Parotid Fluid Calcium and Phosphate Levels in Patients with Hypercalcemia.* (30609)

R. M. FREEMAN† AND L. G. WELT‡
Department of Medicine, University of North Carolina School of Medicine, Chapel Hill

Recently parotid fluid phosphate levels were measured in patients with hypercalcemia as a possible diagnostic tool in differentiating primary hyperparathyroidism from hypercalcemia of other causes. No differences were found. The salivary phosphate levels of patients with hypercalcemia, however, were significantly higher than those found in controls. Hypercalcemic patients also had higher levels of calcium in the parotid fluid than controls. These data are presented in this paper.

Methods. Parotid gland fluid samples were collected by means of a modified Carlson-Crittenden cup(1). All samples were collected between 6:30 a.m. and 9:00 a.m. in the fasting state. Fluid at 2 different salivary flow rates was obtained. The first sample was stimulated by chewing a small bolus of paraffin wax wrapped in a piece of parafilm. The second sample was stimulated by chewing flavored chicle, which was changed every 5 minutes. Both samples were timed and collected in graduated centrifuge tubes. The paraffin-stimulated sample was collected over a 20-minute interval, while the chicle-stimulated sample required 10 minutes. Rarely longer collection periods were necessary to obtain sufficient fluid for analysis if the flow rate was inordinately slow. Controls were healthy laboratory personnel (20-35 years) with normal serum electrolytes. In some of the normals, only paraffin-stimulated samples were obtained.

In addition, the parotid fluid calcium and phosphate levels of 3 individuals were measured before and after administration of parathyroid extract.

Calcium was measured by the Zeiss spectrophotometer(2). The observed calcium concentrations were found to be constant with variations in the concentration of sodium from 2 to 60 mEq/l.

Phosphate was measured on the autoanalyzer by the Fiske-Subbarow method(3). Proteins were measured by the Biuret method, and sodium and potassium by the Baird flame photometer.

Because of the known influence of flow rate on the salivary levels of calcium and phosphorus(4,5), results were analyzed in 3 separate categories: A) flow rate less than 0.25 ml/min, B) flow rate between 0.25-0.50 ml/min, and C) flow rate between 0.5-1.0 ml/min. When multiple salivary samples were obtained from any individual, the mean values were used for calculations (Table I).

Results. Table I lists the diagnoses and serum values of the 12 patients with hypercalcemia. Eight of these patients were also hypophosphatemic at time of examination (serum phosphate, less than 2.6 mg%).

Table II shows the mean salivary phosphate levels at the 3 different flow rates. The mean salivary phosphate decreased with increasing flow rates in both normal and hypercalcemic patients. However, the mean parotid fluid phosphate values of the hypercalcemic patients were greater than normal at all rates of flow. No differences were found in the salivary protein, sodium, potassium or chloride levels between these two groups. There was no correlation between degree of hypercalcemia and level of salivary phosphate in these patients.

Table III demonstrates the increased salivary calcium levels found in hypercalcemic patients at all flow rates when compared to normal.

Table IV shows the apparent lack of in-

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† Work done during tenure of postdoctoral research fellowship from The National Foundation. Current address: U. S. Army Surgical Research Unit, Brooks Army Med. Center, Fort Sam Houston, Texas.

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fluence of parathyroid extract on parotid fluid calcium and phosphate levels. After 3 days' administration of parathyroid extract, hypercalcemia was striking in all 3, and the serum phosphate level had decreased in 2.

Discussion. Relatively few studies on the salivary electrolyte composition in disease states have been performed, and to our knowledge the increased salivary phosphate level in hypercalcemic states has not been previously documented. Frawley and Thorn (6) mentioned increased salivary calcium levels in primary hyperparathyroidism, but no figures are given. It has been previously demonstrated in dogs that administration of parathyroid extract and vitamin D both increased the parotid saliva calcium levels(7). Measurement of salivary calcium was sug-

