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Parotid gland
pancreatic insufficiency
pancreatin
rats
salivary flow rates
submaxillary glands

Salivary Gland Enlargement and Functional Changes During Feeding of Pancreatin to Rats

(Possible Relation to Functional Changes in Cystic Fibrosis)

JOHN A. MANGOS[33], PAUL J. BENKE, and NONA MCSHERRY

The Departments of Pediatrics and Oncology, University of Wisconsin Medical School, Madison, Wisconsin, USA

Extract

Enlargement and functional alterations of the salivary glands were studied in male rats of the Sprague-Dawley strain fed a diet containing 4% pancreatin for 3 weeks. The weight of the parotid and submaxillary glands increased 1.7 and 2.7 times, respectively. When rats were fed 4% pancreatin heated to 100° for 3 h no enlargement of the glands occurred; similarly, gastric intubation of the 4% pancreatin preparation had no effect on the size of the salivary glands. A marked increase in enlargement of the salivary gland occurred in rats fed 4% pancreatin and given subcutaneous injections of 5 mg theophylline twice daily. The functional alterations of the salivary glands included: a) parotid: salivary flow rates in response to the intravenous injection of a standard dose of pilocarpine (0.1 mg/100 g body weight) were slightly lower in experimental rats than those observed in the parotid glands of control rats; sodium and calcium concentrations were higher in the saliva of experimental rats than in the saliva of controls; potassium concentrations did not differ; rats fed heated pancreatin had no functional changes; b) submaxillary: the salivary flow rates in experimental animals following administration of the standard dose of pilocarpine were significantly lower than those in the control rats; sodium, potassium and calcium concentrations were higher in the saliva of experimental rats than in the saliva of control rats at comparable flow rates; the salivary protein concentrations were higher. In rats fed the 4% pancreatin preparation, the saliva of both glands exhibited marked changes in the electrophoretic patterns of excretion of basic secretory proteins.

The similarities of these structural and functional changes observed in rats fed pancreatin and those observed in patients with cystic fibrosis of the pancreas (CFP) suggest that a similar mechanism may be responsible in both cases. Study of this mechanism, particularly the basic biochemical events of intracellular transmission of neurogenic stimuli and their chemical mediators such as cyclic AMP, may help clarify the pathogenesis of CFP.

Speculation

The sialadenotrophic and functional effects observed in the salivary glands of rats when pancreatin was added to the feed are similar to the changes observed in the salivary glands of patients with cystic fibrosis of the pancreas (CFP). The action of these preparations depends on a neural reflex mechanism involving the autonomic nerve supply to the glands. A similar alteration involving the transmission of stimuli from the autonomic nervous system to the secretory elements of exocrine glands may be part of the mechanism of pathogenesis of CFP.

Introduction

Enlargement of the submaxillary salivary glands of rats fed dessicated and defatted pancreas, pancreatin, raw pancreas and various proteolytic enzymes has been previously reported [7, 8, 22, 23]. The wet and dry weight of the glands increased indicating that the enlargement was due to true nonaqueous cellular material. Histologically, the cells of the glands exhibited marked enlargement of the acini with the cytoplasm developing a mucoid appearance, in contrast to the appearance of the cells of the submaxillary glands of control rats, which had smaller acini and coarsely granular cytoplasm. Feeding desiccated and defatted raw liver, duodenum or spleen affected neither the size nor the microscopic appearance of the glands. There was a partial loss in gland weight when pancreatin feedings were terminated. WELLS and his associates [22, 23] suggested that the sialadenotrophic action of these preparations depends on a neural reflex arc: the afferent arm consists of the taste receptors innervated by the glossopharyngeal nerves, while the efferent arm is composed of both branches of the autonomic nervous supply to the glands. Studies of possible functional alterations of the enlarged glands have not been performed.

Since enlargement and functional changes of the salivary glands are regularly found in patients with cystic fibrosis of the pancreas (CFP) [1–3, 5, 6, 11], the present study of the effects of pancreatin feeding on the size and the function of the submaxillary and parotid glands of the rat was performed. Enlargement of both glands and changes in the secretory activity, in the handling of electrolytes, and in excretion of protein in the saliva were observed. These changes resembled those observed in the salivary glands of patients with CFP.

