2. Hober, R., *Physical Chemistry of Cells and Tissues,* 1945, Blakiston, Phila., Pa.

3. Mraz, F. R., LeNoir, M., Pinajian, J., and Patrick, H., *Arch. Biochem. and Biophys.,* 1957, v66, 177.

4. Mraz, F. R., and Patrick, H., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 409.

5. Miller, H. G., *J. Biol. Chem.,* 1923, v55, 61.

6. ———, *ibid.,* 1926, v70, 587.

7. Mraz, F. R., and Patrick, H., *J. Nutrition,* 1957, v61, 535.

Received September 3, 1957. P.S.E.B.M., 1957, v96.

Concurrent Concentrations of Human Salivary Buffer Components in Serum and Saliva.* (23519)

SAMUEL DREIZEN, HENRY A. SPIES, JR., JO G. DREIZEN, AND TOM D. SPIES
Department of Nutrition and Metabolism, Northwestern University

The buffer systems in human saliva constitute a major natural defense mechanism against dental decay. Quantitatively, the buffer concentration of saliva varies with glandular activity, stimulated saliva having an appreciably higher buffer level than unstimulated saliva(1).

Numerous investigators have shown that the salivary buffer capacity of caries-resistant persons exceeds that of caries-active individuals(2,3,4,5). In a given subject under physiologic conditions, the buffer concentration of saliva varies only slightly from hour to hour and from time to time. It has been stated that the buffering power of saliva is directly dependent upon that of blood(6). Experiments in this laboratory have shown that metabolic acidosis depresses the salivary buffer capacity and that a similar reaction follows the administration of 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide (Diamox) a carbonic anhydrase inhibitor(7).

Materials and methods. Specimens of venous blood and paraffin stimulated saliva were obtained simultaneously from 34 patients of the Nutrition Clinic, Hillman Hospital. The samples were collected in sterile tubes under paraffin oil in the morning before breakfast and before brushing the teeth. The serum was drawn off after centrifugation and saved for analysis under paraffin oil. Neither the paraffin blocks which served as secretory stimulants nor the paraffin oil contained detectable amounts of any of the constituents tested. The buffer capacities of samples of serum and saliva through the pH range 7 to 6 were determined by the method of Dreizen, Mann, Cline and Spies(8) using 0.1 N lactic acid for the development of the titration curves. The sodium and potassium content of each sample was assayed with a Perkin-Elmer Model 52-A flame photometer by the procedure of Bills, McDonald, Niedermeier and Schwartz applying the internal standard technic(9). The serum and saliva concentrations of bicarbonate, phosphate and protein were measured by the methods of Van Slyke and Neill(10), Fiske and SubbaRow(11) and Wolfson, Cohn, Calvary and Ichiba(12) respectively. The values for each constituent were converted into milliequivalents per liter using the factors described in a previous publication(1). The data were then subjected to statistical analysis.

Results. The ranges and mean concentrations of the anionic and cationic constituents of the buffer systems measured in the serum and saliva of the 34 patients are shown in Table I. The ranges in saliva exceeded those in serum for each ion except protein. Sodium had the widest range in saliva and bicarbonate in serum. The mean levels in milliequivalents/liter were: Na^+, 32.80 in saliva and 145.72 in serum; K^+, 20.40 in saliva and 5.10 in serum; HCO_3^-, 15.60 in saliva and 27.04 in

* Northwestern University studies in nutrition at Jefferson-Hillman Hospital, Birmingham, Ala. These studies were supported by grants from the Avondale Educational and Charitable Fn., Metropolitan Fn. of Atlanta, and Republic Steel Corp.

TABLE I. Comparison of Buffer Components of Serum and Saliva in 34 Patients.

Components	Mean		σ*		r†	σ_r‡
	Saliva	Serum	Saliva	Serum		
Sodium (meq/l)	32.80	145.72	17.44	3.03	.255	.160
Potassium "	20.40	5.10	4.04	.59	.112	.157
Bicarbonate "	15.60	27.04	2.13	1.95	.035	.171
Phosphate "	10.17	2.73	2.97	.71	−.019	.171
Protein "	.94	16.19	.33	.70	−.208	.164
Buffer capacity (ml 0.1 N lactic acid)	.68	1.14	.17	.16	.392	.145

* σ = Stand. dev. † r = Coef. of correlation. ‡ σ_r = Stand. error of coef. of correlation.

serum; $HPO_4^=$, 10.17 in saliva and 2.73 in serum; and protein$^-$, 0.94 in saliva and 16.19 in serum. The mean buffer capacities through pH range 7 to 6 were 0.68 and 1.14 ml 0.1 N lactic acid for saliva and serum respectively. In each of the 34 patients the saliva potassium and phosphate concentrations exceeded the serum levels whereas the serum sodium, bicarbonate and protein values surpassed those of saliva. The buffer capacity of serum through pH range 7 to 6 was greater than that of stimulated saliva in the same pH range in every patient. The specific values are shown in the scattergram contained in Fig. 1.

The coefficients of correlation (r) developed from a comparison of the concentrations of each of the five buffer components in serum and saliva were: 0.255 for Na^+, 0.112 for K^+, 0.035 for HCO_3^-, −0.019 for $HPO_4^=$, and −0.208 for protein$^-$. The correlation coefficient for the buffer capacity of serum and saliva in the pH range 7 to 6 was 0.392. In no instance did the coefficients of correlation surpass the standard error of the coefficient of correlation (σ_r) by the more than 3 times required for statistical significance.

