without transplants. 2. When the adrenal transplants were excised from surviving hosts, after 67 to 154 days in the cheek pouch, the hosts died within 6 days. 3. Microscopic appearance of healthy cortical cells with some indication of adult arrangement in zones and cords, supported the physiological evidence for growth, differentiation and function of the adrenal transplants. This is the first known instance of successful adrenocortical transplantation in the hamster.

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Human Parotid Gland Secretion: Flow Rate and Interrelationships of pH and Inorganic Components.*† (23798)

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Limited studies by Hildes and Ferguson (1) and Thaysen, Thorn, and Swartz(2) have indicated that pH, sodium, bicarbonate, and chloride levels of parotid saliva are directly related to rate of secretion, in contrast to potassium and phosphorus concentrations which were independent of flow-rate. The secretory rate is influenced by numerous local and systemic factors which include: method of stimulation, diet, sleep, dehydration, infectious diseases, emotional factors, psychological abnormalities, nervous disorders, and presence of various drugs. Thus any attempt to comprehend metabolic processes and secretion-mechanism of the salivary glands demands that the effect of all possible affecting variables be predetermined and controlled.

In a previous study(3), analysis of parotid saliva from 50 individuals permitted a preliminary establishment of normal mean and dispersion values for sodium, potassium, calcium, bicarbonate, chloride, phosphorus, and pH. The present publication reports the effect of various stimuli on secretory rate of the parotid gland and the interrelationships between rate of secretion and salivary composition.

Materials and methods. Parotid saliva was collected using a vacuum cup(4). Graduated collection tubes were immersed in iced water and mean flow-rate (ml/min) calculated by recording the time required for secretion of a fixed volume. The saliva was analyzed for sodium, potassium, calcium, chloride, and phosphorus. Samples for determination of pH and bicarbonate were collected under paraffin oil. Paraffin (masticatory), lemon flavor‡ (gustatory), and flavored chicle§ (masticatory and gustatory) were used to study effect of different stimulatory agents. *Analytical procedures*: Sodium and potassium values were determined with an internal standard (Li) flame photometer; calcium, by the Clark-Collip(5) modification of the Kramer-Tisdall principle; chloride, using Benotti's(6) modification of the Van Slyke prin-

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‡ Lemon Life Savers were used without any sucking or masticatory action.

§ Dentyne Gum.

TABLE I. Comparative Effect of Different Stimuli on Rate of Parotid Gland Secretion.*

Stimulus	1st sample	2nd sample	3rd sample	Avg rate, 3 samples
	ml/min.			
Paraffin	.58	.54	.62	.58
Lemon flavor†	.55	.64	.93	.71
Flavored chicle‡ (bolus renewed)	.72	.82	.79	.78

* Above data based on mean secretion rates from 5 subjects.

† Lemon Life Savers were used without any sucking or masticatory action.

‡ Dentyne gum.

ciple; bicarbonate, by titration method of Van Slyke(7); and phosphorus, by modification(3) of the King procedure. pH was measured in a Model G Beckman pH Meter.

Results. Effect of various oral stimuli: When a single bolus of gum§ (4.33 g) was chewed for a sustained period (during collection of 3 successive 5 ml saliva samples), secretion rate of successive samples was significantly decreased. When the bolus was discarded and a new portion of gum introduced every 5 minutes, flow rate of 3 successive 5 ml samples was more consistent. Similar results were obtained when "sweetened chicle" which contained sugar but no flavoring agent was employed. Examination of mean flow rate of 3 successive 12.5 ml saliva samples (Table I) showed that paraffin and flavored chicle (bolus renewed) evoked fairly consistent rates of secretion. Lemon flavor produced a highly variable inter-sample flow rate. Thus, if the inherent variability in flavored chicle was controlled by subjects chewing a fresh bolus every 5 minutes, the chicle was capable of producing a greater effect than either paraffin or lemon flavor.