Materials and Methods

Male albino rats of the Sprague-Dawley strain, weighing 35–160 g, were obtained from a commercial source [25]. The rats in each experiment had been born on the same day and were assigned to groups at random. The experimental diet consisted of a mixture of ground rat ration [26] and one of three pancreatic enzyme preparations: pancreatin powder (preparation 1) [27]; pancrelipase (preparation 2) [28]; and pancreatin granules (preparation 3) [29]. The mixture, pancreatin+ground rat meal, was prepared daily in a ratio of 4 g of the enzyme preparation to 100 g of ground rat meal. Control animals received only the ground rat meal (basal diet); fresh water was supplied *ad libitum*. Most experiments were performed after the rats had been fed the pancreatin for 3 weeks.

On the day of the experiment, each rat was anesthetized by intraperitoneal injection of sodium pentobarbital (approximately 8 mg/100 g body wt). Tracheostomy and cannulation of the left jugular vein were performed. The duct of the right parotid was dissected through a skin incision 1–2 mm posterior to the corner of the mouth and cannulated with a fine polyethylene tubing (80–120 μ in diameter). The duct of the right submaxillary was cannulated [24]. The glands were stimulated to secrete saliva by a standard dose of pilocarpine (0.1 mg/100 g body wt), injected intravenously. Saliva collected in the first 2 min was discarded. Serial collections of saliva at progressively decreasing flow rates were made until the salivary flow stopped. The saliva was collected under mineral oil in tared polyethylene vessels. Following each experiment, the glands were dissected and weighed. The size of the salivary sample was measured gravimetrically. The salivary flow rates were expressed as μl/min/g of wet gland tissue; it was assumed that 1 μl of saliva weighed 1 mg. Sodium and potassium concentrations in saliva were measured by flame photometry [30]. Calcium concentrations in the saliva were measured by atomic absorption spectrophotometry.

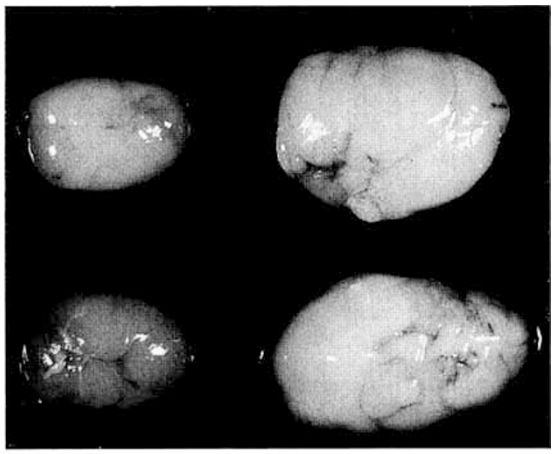
Saliva from the parotid and submaxillary glands was made hypertonic by the addition of sucrose granules. Equal amounts of salivary protein (50–300 μg) were layered over 7.5% polyacrylamide gels, pH 4.5, and electrophoresis was performed by a modification of the method of RIESFIELD *et al.* [18]. Gels were fixed in 10% trichloroacetic acid (TCA) and stained for protein with 0.05% Coomassie blue in 20% TCA [4]. Protein was determined by the method of LOWRY *et al.* [12].

Since the animals used in this investigation had intact pancreatic function, the question of whether or not a sialadenotrophic effect could be induced in animals with pancreatic insufficiency was then studied. Ligation of the pancreatic duct was performed on a group of nine rats; 1 week after pancreatectomy the animals were fed a diet containing 4% pancreatin powder.

Another experiment was designed to investigate whether pancreatin powder can produce a sialadenotrophic effect when administered by gastric tube. Rats received 0.5 g of pancreatin powder dissolved in 2.0 ml water twice a day. The control rats received 2 ml of normal saline.

Results

As shown in figure 1, feeding rats a diet containing 4% pancreatin (preparation 1) resulted in marked enlargement of the submaxillary glands. The weight of the submaxillary glands of the experimental rats was 2.7 times that of the glands of control rats (table I, exp. 1).