TABLE I. Diagnoses and Serum Values in Hypercalcemic Patients.*

	Age	Diagnosis	Calcium	Phosphorus	Total protein	Albumin
			mg %		g %	
R.T.	26	Primary hyperparathyroidism, surgically proved	13.9	2.4	7.2	4.8
L.R.	49	*Idem*	13.4	2.2	6.9	4.3
I.J.	41	Primary hyperparathyroidism, probable, not surgically proved	13.0	2.3	8.5	4.9
B.C.	40	*Idem*	13.1	2.2	8.2	4.3
L.W.	53	Multiple myeloma	16.6	3.2	12.4	2.7
S.F.	53	*Idem*	15.2	2.8	6.9	4.6
A.G.	55	"	16.6	3.9	13.5	1.8
H.C.	19	Sarcoidosis	11.8	4.6	9.8	3.9
L.J.	58	"	13.8	4.9	7.2	3.2
P.R.	47	Carcinoma of esophagus	13.0	2.3	7.8	3.4
J.W.	62	Metastatic carcinoma of ileum; diagnosed by supraclavicular lymph node biopsy; origin unknown	14.8	2.3	7.1	4.4
J.J.	18	Pulmonary tuberculosis; adrenal insufficiency	14.8	3.3	10.4	3.4

* All values in mg/100 cm^3.

TABLE II. Parotid Fluid Phosphate Levels in Hypercalcemia.*

	Hypercalcemics	Controls	"t" value	P value
Flow rate less than .25 ml/min				
Mean flow rate (ml/min)	.17	.17		
Mean salivary phosphate (mg/100 ml)	19.8 (8)	15.9 (9)	2.48	$.02 < P < .05$
Flow rate .25-.50 ml/min				
Mean flow rate (ml/min)	.36	.42		
Mean salivary phosphate (mg/100 ml)	16.8 (10)	13.4 (11)	2.86	$.001 < P < .01$
Flow rate .50-1.00 ml/min				
Mean flow rate (ml/min)	.65	.67		
Mean salivary phosphate (mg/100 ml)	13.6 (6)	11.3 (10)	2.30	$.02 < P < .05$

* No. in parentheses is No. of individuals.

TABLE III. Parotid Fluid Calcium Levels in Patients with Hypercalcemia.

	Hypercalcemics	Controls	"t" value	P value
Flow rate less than .25 ml/min				
Mean salivary calcium (mg/100 ml)	4.36 (8)	2.88 (9)	4.24	$< .001$
Flow rate .25-.50 ml/min				
Mean salivary calcium (mg/100 ml)	4.50 (10)	2.82 (11)	3.51	$.001 < P < .01$
Flow rate .50-1.00 ml/min				
Mean salivary calcium (mg/100 ml)	4.92 (6)	3.24 (11)	3.72	$.001 < P < .01$

TABLE IV. Effect of Parathyroid Extract (PTE) on Parotid Fluid Electrolytes.*

	Parotid fluid values						Serum values	
	Paraffin stimulated			Chicle stimulated				
	F.R.	Calcium mg/100 ml	Phosphate mg/100 ml	F.R.	Calcium mg/100 ml	Phosphate mg/100 ml	Calcium mg/100 ml	Phosphate mg/100 ml
J.T. (vit D resistant rickets)								
Before PTE	.25	2.88	11.6	.55	3.84	11.9	9.4	3.5
After PTE	.23	2.70	11.1	.47	3.28	10.5	13.4	2.8
H.B. (vit D resistant rickets)								
Before PTE	.40	2.20	9.0	.83	2.70	8.6	10.8	2.6
After PTE	.35	2.36	9.6	.92	2.72	8.4	13.6	2.7
J.H. (healthy control)								
Before PTE				.66	3.56	9.5	10.4	2.8
After PTE				.75	3.50	10.7	12.1	2.3

* Parotid fluid was obtained before and 3 days following parathyroid extract (Eli Lilly)—200 units every 6 hr intramuscularly. F.R. = Flow rate in ml/min. Both calcium and phosphate expressed in mg %.

gested as a method for testing potency of parathyroid extract in these animals. Increased parotid fluid calcium levels have also recently been documented in patients with cystic fibrosis of the pancreas(8).

The interdependence of salivary electrolyte composition with salivary flow rate has now been well documented(4,5). Salivary phosphate levels are extremely high at flow rates of less than 0.1 ml/min, decrease with increasing flow rates, and then tend to stabilize at rates above 0.5 ml/min. Conversely, parotid fluid calcium levels increase with increasing flow rates. It must be emphasized that not only are timed collections essential, but that constancy of stimulation is necessary in order to obtain interpretable results.

