholine (1)	353.3	230.6	65.2	0.82
Control (2)	371.9	215.6	57.8	0.22
Nor-adrenalin (2)	402.9	276.8	68.6	0.31

+All values are means.
*Activity expressed in anthrone units.

Acetylcholine increased the submaxillary amylase activity but the histology of the glands was very similar to that of control animals. Granulation in the terminal tubules was not lost (Figures 3 and 4).

PLATE I

Slides were stained with Mallory's Triple Connective Tissue stain. Magnification 348 x.

Figure 3. Submaxillary gland from a male rat injected with saline. The density of granulation in this gland could be considered normal.

Figure 4. Submaxillary gland from a male rat injected with acetylcholine. The granulation of the terminal tubules appears normal.

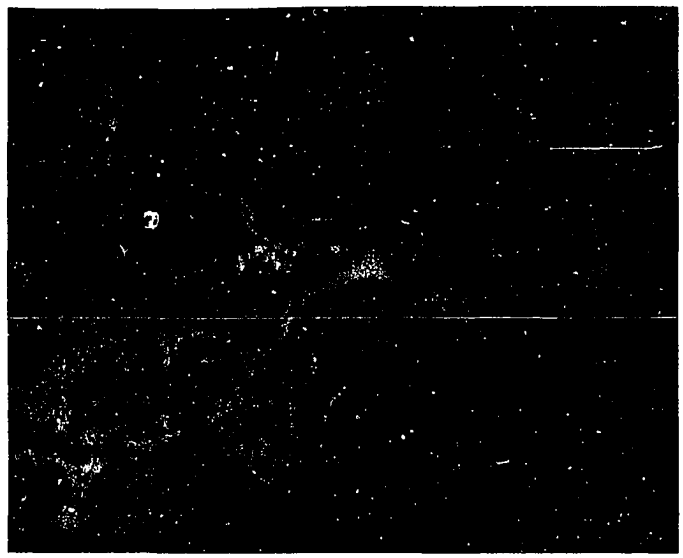


FIG. 3

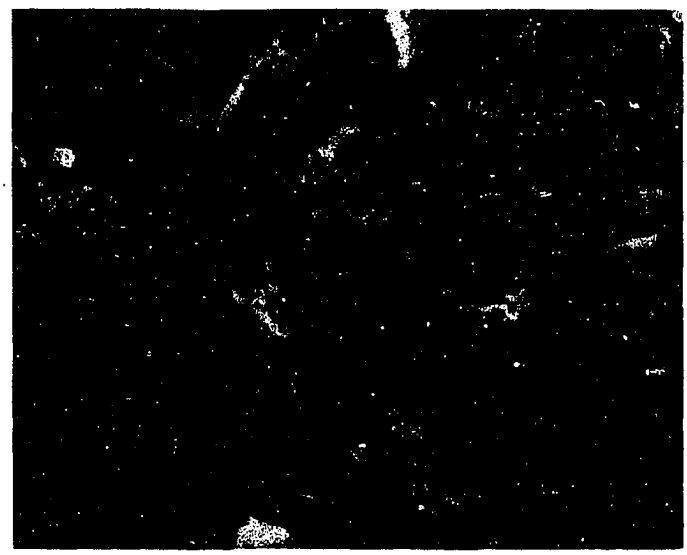


FIG. 4

Table 6

Effect of Neurohumors on Parotid Enzyme Activity+

Group (Colony)	Body Wt. (gm)	Activity/100 mg Tissue*
Control (1)	322.5	5,370
Acetylcholine (1)	353.3	7,092
Control (2)	371.9	3,600
Nor-adrenalin (2)	402.9	2,497

+All values are means.
*Activity expressed in anthrone units.

Transplantation tested the effect of removal of tonic stimulation of the parotid gland. Parotid tissue normally is about 20,000 times higher in enzyme activity than the submaxillary gland as can be seen in a comparison of the glands of control animals. The submaxillary gland had a mean value of 0.25 units (Table 5) of amylase activity while the activity of the parotid was 5,370 units (Table 6) of activity per 100 mg of tissue. The normal amylase activity of the submaxillary is about the threshold of the sensitivity of the amylase test employed. The parotid has such a high normal activity that any decrease in amylase would be much more obvious than in the submaxillary.

Parotid tissue was transplanted in twenty-six animals. Six of the transplants were used only for histological study. Transplants could not be located in five animals. The remaining fifteen transplants were recovered after a period of not less than seven or more than fourteen days and used for enzyme studies. The recovered transplants lost their amylase activity (Table 7). Five of the animals with transplants were injected with acetylcholine but no increase in amylolytic activity of the transplanted tissue was evoked.

Table 7

Influence of Transplantation on Parotid Amylase Activity

Activity on Day of Transplantation (Units/100 mg Tissue)	Activity of Transplant After Seven Days (Units/100 mg Tissue)
5,400	0
10,820	3,000
14,320	0
8,820	5,600
7,360	80
6,840	220
7,560	0
7,600	0
7,800	0
7,200	0
5,080	0*
8,300	80*
6,160	40*
6,920	0*
3,660	0*

*Received 1 mg of acetylcholine twenty minutes prior to removal of the transplant.

The histological change in the transplanted parotid tissue was characterized by dense connective tissue at the periphery of the transplants and fibers intermingled with the acinar tissue (Figures 5 and 6). The ducts of the transplanted tissue were readily distinguishable but the acinar cells were shrunken and the tissue was disoriented so that it was difficult to discern the boundaries of acini. The vascularization of the transplants was well established and some slides revealed the presence of large blood vessels. The parotid gland is lacking in terminal tubules and acidophilic granules which are used as criteria of change in submaxillary gland histology. The nature of the histological changes in transplants made measurement of cells or any quantitation of these changes impossible.