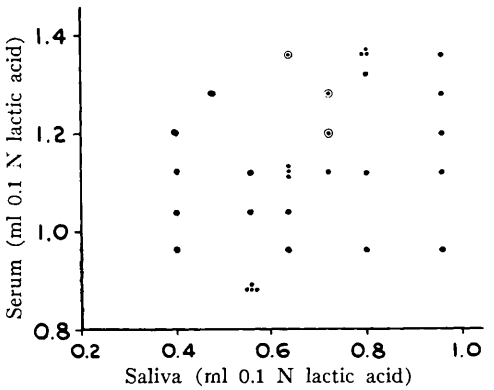

FIG. 1. The buffer capacities of serum and saliva in 34 patients (pH range 7.0-6.0).

Discussion. The most important buffer systems in blood and saliva are salts of weak acids and strong bases. In each, the main constituents are sodium, potassium, bicarbonate, phosphate and protein. At the normal pH of blood, the greatest proportion of buffer action in serum is contributed by bicarbonate with protein next in importance (13); at the pH of stimulated saliva, bicarbonate is the principal buffer with minor contributions from the phosphates(14,15). In each of the 34 patients the concentration of bicarbonate in serum exceeded that of stimulated saliva when the samples were collected concurrently. The difference in the bicarbonate content was reflected in the uniformly greater buffer capacity of serum in contrast to saliva in the pH range 7 to 6. This range includes the pK_1 of bicarbonate, the level at which the buffer action of this system operates at maximum efficiency.

The lack of statistically significant correlation between the buffer capacities of stimulated saliva and serum indicates that the composition and level of salivary buffers is dependent upon the biochemical activity and selectivity of the salivary glands. By such selective action on blood electrolytes, the salivary glands produce a secretion which is hypotonic to serum, a property unique among the major glandular secretions of the alimentary tract. The recent demonstration that oral microbial flora and saliva sediment contain buffer substances(16) indicates that both glandular and extraglandular sources contribute to the total buffering capacity of saliva.

Summary. The present study is concerned with a comparison of the buffer components and buffer capacities (pH range 7 to 6) of

paraffin stimulated whole saliva and blood serum in 34 patients from whom samples were collected simultaneously. 1) In every patient, the buffer capacity of serum exceeded that of saliva in the pH range tested; in each, saliva contained higher concentrations of potassium and phosphate than serum, and serum contained greater concentrations of sodium, bicarbonate and protein than saliva. 2) There was no statistically significant correlation between the buffer capacities and the main buffer constituents of serum and stimulated whole saliva. 3) Thus, the quantitative composition of the salivary buffers which act to protect against dental caries is determined primarily by glandular activity and not by simple ultrafiltration.

1. Dreizen, S., Reed, A. I., Niedermeier, W., and Spies, T. D., *J. D. Res.*, 1953, v32, 497.

2. Mann, A. W., Dreizen, S., Spies, T. D., and Hunt, F. M., *J. Am. Dent. Assn.*, 1947, v34, 244.

3. Ericsson, Y., *Acta Odont. Scandinav.*, 1949, v8, Suppl. 3, 1.

4. Turner, N. C., and Anders, J. T., *J. D. Res.*, 1956, v35, 385.

5. Muracciole, J. C., *idem.*, 1955, v34, 387.

6. Hafer, H., *Zahnartz, Praxis*, 1956, v7, 3.

7. Niedermeier, W., Stone, R. E., Dreizen, S., and Spies, T. D., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 273.

8. Dreizen, S., Mann, A. W., Cline, J. K., and Spies, T. D., *J. D. Res.*, 1946, v25, 213.

9. Bills, C. E., McDonald, F. G., Niedermeier, W., and Schwartz, M. C., *Anal. Chem.*, 1949, v21, 1076.

10. Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, v61, 523.

11. Fiske, C. M., and SubbaRow, Y., *ibid.*, 1925, v66, 375.

12. Wolfson, W. Q., Cohn, C., Calvary, E., and Ichiba, F., *J. Clin. Path.*, 1948, v18, 723.

13. Viikari, S. J., Harjola, P., and Maamies, T., *Scandinav. J. Clin. and Lab. Investigation*, 1954, v6, 122.

14. Wah Leung, S., *J. D. Res.*, 1951, v30, 403.

15. Sellman, S., *Acta Odont. Scandinav.*, 1949, v8, 244.

16. Lilienthal, B., *Oral Surg., Oral Med., Oral Path.*, 1955, v8, 828.

Received September 27, 1957. P.S.E.B.M., 1957, v96.

Role of Enzymatic Adaptation in Production of Experimental Alkaptonuria.* (23520)

E. C. C. LIN AND W. E. KNOX (Introduced by Shields Warren)

Cancer Research Institute, New England Deaconess Hospital, and Department of Biological Chemistry, Harvard Medical School, Boston

Papageorge and Lewis reported that rats maintained on high phenylalanine diets began, after a lag period of as long as 3 weeks, to excrete homogentisate in their urine(1). After this demonstration it was no longer possible to consider, as Dakin had, that homogentisate was an abnormal metabolite formed only by individuals with the rare hereditary disease of alkaptonuria. It has since been well established that homogentisate is normally formed in mammalian liver in the course of the metabolism of tyrosine and phenylalanine (2). Biochemical genetics has suggested a mechanism for hereditary alkaptonuria: the inactivity of the genetically controlled enzyme that degrades homogentisate. No mechanism has been suggested for the dietary alkaptonuria, but the lag period before its appearance suggests that some metabolic adaptation (11) must occur. In the present studies, the activities of the several enzymes acting successively to form and degrade homogentisate have been measured during the production of experimental alkaptonuria in rats. It was expected that adaptive changes in the amount of one enzyme relative to another would occur which would account for the accumulation of homogentisate. This expectation was con-

* This investigation was supported by grant from the Natl. Inst. of Arthritis and Metabolic Diseases, U. S. P. H. S., and by U. S. Atomic Energy Com. contract with the New England Deaconess Hospital.