Comparison of secretion from both parotid glands: No differences were evidenced in secretion rate and composition of saliva samples collected before and after breakfast (5 subjects). Above measurements were made on saliva from the right gland only. Three persons were used for comparison of flow rate and composition of saliva from both parotid glands. Samples were collected simultaneously from both glands using flavored chicle and alternating the side of mastication. The analysis (Table II) showed only slight differences in flow rate, pH, potassium, calcium, bicarbonate, and phosphorus titers of right and left glands. In contrast, a marked difference was observed in sodium and chloride levels of the 2 glands of certain subjects.

Effect of methacholine: Two subjects were given intramuscular injections of methacholine (10 mg). Chicle stimulated saliva samples were collected before and after administration of the drug (Table III). There was a marked increase in flow rate, chloride, sodium, and calcium levels. The latter 2 components were several times greater than the mean value found with 50 "normal" subjects and were greater than the maximum of "normal" range(3).

Secretion rate and salivary constituents: Parotid saliva samples were secured from 50 fasting subjects (before breakfast) using flavored chicle as stimulatory agent. Twenty-five ml of saliva (two 12.5 ml samples) were collected from each individual for 2 successive days. Samples A_1 and A_2 were collected on first day, while B_1 and B_2 were obtained the second day. Paired data analysis of the difference between individual mean flow rates of 4 specimens indicated that fraction A_1 had a significantly lower rate than the other 3 fractions. However, there was no

TABLE II. Comparison of Composition and Secretion Rate of Both Parotid Glands of 3 Subjects.

	Flow rate, ml/min.	pH	Na	K	Ca	Total cations	HCO_3	Cl	P	Total anions
			meq/l							
Right gland	.58	7.29	20.9	22.3	1.0	44.2	15.7	24.0	4.4	44.1
Left "	.58	7.28	19.1	22.8	1.0	42.9	15.4	20.2	4.1	39.7
Right gland	.75	7.40	41.8	19.7	1.6	63.1	18.6	38.6	4.2	61.4
Left "	.73	7.46	23.5	18.7	1.5	43.7	19.3	20.0	4.9	44.2
Right gland	.83	7.42	31.8	18.2	1.7	51.7	20.0	29.9	4.0	53.9
Left "	.86	7.50	39.2	19.7	1.9	60.8	23.2	27.1	3.9	54.2

TABLE III. Effect of Methacholine on the Parotid Secretion.

	Patient	Flow rate, ml/min.	Na	K	Ca	Total cations	Chloride
			meq/l				
Before admin.	G	.23	3.51	19.7	1.3	24.5	8.2
	P	.33	13.01	20.7	1.4	35.2	42.0
After "	G	4.44	47.0	16.0	3.3	66.3	11.2
	P	1.33	58.3	21.8	3.8	83.9	61.9

significant difference between fractions A_2, B_1, and B_2. Phi coefficient analysis of the relationship between rate of secretion and composition indicated a positive correlation between flow rate and sodium (+ 0.50), calcium (+ 0.38), bicarbonate (+ 0.55), and pH (+ 0.52). Only the potassium titer exhibited a negative correlation (–0.30), with rate of secretion. A positive correlation was noted between pH and sodium (+ 0.56), calcium (+ 0.36), bicarbonate (+ 0.64), and chloride (+ 0.28) levels. Intercorrelations between various parotid saliva electrolytes were also observed (Table IV). The above stated correlations were all significant: having a probability (P) of less than 0.05.

Discussion. Preparations which contain both masticatory and gustatory stimuli are most effective for eliciting secretion of saliva. However, if the gustatory stimulus is sugar, the effect is transitory; diminishing as concentration of available gustatory agent decreases. On the other hand, if total stimulus is renewed so that an optimal level of the gustatory agent (sugar) is present, the initial degree of maximal stimulation can be maintained.

Samples of parotid saliva collected from the same subjects in a fasting and postprandial state (A.M.) showed no significant differences in secretion rate, pH, or electrolyte composition. It was disclosed that the composition of parotid saliva can be markedly different in secretion from the 2 glands of the same person as well as between individuals.