Administration of Pancreas Tissue and Isoproterenol

The amylolytic activity of the submaxillary gland increases subsequent to the feeding of desiccated, defatted pancreas tissue (pancreas powder) as a diet supplement or injections with the catecholamine, isoproterenol (Table 8, Figures 7 and 8).

Feeding of desiccated pancreas tissue and injection of isoproterenol serve to cause hypertrophy of the

PLATE II

Slides were stained with Mallory's Triple Connective Tissue stain. Magnification 410 x.

Figure 5. Parotid gland from control male rat.

Figure 6. Transplanted parotid tissue. Ducts remain distinguishable but the boundaries of acini are obliterated by connective tissue.

FIG. 5

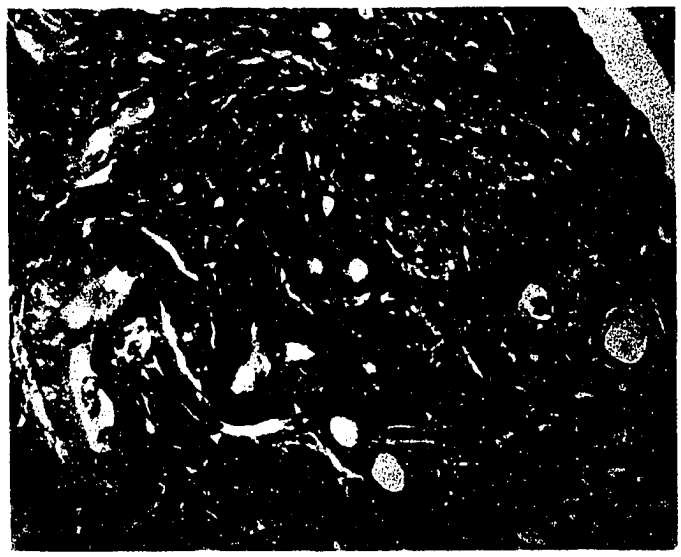


FIG. 6

Table 8

Salivary Gland Mean Enzyme Activity Accompanying Hypertrophy+

Group (Colony)	Body Wt. (gm) Initial	Final	Submax. Wt. (mg)	Submax. Gland Mg/100 gm Body Wt.	Submax. Activity/ 100 Mg Tissue*	Parotid Activity/ 100 Mg Tissue*
Control Diet (2)	414	397.8	267.9	67.4	0.31	5,708
Viokase (14 days) (2)	406	331.8	370.7	109.9	1.61	10,732
Viokase (28 days) (2)	434	331.6	307.0	92.5	1.49	7,208
Control (1)	369	361.6	245.2	67.4	0.30	8,288
Isoproterenol 5 mg/day (1)	337	324.4	517.5	158.9	2.99	1,755
Control (1)	147	190.2	124.4	65.1	0.59	5,564
Isoproterenol 50 mg/day (1)	144	182.3	738.0	422.0	11.26	1,442
Control (1)	172	199.5	174.2	87.7	3.03	9,650
Raw Pancreas Diet Supplement (1)	173	224.2	184.5	82.4	1.10	8,253

+All values are means.

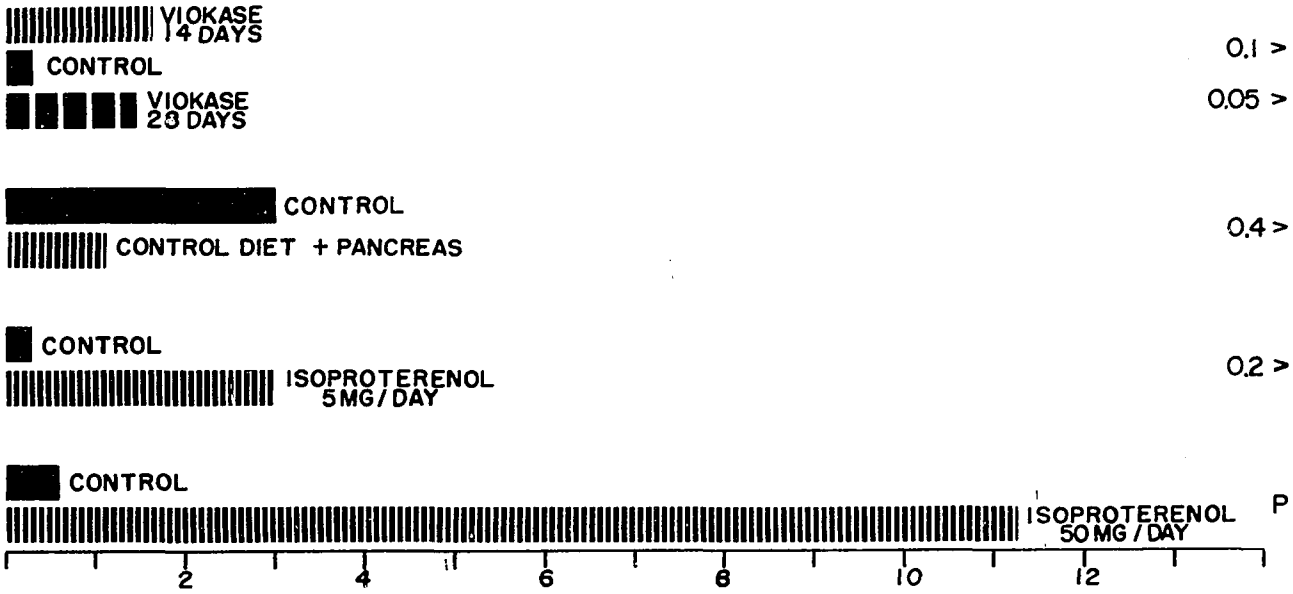
*Activity expressed in anthrone units.

submaxillary glands. The greatest enlargement of the glands caused by feeding desiccated pancreas tissue was seen in the group which was fed for the shorter time (14 days). The greatest submaxillary hypertrophy resulting from injection of isoproterenol was observed in the group given the higher dosage (50 mg/day). The mean amylase activity per 100 gm of tissue was also greater in the animals fed desiccated pancreas for the shorter time and in isoproterenol-injected animals given the greater dosage. There was a positive correlation between the increased gland weight and the increased activity per unit gland weight.