The parotid glands of the experimental rats were also enlarged, but weighed only 1.7 times more than those of the control rats. When preparation 1 was heated to 100° for 3 h and then added to the diet, the sialadenotrophic effect disappeared (fig. 1 and table I, exp. 2). In the first two experiments young animals were used. Experiment 3 shows that the same effect was observed when older rats, weighing 140–150 g, were used. Less hypertrophy of the salivary gland was observed when

Fig. 1. Enlargement of the submaxillary glands of rats fed 4% pancreatin for 3 weeks (right upper and lower) as compared with the submaxillary glands of a control rat (left upper) and a rat fed 4% heated pancreatin (left lower).

Table I. Effects of feeding pancreatic preparations[1] on the weight of the parotid and submaxillary glands of the rat

Experimental conditions	Initial body wt, g	Final body wt, g	Parotid gland wt, mg	Submaxillary gland wt, mg
Exp. 1				
Controls (7)[2]	38.4	172.5	192±27	246±36
Rats fed 4% preparation 1 (10)	36.7	161.3	318±55	654±129
Exp. 2				
Controls (6)	56.5	178.4	174±28	247±17
Rats fed 4% heated preparation 1 (13)	55.5	184.1	173±21	246±35
Exp. 3				
Controls (6)	141.7	300.1	212±48	308±15
Rats fed 4% preparation 1 (6)	142.3	278.3	348±35	717±77
Exp. 4				
Controls (10)	46.3	166.6	154±22	210±22
Rats fed 4% preparation 2 (10)	44.8	156.1	247±36	352±66
Rats fed 4% preparation 3 (10)	43.5	168.1	244±30	289±29
Exp. 5				
Controls (6)	142.6	269.4	306±19	476±31
Rats fed 4% preparation 1 (9) (after pancreatic duct ligation)	145.8	310.5	524±28	954±57
Exp. 6				
Controls (6)	57.4	252.9	207±41	257±49
Rats fed 4% preparation 1 for 3 weeks (6) and returned to control diet for 3 weeks	55.8	285.4	249±29	402±48
Exp. 7				
Controls (6)	59.3	161.2	198±33	205±10
Rats fed preparation 1 by gastric tube (6) for 2 weeks	57.8	165.2	200±24	211±14

[1] Preparation 1, pancreatin powder [27]; preparation 2, pancrelipase [28]; preparation 3, pancreatin granules [29].

[2] Numbers in parentheses indicate number of rats studied.

the two other pancreatic enzyme preparations were used (table I, exp. 4). As shown in table I (exp. 5), hypertrophy of the salivary gland was induced in pancreatectomized animals. Following termination of the experiment, destructive changes of the ligated pancreas were demonstrated in all animals. Experiment 6 shows that the sialadenotrophic effect of preparation 1 persisted 3 weeks after the return of the animals to the control diet. Experiment 7 revealed (table I) that intragastric administration of pancreatin did not affect the size of the salivary glands.

Functional Changes of the Enlarged Glands

Parotid gland. As shown in figures 2 and 3, the parotid glands of rats fed a diet containing 4% pancreatin powder (preparation 1) for 3 weeks responded to the standard dose of pilocarpine by excreting saliva at flow rates slightly lower than those of the parotids of control rats. Although there was some overlap of values, particularly at very low flow rates, the saliva of the experimental rats contained sodium at significantly higher concentrations than those of the saliva of control rats. Potassium concentrations in the saliva of the experimental rats did not differ from those of the control rats. The flow rates and sodium and potassium concentrations in the parotid saliva of rats fed the diet containing 4% preparation 1 were the same as those of the control rats (figs. 4 and 5).

In experimental rats, calcium concentrations in the saliva of the parotid glands were slightly higher than in saliva from control rats (table II). Concentration of protein did not decrease significantly ($p < 0.1$) as shown in table III. This was associated with the disappearance of bands 1, 3, and 5 and with differences in the relative basic protein concentrations in bands 5–9 of the electrophoretic pattern of the secretory proteins (fig. 8).

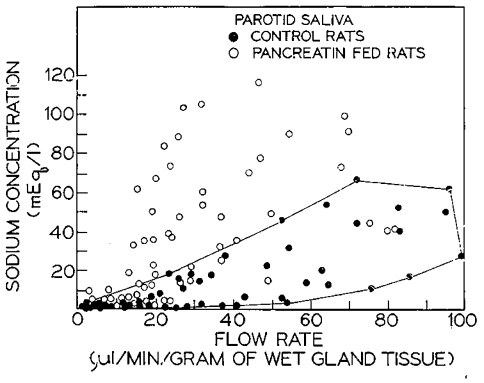


Fig. 2. Relation of flow rate to sodium concentration in the parotid saliva of eight control rats (●) and eight rats fed 4% pancreatin powder for 3 weeks (○).