The reason for the increased phosphate levels in hypercalcemic states is not apparent from our data. Parathyroid hormone does not appear to be responsible for this finding for the following reasons: a) The parotid fluid phosphate levels were no higher in those 2 patients with documented primary hyperparathyroidism than in patients with hypercalcemia of other causes; b) No changes in salivary phosphate were noted in 3 patients who were given parathyroid extract over a 3-day period. The influence of dietary factors on the electrolyte composition of saliva is unknown. Since there was no attempt to keep either patients or controls on a constant diet, this may be a pertinent factor. However, preliminary studies in patients with kidney stones have not suggested that changes in dietary calcium and phosphate intake over short periods (3 to 5 days) significantly influence the parotid fluid levels of calcium and phosphate. Earlier suggestions that carbohydrate ingestion lowers salivary phosphate (9) have not been confirmed under more standardized conditions(10).

The increased salivary calcium levels of patients with hypercalcemia are no better understood. Although the 2 patients with primary hyperparathyroidism had the highest salivary calcium levels observed, this may have been a fortuitous observation.

Summary. Parotid saliva was collected from normocalcemic and hypercalcemic subjects, and it was found that the latter had higher parotid fluid calcium and phosphorus levels than the controls. The observations did not permit discrimination as to the etiology of the hypercalcemia. The reasons for the elevated levels of phosphorus have not been established.

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An Effect of Growth Hormone on Hexose Phosphate Metabolism.* (30610)

RUDOLF WEIL, P. P. HO AND L. E. SCHAEFER (Introduced by A. B. Gutman)
Nutrition Laboratory, Mount Sinai Hospital, New York City

In the course of studies on the effect of pituitary growth hormone (GH) on the intermediary metabolism of carbohydrate(1,2,3), its influence on the concentration of hexose-6-phosphate in muscle was examined. An increase in hexose-6-phosphate has been shown to occur in muscle after administration of epinephrine(4,5,6) and cortisol(7), and also in starvation and in diabetes(4,8,9). Recently, an accumulation of hexose-6-phosphate compounds has been found in the heart muscle of hypophysectomized diabetic rats treated with bovine growth hormone (BGH) and cortisol, while BGH alone did not induce any significant changes in these metabolites(7).

Levels of glucose-6- and fructose-6-phosphate were determined in the skeletal muscle of 4 groups of rats. These groups included normal animals, normal animals receiving GH, hypophysectomized rats, and hypophysectomized rats receiving GH. The rats were given an intraperitoneal glucose load, in the fasting state, to provide an adequate amount of carbohydrate. This procedure appears indispensable for detection of an increase in hexose-6-phosphate. Since preliminary experiments in the laboratory(10) have shown that the highest concentration of hexose-6-phosphates occurred one hour after the glucose injection, the 1-hour level was used.

Materials and methods. Normal female rats weighing 200 g, and hypophysectomized female rats weighing 150 g, were fasted for 18 hours and injected with 0.5 g of glucose per 100 g of body weight, in the form of 10% glucose in water. One hour later the rats were killed by a blow on the head, and 1 g of leg muscle tissue was rapidly removed and frozen in a Wollenberger clamp cooled in liquid nitrogen(11). The muscle was pulverized in a chilled mortar and extracted with perchloric acid, after which the supernatant solution was neutralized to pH 7.4(4). Glucose-6-phosphate was determined by adding glucose-6-phosphate dehydrogenase to the assay mixture(12) and by measuring the change in optical density at 340 mμ. Fructose-6-phosphate was assayed by estimating the increase in absorbance after addition of phosphoglucose-isomerase(12). Other groups of normal and hypophysectomized rats were injected intramuscularly with GH† (1 mg/100 g) for 4 days (the last injection 6 hours before death) after which glucose-6-phosphate and fructose-6-phosphate were assayed in the same manner.

Results and discussion. The results of the tests show a significant elevation of the glucose-6-phosphate level, one hour after glucose injection, in the skeletal muscle of normal rats after GH treatment, when compared with the glucose-6-phosphate content of the muscle of non-treated controls. The differences in the concentration of glucose-6-phosphate were consistent and striking, and the

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