Isoproterenol and pancreas powder did not have the same effect on parotid amylase as they had on submaxillary amylase. Pancreas powder caused an increase in mean parotid amylase activity which was greater after 14 days of feeding than after 28 days. Isoproterenol lowered the amylolytic activity of the parotid.

After the first 14 days of the feeding period the desiccated pancreas had no further effect in increasing submaxillary gland size or in increasing amylolytic activity. Then the trend was a return of the gland to normal. No weight was gained in the second 14 days of feeding and after 28 days the body weight was the same as after the first 14

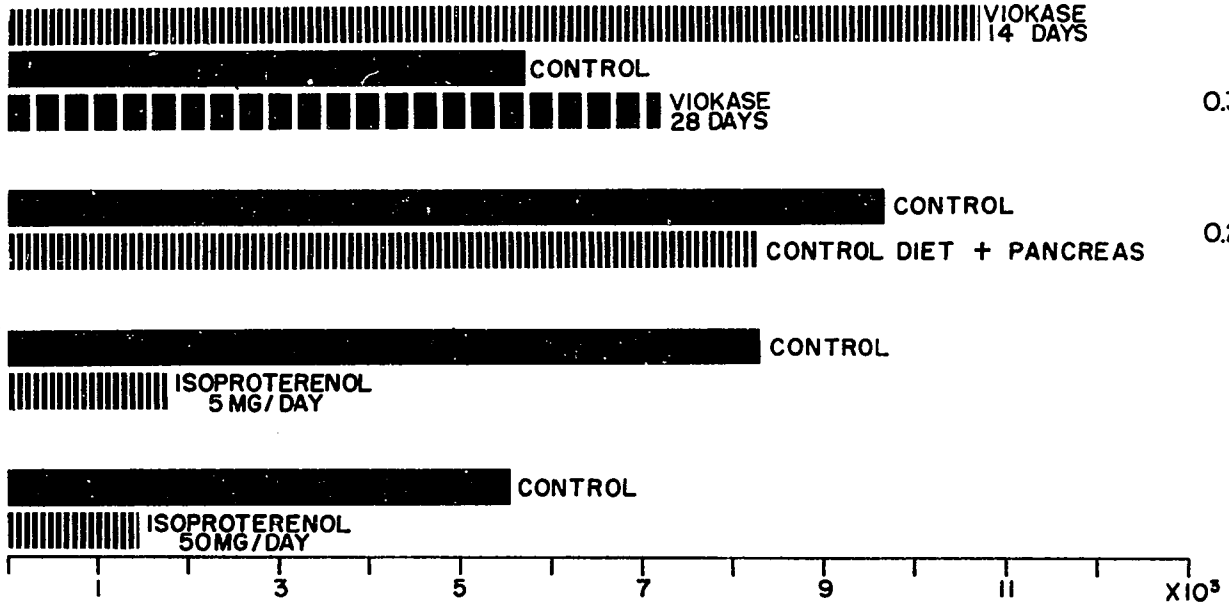
FIG. 7 MEAN SUBMAXILLARY AMYLASE LEVELS



UNITS ACTIVITY PER 100 MG TISSUE

	PROBABILITY (t-TEST)	NUMBER OF ANIMALS	RAT COLONY
VIOKASE 14 DAYS		5	
CONTROL	0.1 > P > 0.05	10	2
VIOKASE 28 DAYS	0.05 > P > 0.02	5	
CONTROL	0.4 > P > 0.3	6	1
CONTROL DIET + PANCREAS		6	
CONTROL	0.2 > P > 0.1	5	1
ISOPROTERENOL 5MG/DAY		4	
CONTROL	P < 0.01	6	1
ISOPROTERENOL 50MG/DAY		5	

FIG.8 MEAN PAROTID AMYLASE LEVELS



UNITS ACTIVITY PER 100 MG TISSUE

	PROBABILITY (t-TEST)	NUMBER OF ANIMALS	RAT COLONY
VIOKASE 14 DAYS	P < 0.01	5	
CONTROL		10	2
VIOKASE 28 DAYS	0.3 > P > 0.2	5	
CONTROL	0.2 > P > 0.1	6	1
CONTROL DIET + PANCREAS		6	
CONTROL	P < 0.01	5	1
ISOPROTERENOL 5 MG/DAY		4	
CONTROL	P < 0.01	6	1
ISOPROTERENOL 50MG/DAY		6	

days of feeding.

The terminal tubules of the rat submaxillary gland may be distinguished from the remainder of the duct system by the smaller lumen, the basal position of the nucleus, and the presence of numerous granules in the cytoplasm (Grad and Leblond, 1949). The normal submaxillary gland tubule arrangement is shown in Figure 9. The histology of the submaxillary glands of animals given isoproterenol or pancreas powder was compared with that of normal animals with special emphasis on any changes in the terminal tubules or the granulation of these tubules.