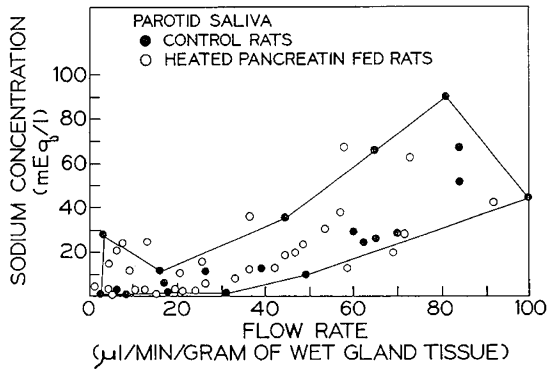


Fig. 4. Relation of flow rate to sodium concentration in the parotid saliva of five control rats (●) and five rats fed 4% heated pancreatin powder for 3 weeks (○).

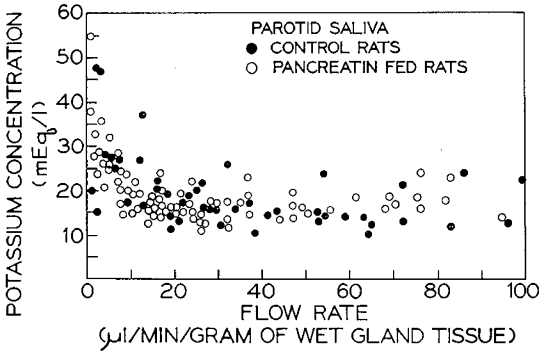


Fig. 3. Relation of flow rate to potassium concentration in the parotid saliva of eight control rats (●) and eight rats fed 4% pancreatin powder for 3 weeks (○).

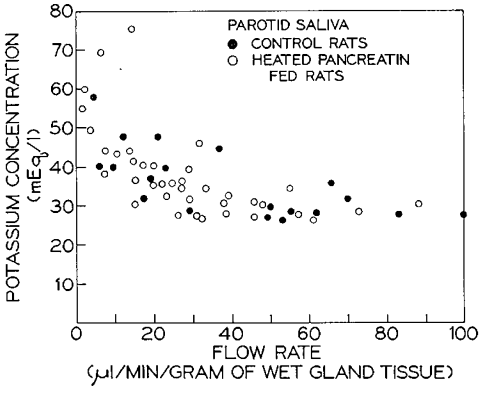


Fig. 5. Relation of flow rate to potassium concentration in the parotid saliva of five control rats (●) and five rats fed heated pancreatin powder for 3 weeks (○).

Table II. Calcium concentrations in the saliva of control rats and those fed 4% preparation 1[1]

Experimental conditions	Calcium, mg/100 ml saliva	
	Parotid gland	Submaxillary gland
Controls (18)[2]	5.7±0.9[3]	6.4±2.1[3]
Rats fed 4% preparation 1 (14)	6.3±1.2	14.1±1.1

[1] See table I, footnote 1.
[2] Numbers in parentheses indicate number of rats studied.
[3] ±SE.

Table III. Protein concentrations in the saliva of control rats and those fed 4% preparation 1[1]

Experimental conditions	Protein, mg/100 ml saliva	
	Parotid gland	Submaxillary gland
Controls (9)[2]	382±129[3]	106±38[3]
Rats fed 4% preparation 1 (6)	214±97	267±112

[1] See table I, footnote 1.
[2] Numbers in parentheses indicate number of rats studied.
[3] Mean ±SE.

Submaxillary gland. Marked functional changes occurred in the enlarged submaxillary glands of the experimental animals. Following administration of the standard dose of pilocarpine to the experimental rats, the flow rates were significantly lower than those of the control rats. Even after the administration of two to five times the standard dose of pilocarpine, flow rates could not be obtained that were comparable to those of the control rats. Sodium and potassium concentrations in saliva were higher in the experimental animals than in the control rats, but there was significant overlap of the values. In experimental rats, the calcium concentrations in the saliva secreted from the submaxillary glands were significantly higher than the concentrations in the saliva secreted by control glands (table II). The total secretory protein was also elevated (table III). Polyacrylamide gel electrophoresis of submaxillary saliva from experimental rats showed disappearance of band 2 and an alteration in the electrophoretic motility of band 3.