TABLE IV. Intercorrelation between Parotid Saliva Components.

Component	Phi correlation coef.
Bicarbonate *vs*	
Na	+.44
Ca	+.48
Chloride *vs*	
Na	+.41
K	–.29
Sodium *vs* K	–.37

Marked changes in salivary composition were noted after injection of methacholine. This demonstrates the necessity that type of stimulant be one which elicits normal responses without introducing artifacts which might occur when stimulatory agent also produces vasodilatation or intense stimulation of post-ganglionic nerve fibers.

Statistical analysis of flow rates of 4 samples of parotid saliva collected from 50 normal subjects for a 2-day period showed that, except for initial sample, the parotid gland secretory rate was quite consistent when stimulus of constant intensity was employed. The lower rate of secretion in the initial sample apparently was due to apprehension by test subjects who were not familiar with the collection procedure. This variation was not encountered when subjects were given a pretesting "acquaintance session"(8).

Among constituents and properties of saliva reputed to be affected by rate of secretion are sodium, bicarbonate, chloride, and the pH (1,2). Our results confirmed these observations for sodium, bicarbonate, and pH. In addition, a positive correlation was found between flow rate and calcium titer, while the potassium titer was inversely related to flow rate. Only chloride and phosphorus levels showed no significant correlation with secretory rate. Intercorrelations between certain parotid saliva components were also observed.

It can be postulated from our findings that these relationships are, in part, due to carbonic anhydrase in the parotid glands(9,10). Under the influence of this enzyme the carbon dioxide and water, produced by metabolic processes of cells, would be converted into

carbonic acid with subsequent ionization into a hydrogen ion and a bicarbonate ion. The assumption that hydrogen ions so formed are transferred to blood or extravascular fluid while sodium ions from blood are transferred to cells, to become associated with bicarbonate ions and be secreted in the saliva, would account for the rise in pH, sodium, and bicarbonate levels produced by increasing secretion rates.

Summary. Stimulated parotid saliva was collected from 50 individuals using flavored chicle as the stimulatory agent. Analysis of the flow rate and composition indicated a significant positive correlation between the flow rate and the pH, sodium, calcium, and bicarbonate contents. Intercorrelations between the various salivary constituents were also noted. No differences were found in the composition of saliva samples collected in the fasting and postprandial state. The composition of the parotid secretion was found to vary not only from person to person but between the two glands in the same person.

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Effect of Glucagon on Growth of Chick Embryo. (23799)

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It has been reported that glucagon causes a significant increase in the weight of Leghorn chick embryos and this finding has been used as evidence that glucagon is a growth-stimulating factor(1). In these experiments a single dose of glucagon (5 or 25 μg) was injected "in" the chorioallantoic membrane on the ninth day of incubation, and the embryos weighed 4, 7 or 9 days later. Since relatively crude preparations of glucagon* were used in these studies, it was felt that the experiments should be repeated using highly purified, crystalline glucagon.

Methods. Five experiments were done using a total of 113 control and 111 experimental White Leghorn x Delaware (Hyline strain) chick embryos, incubated at 37°C in a forced-draft incubator. The doses of crystalline glucagon† per embryo ranged from 5 to 80 μg administered in 1 to 5 injections over the course of 1 to 9 days. The injections were done according to the following technics: After puncturing the air space with a sharp needle, a hole was carefully drilled through the shell over the embryo. The shell membrane was moistened with sterile saline and carefully torn with a needle. After ascertaining that the chorioallantoic membrane had dropped, the tip of a hypodermic needle was inserted into the opening and the appropriate volume of solution delivered. The openings in the shell were sealed with paraffin, after

* Lot No. 208-108B-234: contains 20% of the activity of crystalline glucagon.

† Prepared by Dr. A. Staub according to the method of Staub *et al.*(3).