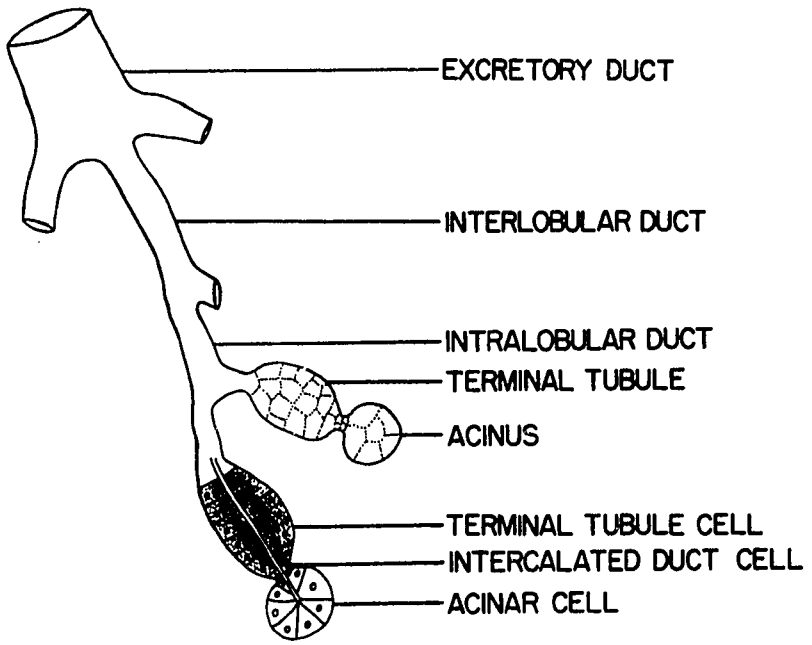


Fig. 9. Diagram of submaxillary gland tubules of the laboratory rat.

Submaxillary glands of animals given desiccated pancreas or isoproterenol showed histological effects from either of these treatments including decreased granulation of the terminal tubules (Figures 10, 11, 12, 13, 14, and 15) and decreased area of the granule-containing terminal tubules in the experimental animals (Table 9).

Table 9

Effect of Hypertrophy on Area of Submaxillary Terminal Tubules

Treatment	Mean area terminal tubules ($mm^2/100\ mm^2$)	Range (mm^2)
Viokase controls	37.8	33.1-43.3
Viokase (14 days)	8.1	0.0-21.7
Viokase (28 days)	24.0	16.3-35.0
Isoproterenol controls	33.1	22.9-49.1
Isoproterenol (5 mg/day)	9.6	1.9-20.0
Isoproterenol (50 mg/day)	0.0	0.0

A comparison of the area of terminal tubules showed that the feeding of pancreas powder affected the tubules markedly during the first two weeks of feeding but during the second two weeks there was a return toward normal tubule size. These results are paralleled by the changes found in

PLATE III

Slides were stained with Mallory's Triple Connective Tissue stain. Magnification 370 x.

Figure 10. Submaxillary gland from control, male rat. The granules are a prominent feature of the terminal tubules.

Figure 11. Submaxillary gland from male rat injected with isoproterenol (5 mg/day). The granule content of the terminal tubules is decreased.

Figure 12. Submaxillary gland from male rat injected with isoproterenol (50 mg/day). There is no visible terminal tubule granulation.