Discussion

This study demonstrates that addition of desiccated and defatted raw pancreas or pancreatic enzymes to the diet of rats results not only in enlargement of the submaxillary glands, as previously reported [7, 8, 22, 23], but also in enlargement of the parotid glands of the animals. In addition to the changes in size and weight, marked functional alterations occur in the glands exhibiting this sialadenotrophic effect of pancreatic preparations. In all the previous studies of this effect, young animals were used [7, 8, 22, 23]. The

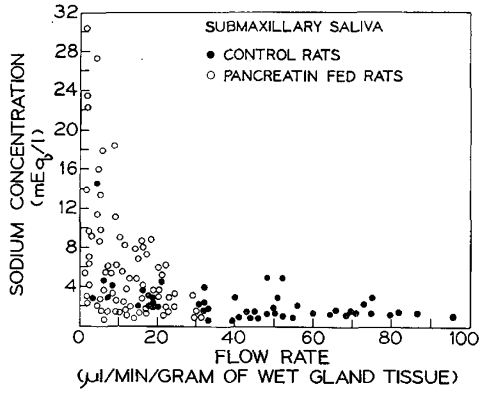


Fig. 6. Relation between flow rate and sodium concentration in the submaxillary saliva of six control rats (●) and six rats fed 4% pancreatin powder for 3 weeks (○).

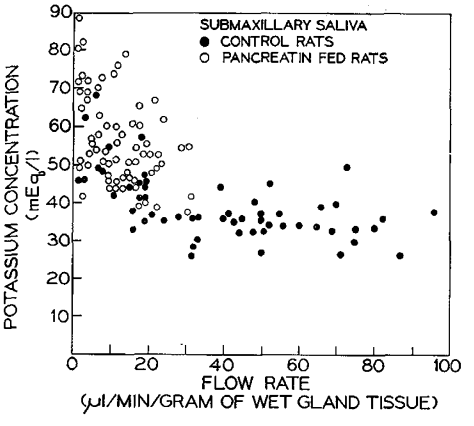


Fig. 7. Relation between flow rate and potassium concentration in the submaxillary saliva of six control rats (●) and six rats fed 4% pancreatin powder for 3 weeks (○).

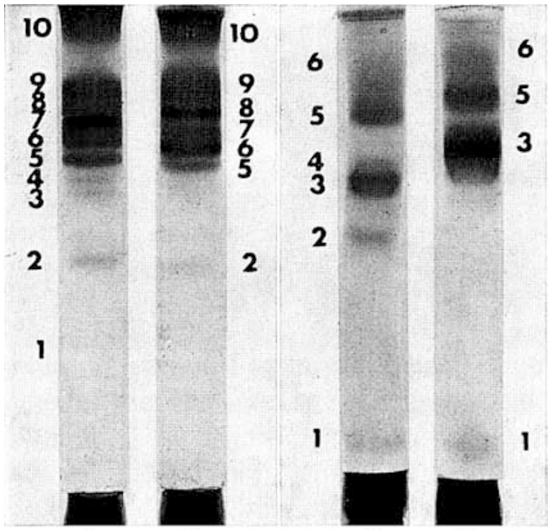


Fig. 8. Polyacrylamide gel electrophoresis, pH 4.5, of salivary proteins. A and B: saliva from parotid glands of control rats and rats fed 4% pancreatin, respectively (130 μg of salivary protein applied to each gel). C and D: saliva from submaxillary glands of control rats and rats fed 4% pancreatin, respectively (100 μg of salivary protein applied to each gel). Migration toward the bottom (cathode).

present experiments show that this effect is not limited to the young, rapidly growing animals but can also be induced in older animals. It was also shown that the sialadenotrophic effect can be produced in animals devoid of exocrine pancreatic function resulting from ligation of the ducts. Our inability to produce this effect by the intragastric administration of pancreatin to the rats confirms the similar observations of WELLS *et al.* [22, 23] and adds support to the proposed mechanism of action involving a reflex arc with the taste receptors-glossopharyngeal nerve as the afferent and the autonomic nervous supply to the glands as the efferent arm of this arc.