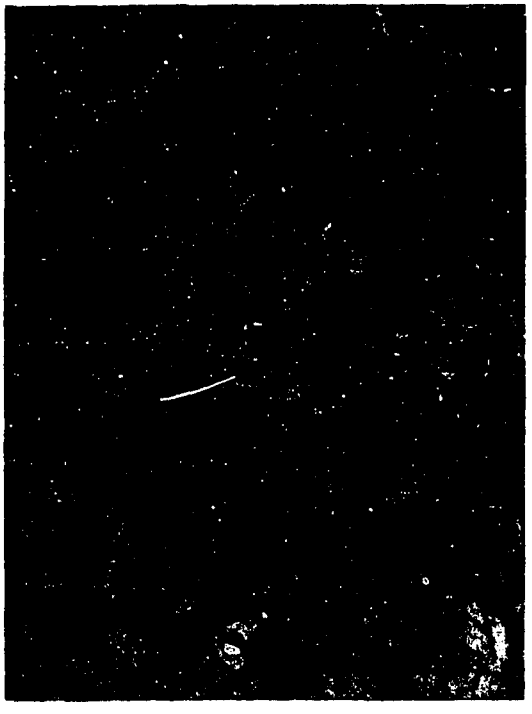


FIG.10

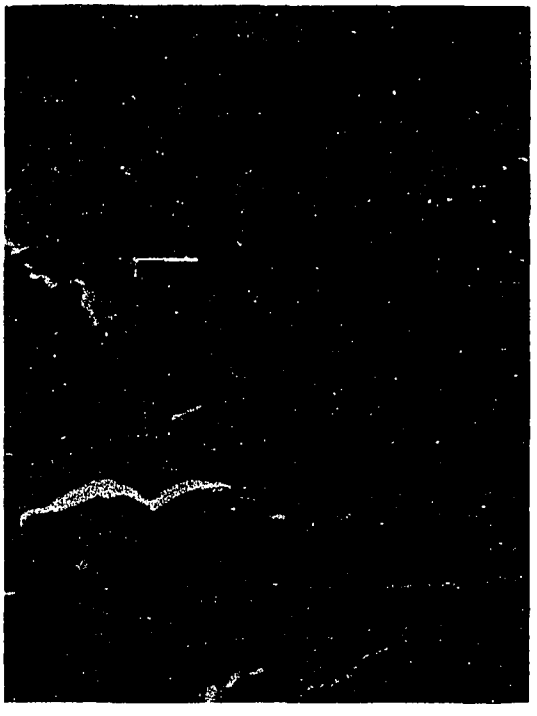


FIG.11

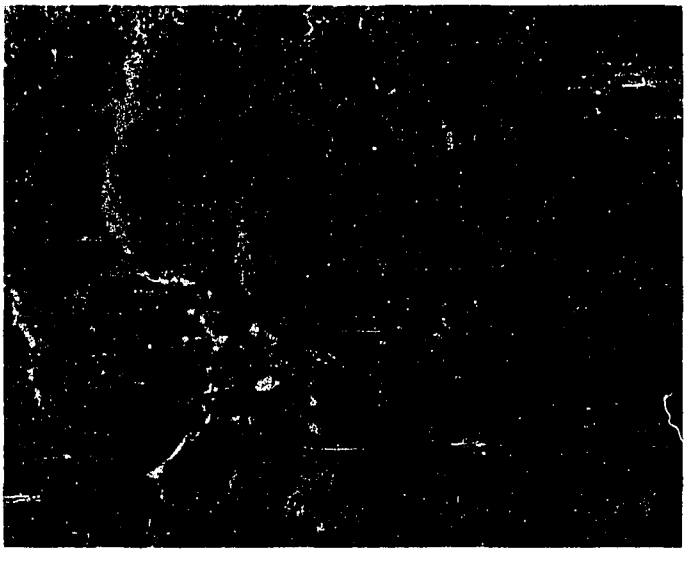


FIG.12

PLATE IV

Slides were stained with Mallory's Triple Connective Tissue stain. Magnification 370 x.

Figure 13. Submaxillary gland from male rat fed control diet.

Figure 14. Submaxillary gland from male rat fed diet supplemented with desiccated pancreas tissue for 14 days. This slide shows loss of terminal tubule granulation, decreased tubule cell height, and increased tubule lumen diameter.

Figure 15. Submaxillary gland from male rat fed diet supplemented with desiccated pancreas tissue for 28 days. The granule content of the tubules is less than that of control animals.

FIG.13

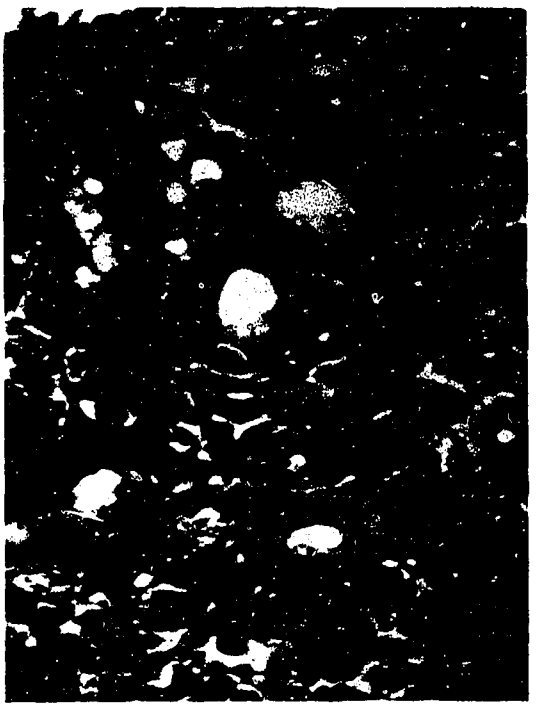


FIG.14

FIG.15

the submaxillary amylase activity evoked by feeding pancreas powder. In addition to the changes in tubule granulation the pancreas powder caused an enlargement of acini and the stained acinar tissue took on a bluer color indicating the presence of more mucoid material.

Animals injected with isoproterenol showed a complete loss of terminal tubule granulation when given a daily dosage of 50 mg but a more moderate dosage of 5 mg per day decreased the terminal tubule granulation without the extinction of the granules. The lower dosage of isoproterenol resulted in histological changes more nearly like those observed when pancreas powder was fed for two weeks as is reflected by the tubule area determination.

The feeding of raw pancreas tissue did not increase salivary gland size or enzyme activity as did the feeding of desiccated pancreas tissue.

CHAPTER IV

DISCUSSION

A variety of stimuli similar to those known to affect the pancreas were given to attempt to alter the amylase production of the submaxillary and the parotid glands. The gland histology was studied in relation to alterations in enzyme content of the glands.

Feeding of Special Diets

Both a high carbohydrate and high protein diet were fed to determine whether either would alter the salivary amylase levels as they alter the pancreatic amylase. In animals fed a high carbohydrate diet the pancreas responds with an increase in amylase content of pancreas homogenate or pancreatic juice (Grossman, et al., 1942-43). The amylase content of the pancreas is lowered after feeding a diet low in carbohydrate or high in protein.

Neither diet effected a significant change in the amylase content per mg of submaxillary gland. The amylase

level of the parotid tissue decreased when the animals were fed a diet high in carbohydrate. This was the reverse of results obtained by Grossman, et al. with similar experiments on the pancreas. The pancreatic amylase decreases a few hours after animals are fed a high carbohydrate diet and then rises to the original level (Smith, 1958). The parotid amylase changes are more in agreement with these results. A high protein diet was not effective in changing parotid amylase activity.

These results point to the fact that one of the salivary glands may respond to a stimulus which does not necessarily affect the enzymatic activity of the other salivary glands.

Grossman, et al. (1942-43) did not investigate weight changes in the pancreas in relation to changes in the diet. The activity per unit weight of submaxillary tissue was the same as that of the controls but since the submaxillary gland increased in size in the carbohydrate fed animals the result was an increase in total gland enzyme activity. Total weights of parotid glands were not determined since the glands are rather diffuse so it is not known if this weight change occurred in salivary glands other than the submaxillary.

Hormone Administration

The effects of the hormones on pancreatic secretion are established as primarily hydrelatic from secretin while pancreozymin is ecbolic (Harper and Raper, 1943). Secretin increases the volume output of the pancreas without increasing total enzyme production. Pancreozymin increases the enzyme production of the pancreas without increasing the volume of pancreatic secretion.

As in the pancreas secretin does not affect the enzyme activity of the salivary homogenates. Only enzyme concentration was determined and any increased secretory volume such as secretin causes in the pancreas would not have been detected. Pancreozymin, however, does not alter salivary amylase as it alters pancreatic amylase production.

Neither hormone caused any histological changes in the salivary glands.

Neurohumor Administration

Pancreatic amylase activity has been increased with several parasympathomimetics (Lin and Ivy, 1957). Among these was acetylcholine.

Acetylcholine injection brings about a similar increase in amylolytic activity of the submaxillary glands but

there is no change in parotid amylase activity.

Although there was a significant increase in the amylase content of the submaxillary gland homogenate following the injection of acetylcholine, the histological picture of these glands seemed quite normal. Any change in the granulation of the terminal tubules of the submaxillary gland after acetylcholine injection was not apparent. Acetylcholine increased the amylase content of the gland but the increase was not so pronounced as that caused by the administration of the non-neurohumoral agents, desiccated pancreas and isoproterenol. These latter treatments also caused extreme histological changes in the glands. Therefore, the enzyme test may be more sensitive to changes occurring within the gland than is the histological response.

It is conceivable, however, that tubule granules could be involved in enzyme changes without observable histological differences. If the granule disappearance equaled the rate of granule formation, there would be no histological change noticeable. Yet a change in the rate of the granule formation and disappearance could affect the enzyme levels.

Nor-adrenalin was used for testing the effect of sympathomimetic administration. The parotid gland did not respond with a change in enzyme activity. The submaxillary

gland increased in size after nor-adrenalin injection and increased its total enzyme activity even though the amylase per mg of tissue was not increased. There was no change in gland histology.

A decreased saliva flow usually resulting from sympathetic stimulation by drugs and the subsequent effects on enzyme content of the glands could not be speculated upon since the quantity of saliva produced was not measured.

Transplantation with its consequent loss of tonic stimulation to tissue further tested the neurohumoral effect by reducing parasympathetic stimulation. The transplanted tissues lost their enzymatic activity although in most cases the transplants seemed to live and develop an adequate blood supply. The connective tissue infiltrating the transplants appeared to be sufficient in quantity to cause a decrease in the enzyme activity per mg of otherwise normal parotid tissue since approximately only a quarter of the transplant consisted of acinar cells. Gland tubule changes were masked by the striking changes in the acinar tissue.

Normal parotid tissue from this strain of animals exhibits high amylase activity of more than 5,000 units of activity per 100 mg of tissue. If a quarter of the acinar cells remain and are able to produce enzyme, the activity

would be greater than 1,000 units per 100 mg of tissue. The transplanted tissue contained more connective tissue than control parotid glands but some acinar tissue remained. Yet the tissue had no amylolytic activity.

One possibility is that the transplanted cells cannot produce enzyme in the absence of their nerve supply. The fact that enzyme activity was lost while some acinar tissue remained in evidence lends some support to this idea.

A second possibility is that the transplant did not become established at the new site and did not produce enzymes for reasons other than loss of innervation. The presence of a good blood supply to the transplant and the presence of some acinar tissue seems to indicate the transplants were not totally dead.

It is easier to obtain functioning homographs of ovary than any of the endocrine tissues transplanted, but even in this tissue there is common central necrosis (Brooks, 1962). The latent period to onset of estrus following autotransplantations varies but 6-8 days is average. Autotransplants are functional earlier than grafts in a strain or between strains. The salivary glands were autotransplantations so the time the transplants remained in the new location should have been sufficient.

Autotransplantations of the pancreas beneath the mammary glands in dogs (Ivy and Farrell, 1926) caused gradual deterioration to about 50 percent of the exocrine tissue as judged by external secretion. There was not a normal nerve supply in the new environment. The reduction in secretion was attributed to a loss of nerve stimulation and the remaining external secretion was attributed to humoral stimulation. The salivary transplants would seem to be a similar situation in that loss of nerve supply was accompanied by loss of secretion products. If the salivary glands are more dependent on nerve stimulation than the pancreas tissue, it would then be expected that a greater atrophy of the salivary tissues would occur.

Acetylcholine injected prior to removal of the transplant did not serve to elevate the amylase levels. In view of the decreased size of the acini and presence of connective tissue in the transplant it seems unlikely that it could produce enzymes if it were normally quite responsive to parasympathetic stimulation.

Salivary Hypertrophy and Amylase Activity

Since the hypertrophy of the salivary glands which follows addition of desiccated pancreas to the diet has been

described as similar to that resulting from the injection of isoproterenol (Ershoff and Levin, 1962), it seemed likely that changes in enzymatic activity might be similar after the two treatments. This was not the case, however. The submaxillary amylase increased after either treatment, but the parotid amylase decreased after injections of isoproterenol and increased after feeding of pancreas powder as a diet supplement.

A comparison of the submaxillary glands in animals given 50 mg/day of isoproterenol with glands from animals fed pancreas powder revealed the loss of terminal tubule granulation and increased diameter of acini. These changes were more pronounced and the submaxillary amylase much higher in those animals given isoproterenol. This may result from the difference in routes of administration as well as dosage. Isoproterenol would have been less effective if given orally as was the desiccated pancreas (Ershoff and Levin, unpublished data). The dosage of isoproterenol was reduced to 5 mg/day in a second group of animals in an attempt to duplicate more closely the enzymatic changes evoked by the pancreas powder and then determine whether the enzymatic and histological changes were similar. The lower dosage caused the amylase activity of the submaxillary to be

elevated less than the high dosage, and at the same time reduced the terminal tubule granulation to approximately that response produced by feeding defatted pancreas. A similar response can be evoked if the proper dosage of isoproterenol is given.

The prolonged administration of isoproterenol might, like desiccated pancreas tissue, have decreasing effects after a certain period of time. In order to compare more accurately the effects of administering isoproterenol and desiccated pancreas, it would be desirable to do a prolonged study of the effects of each and make the comparison at the times of greatest gland changes.