The sialadenotrophic effect of pancreatin was associated with increased sodium concentrations in the saliva. The salivary glands of the rat produce a primary secretion with plasmalike concentrations of sodium and potassium [14, 15, 17, 24]. The final concentration of these ions in the saliva is regulated by processes of reabsorption of sodium and secretion of potassium across the duct system of the glands. Thus, the increased sodium concentrations in the saliva of the glands exhibiting the sialadenotrophic effect of pancreatin may be considered an indication of decreased transductal reabsorption of sodium. In a previous study, we demonstrated a marked decrease in the transductal reabsorption of sodium when the luminal side of the duct system of the rat parotid was exposed to dilute solutions of strongly positively charged macromolecular compounds such as polyamino acids, basic proteins and organic polyelectrolytes [16]. In the present study, changes in the excretory pattern of basic macromolecules occurred in the saliva of the glands exhibiting the sialadenotrophic effect of pancreatin feeding. Possibly the observed elevation of sodium concentrations in the saliva may be caused by this change in the secretion of basic macromolecules in the saliva. If such a cause-effect relation is proved, it may offer significant clues as to the pathogenesis of the abnormality of sodium excretion in the exocrine glands of patients with CFP.

The results of this study demonstrate that the enlarged salivary glands of the experimental rats respond to a standard stimulus of pilocarpine with flow rates significantly lower than those of the control rats. This decrease in flow rate was pronounced in the submaxillary and moderate in the parotid glands. Since there is no substance available that could be used for the measurement of secretory activity in salivary glands, as inulin is used to measure glomerular filtration rates in the kidney, the flow rates are expressed as volume of saliva per unit time per unit weight of the gland (μl/min/g of wet gland tissue). This can be easily done in rats since after the termination of the experiments the glands are removed and weighed. In the human, however, the weight of the gland undergoing functional studies is not known. Thus, in studies of the function of human salivary gland, the salivary flow rates are expressed as volume of saliva per unit time per gland. Since variations in gland size are common, this limitation could introduce a significant error in comparing the results from one individual with another. The situation becomes particularly difficult when control subjects are compared with patients having diseases that cause enlargement of the salivary glands such as CFP. If our measurements of flow rates in the rat salivary glands were expressed per individual glands rather than per gram of gland tissue, then the reported flow rates should be multiplied by a factor that is derived from the ratio:

$$\frac{\text{weight of experimental gland}}{\text{weight of control gland}}.$$

Such a factor would be 2.7 and 1.7 for submaxillary and parotid glands, respectively. Inspection of figures 2, 3, 6, and 7 indicates that if such a change is introduced, the individual points of flow rates would shift to the right. In such a case, the flow rates per gland would be different. In experimental animals, flow rates of the submaxillary glands would be equal to flow rates of submaxillaries from control rats, and flow rates

Table IV. Comparison of functional changes between salivary glands of rats fed 4% preparation 1[1] and salivary glands of patients with cystic fibrosis

	Enlarged salivary glands of rat due to feeding 4% preparation 1		Cystic fibrosis	
	Parotid	Submaxillary	Parotid	Submaxillary
Flow rate	Increased	Moderately decreased	Increased	Decreased
[Na]	Increased	Increased	Increased	Moderately increased
[K]	No change	Moderately increased	No change	No change
[Ca]	No change	Increased	No change	Increased
Total protein	No change	Increased	No change	Increased

[1] See table I, footnote 1.

Table V. Effects of feeding a pancreatic preparation and injecting theophylline for 10 days on the weight of the parotid and submaxillary glands of the rat[1]

Group no.	No.[2]	Diet	Theophylline	Body wt, g		Gland wt, mg	
				Initial	Final	Submaxillary	Parotid
1	6	Basal	—	62.30	108.0	201±17[3]	141± 7[3]
2	6	Basal	+	61.5	102.3	257±21	179±13
3	6	Basal+4% preparation 1[4]	—	62.1	99.5	390±10	217±13
4	6	Basal+4% preparation 1	+	60.9	96.1	560±23	321±21

[1] Theophylline injected subcutaneously twice daily, 10 mg/24 h.
[2] Number of animals in group.
[3] ±SE.
[4] See table I, footnote 1.

of the parotid gland in experimental rats would exceed flow rates from parotids of control rats.