Since pancreas powder and isoproterenol increase the submaxillary gland weight as well as the amylase activity per unit gland weight, together these two factors compound the increase in enzymatic activity of the total submaxillary gland. This is particularly true after the administration of isoproterenol. The gland was bigger and there was an increase in activity per mg of tissue, the total gland activity in animals given 50 mg/day being 113 times as great as that of the controls.

The effects produced by isoproterenol and desiccated pancreas on body weight are quite different. Isoproterenol

did not affect tht total body weight while causing an increase in salivary gland size whereas pancreas powder caused a marked decrease in body weight concurrent with the increase in gland size. The weight loss resulting from the feeding of pancreas powder caused the increase in relative submaxillary gland weight to appear more pronounced than it would had the body weight remained normal.

The effects of administration of desiccated pancreas tissue were an elevation of amylolytic activity in both the submaxillary and parotid glands and decreased body weight. Isoproterenol also increased the submaxillary amylase activity but decreased parotid activity and there was no change in body weight. Since the parotid gland and body weight responses resulting from the administration of desiccated pancreas were different from those caused by isoproterenol, it would seem the active principle of the two compounds must differ. Had the whole array of responses been similar after the two treatments as the submaxillary responses were, it would seem more likely the active principle of the compounds might be the same.

Two groups of animals were fed desiccated pancreas for two different periods of time. One group was fed the material for 14 days and the other for 28 days. Those fed

for the shorter time had the greater hypertrophy of the salivary glands. This was followed by a decrease in gland size so that those animals fed for 28 days had smaller submaxillary glands than those fed 14 days although they were still larger than controls. The general tendency to an increase in gland size following this treatment was well established by Ershoff and Levin (1962). Examination of experimental animals after successive time intervals was not done, however, so that the tendency for a slight decrease in gland size during the second two-week period seems not to have been observed before.

Enzymatic activity showed its greatest increase during the first 14 days, and a very slight change between days 15 and 28. Two-tailed t-tests demonstrated that the increase at day 14 had reached only the borderline of significance ($P=0.09$), but established the difference at day 28 as conventionally significant ($0.05>P>0.02$). In retrospect, therefore, there is adequate justification for interest only in the single direction of change up to day 14, which may therefore be regarded as having a 1-tailed significance of 0.045.

There is good correlation between the histological and enzymatic changes and the time at which they occur after feeding desiccated pancreas. The precise length of time

which is required for the maximum change to occur was not established in this study. The magnitude of the changes which occur was originally reported as a function of the quantity of the pancreas powder fed per day but as this study shows it is also related to the time over which it is fed.

Selye, et al. (1961) described the hypertrophy of the salivary glands resulting from mitotic proliferation of serous, mucous, and duct cells subsequent to isoproterenol injection. The gland changes were similar to those obtained by Ershoff and Levin (1962) after feeding desiccated pancreas tissue to rats. No mention was made of any change in the terminal tubules or in the granulation of these tubules. Selye, et al. mentioned microscopic evidence of increased secretory activity but gave no indication of the nature of this evidence.

In order that the histological changes might be more specifically correlated with the enzyme changes mediated by treatment of the experimental animals, the area occupied by granule-containing terminal tubules was measured. There was good correlation between the change in granule content of the tubules and the change in enzyme content of the glands. The decrease in granulation was associated with greater increases

in activity.

Ershoff and Levin (1962) found the feeding of desiccated pancreas tissue caused enlargement of the alveoli and caused the cytoplasm of the cells in the alveoli to become mucoid in appearance. Except for the change in amount of terminal tubule granulation which was not mentioned by Ershoff and Levin, the histological changes of the submaxillary glands were very similar to those they reported.

The epithelium of the smallest excretory ducts in the submaxillary glands has been thought normally to undergo transformation to mucous cells (Maximow and Bloom, 1957). The increase in acinar tissue and the decrease in area occupied by tubules in the submaxillary glands of isoproterenol or desiccated pancreas treated animals may be a case of such cell transformation. The histological change was so great after 10 days the fate of the tubule cells could not be determined, but some intermediate stages in the histological study could very well shed light on the means by which the tubule area changes.

The histological and morphological changes in the salivary glands produced by feeding pancreas powder also occur after feeding raw hog pancreas at a level corresponding to 10% of the diet by weight (Ershoff and Levin, 1962).

Rat pancreas tissue comprising 5% of the diet did not alter the enzymatic activity or size of the salivary glands. One explanation for this may lie in the fact that there is more fat in close association with the rat pancreas and it probably comprised a great deal of the tissue fed. It is also possible that the active portion of the pancreas responsible for salivary gland changes is species specific and is not found in the pancreas of the rat. The rats fed pancreas tissue gained more weight than their control group while defatted pancreas powder caused weight loss.

The animals serving as controls for the group fed pancreas tissue had submaxillary amylase levels higher than most control animals. Histologically these animals showed a decrease in terminal tubule granulation in association with the higher enzyme content. This result is similar to that obtained in some experimental groups in which decreased granulation accompanied higher enzyme levels. The change in granulation evidently accompanies normal enzyme fluctuations in untreated animals.

CHAPTER V

SUMMARY AND CONCLUSIONS

The histology and amylolytic activity of the submaxillary and parotid glands of mature male rats were studied. The effect of diet, hormones and neurohumors on these salivary glands as well as the enzyme activity accompanying hypertrophy were determined using the method of Sobel and Meyers (1953).

Acetylcholine increased the amylase content of submaxillary tissue but did not affect the enzymatic activity of the parotid gland. Sympathetic stimulation (nor-adrenalin injection) had no effect on parotid enzyme activity but increased the total submaxillary amylase. Neither substance changed the gland histology.