With this difference under consideration, a comparison can be made between the present experimental data and those reported in the literature [1, 2, 5, 6, 11] concerning the functional alterations of salivary glands in CFP (table IV). As shown in this table, significant similarities exist between the functional alterations of the salivary glands of rats fed pancreatin and those of patients with CFP. If one is allowed to make comparisons between the salivary glands of these rats and those of patients with CFP, these similarities could indicate that the enlargement and functional changes in the salivary glands of patients with CFP may be due to a pathogenetic mechanism similar to that causing the sialadenotrophic effect of pancreatic enzymes in the rat.

Generally, in the salivary and exocrine glands, the size of secretory cells, synthesis and secretion of macromolecules, secretion of water and electrolytes, and possibly the intraluminal modification of the primary secretory fluid are controlled by the autonomic nerve supply to the glands. The mechanisms of transmission of stimuli from the nerve endings into the secretory cells and their translation into distinct changes of cellular structure and function are unknown. Recent studies, however, indicate a possible mechanism for this action. SUTHERLAND *et al.* [19] have demonstrated that various hormones act upon their target cells by activating the membrane-bound enzyme system, adenyl cyclase, which catalyzes the intracellular synthesis of the cyclic nucleotide adenosine 3′-5′ monophosphate (cyclic AMP) from ATP. Cyclic AMP appears to be the common intracellular mediator, the 'second messenger', of the action of a variety of hormones such as epinephrine, glucagon, and parathormone. Cyclic AMP is rapidly broken down to 3′ AMP by the action

of the enzyme, cyclic nucleotide phosphodiesterase. Epinephrine and isoproterenol stimulate the activity of adenyl cyclase, while theophylline strongly inhibits the activity of phosphodiesterase.

Recently, GRAND [9] showed that cyclic AMP stimulates the protein synthesis in rat parotid gland slices *in vitro*. It has also been shown that chronic administration of isoproterenol to rats causes marked enlargement of the salivary glands [13, 20]. This is due to marked increase in the synthesis of RNA and DNA followed by marked acceleration of the protein synthesis in the secretory cells of the salivary glands. The sialadenotrophic effect observed during feeding of pancreatin to rats may also be mediated by cyclic AMP. If a neurogenic reflex mechanism is basic to this effect, the neural hormones released at the nerve endings of the autonomic nerve supply of the glands may act by stimulating adenyl cyclase activity in the secretory cells. Such stimulation would cause increases in the synthesis of cyclic AMP leading to increased protein synthesis and cell hypertrophy.

Administration of theophylline, an inhibitor of phosphodiesterase activity, should cause intracellular accumulation of cyclic AMP and potentiation of the sialadenotrophic effect of pancreatin. As shown in table V, subcutaneous administration of 5.0 mg of theophylline twice daily alone to a group of rats caused minimal increase in the weight of both parotid and submaxillary glands. When the same amounts of theophylline were given to a group of rats fed 4% pancreatin, a marked increase in enlargement of the salivary gland was observed. This increase was greater than that produced by theophylline or pancreatin alone, and it was also greater than what could be considered an additive effect of theophylline on that of pancreatin. It was concluded that the action of theophylline on the rats fed pancreatin was specific and that its effects might be due to inhibition of cyclic phosphodiesterase activity leading to increased levels of cyclic AMP in the secretory cells. The recent demonstration from this laboratory [13] that the serum of rats exhibiting the sialadenotrophic effect of isoproterenol have ciliary dyskinetic activity similar to that described by SPOCK *et al.* [21] in the sera of patients with CFP and their parents further supports the possibility that CFP may indeed be due to such an error in protein synthesis that is expressed mainly in the exocrine glands.

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29. Pancreatin granules (Panteric Granules), Parke-Davis & Company, Detroit, Mich.
30. Model 143, Instrumentation Laboratory, Inc., Lexington, Mass.
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33. Requests for reprints should be addressed to: JOHN A. MANGOS, M.D., Department of Pediatrics, University of Wisconsin Medical Center, 1300 University Avenue, Madison, Wisconsin 53706, USA.