A high carbohydrate diet lowered the enzyme activity of the parotid gland without affecting the activity of the submaxillary gland per mg of tissue. The diet increased the weight of the submaxillary gland thereby increasing total

gland activity. A high protein diet had no effect on either gland and neither diet changed the submaxillary gland histology.

Neither pancreozymin nor secretin changed the amylase content or the histology of the salivary glands.

The salivary glands were responsive to very few of the stimuli of pancreatic enzyme production. Conversely there were significant enzyme changes associated with hypertrophy of the glands.

Hypertrophy of the salivary glands was caused by the injection of isoproterenol and there was increased amylolytic activity of the submaxillary and decreased activity of the parotid glands. The histological changes in the submaxillary glands were dependent on the dosage injected, with larger dosages mediating more severe changes but in any case terminal tubules were reduced in number, and the acidophilic granulation of the terminal tubules was decreased.

The hypertrophy induced by feeding pancreas powder was related to the length of time over which the material was fed. When fed for two weeks submaxillary and parotid amylase were increased and body weight decreased. These changes were accompanied by loss of terminal tubule granulation in the submaxillary gland. When the desiccated pancreas was fed for

four weeks there was a return toward normal histology and enzyme content by the end of this time.

Although both pancreas powder and isoproterenol caused hypertrophy of the salivary glands, the action of the two was somewhat different as isoproterenol did not affect body weight concurrent with the salivary hypertrophy while desiccated pancreas caused weight loss. Pancreas powder raised parotid amylase while isoproterenol lowered its activity.

The submaxillary and parotid glands do not always respond to the same stimuli or may respond with results which are directly opposed.

The histological studies seemed to bear out that an extreme increase in submaxillary amylase activity was paralleled by a decrease in the granulation and number of terminal tubules of these glands. The animals given 50 mg/day of isoproterenol had the highest submaxillary amylase of any group and had a complete loss of terminal tubule granulation.

There were differences in amylase content of the submaxillary and parotid glands from rats of different strains.

LITERATURE CITED

Babkin, B. P. 1931. The innervation of the salivary glands. Trans. Roy. Soc. Can., 25, Section V, 205.

_____. 1944. Secretory Mechanism of the Digestive Glands. New York: P. B. Hoeber, Inc.

Bensley, R. R. and S. H. Bensley. 1945. Handbook of Histological and Cytological Technique. Chicago: University of Chicago Press.

Brooks, John R. 1962. Endocrine Tissue Transplantation, A Monograph in American Lectures in Living Chemistry. Springfield, Ill.: Charles C. Thomas, Pub.

Crick, Joan, A. A. Harper and H. S. Raper. 1949. On the preparation of secretin and pancreozymin. J. Physiol., 110:367-376.

Ershoff, Benjamin H. and Ezra Levin. 1962. An orally-active heat-labile factor in pancreas which induces salivary gland hypertrophy in rats. Proc. Soc. Exp. Biol. and Med., 110:262-265.

Grad, B. and C. P. Leblond. 1949. The necessity of testis and thyroid hormones for the maintenance of serous tubules of the submaxillary gland in the male rat. Endocrinology, 45:250-266.

Grossman, M. I., H. Greengard, and A. C. Ivy. 1942-43. The effect of dietary composition on pancreatic enzymes. Am. J. Physiol., 138:676-682.

Harper, A. A. and H. S. Raper. 1943. Pancreozymin, a stimulant of the secretion of pancreatic enzymes in extracts of the small intestine. J. Physiol., 102:115-125.

Harvey, Harriet. 1957. The effect of sex hormones on the amylolytic activity of submaxillary, parotid and pancreas of the laboratory mouse. Anat. Rec., 128: 560-561.

Ivy, A. C. and J. I. Farrell. 1926. Autotransplantation of the pancreas. Am. J. Physiol., 77:474-479.

Lin, T. M. and A. C. Ivy. 1957. Relation of secretin to the parasympathetic mechanism for pancreatic secretion. Am. J. Physiol., 189:361-368.

Maximow, Alexander A. and William Bloom. 1957. A Textbook of Histology. Philadelphia: W. B. Saunders Company.

Rawlinson, H. E. 1933. Cytological changes after autonomic and adrenalin stimulation of the cat's submaxillary gland. Anat. Rec., 57:289-296.

_____. 1935. The changes in the alveolar and demilune cells of the simple and the stimulated paralytic submaxillary gland of the cat. J. Anat., 70:143-148.

_____. 1936. The effect on the cells of the cat's submaxillary gland of the augmented secretion. Quart. J. Exp. Physiol., 26(1):79-82.

Richins, C. A. and A. Kuntz. 1953. Role of sympathetic nerves in the regulation of salivary secretion. Am. J. Physiol., 173:471-473.

Selye, H., R. Veilleux, and M. Cantin. 1961. Excessive stimulation of salivary gland growth by isoproterenol. Science, 133:44-45.

Smith, B. W. 1958. Interrelationships of glycogen and amylase in carbohydrate fed rats. Fed. Proc., 17(1): 493.

Sobel, Harry and Sara M. Meyers. 1953. A simplified method for the determination of amylase activity in serum and urine using glycogen and the anthrone reagent. J. Lab. and Clin. Med., 41:655-658.

Sreebny, Leo M., Julia Meyer, Erica Bachem and J. P. Weinmann. 1955. Postnatal changes in proteolytic activity and in the morphology of the submaxillary gland in male and female albino rats. Growth, 19: 57-73.

Wang, C. C., M. I. Grossman and A. C. Ivy. 1948. Effect of secretin and pancreozymin on amylase and alkaline phosphatase secretion by the pancreas in dogs. Am. J. Physiol., 154:358-368.