

plasma albumin is lost from the animal to deplete plasma albumin concentration, elevation of plasma cholesterol will occur whether the protein is lost via a heavy proteinuria, or whether it is withdrawn directly from the blood stream in the presence of substantially normal kidneys. The corollary to this experiment has already been performed in the rat by Rosenman *et al.*(1) when they demonstrated that plasma cholesterol values fell toward normal following the infusion of bovine albumin into nephrotic animals.

Summary. Chronic plasmapheresis in the dog resulted in a fall in plasma albumin and

an elevation of plasma cholesterol concentration.

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Boundary Electrophoresis of Human Parotid Saliva. (23123)

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The ultraviolet spectrum of human parotid saliva has been shown to possess bands characteristic for protein(1). Recently, Pigman *et al.*(2) stated that parotid saliva contained 8 electrophoretic components with a major peak of low mobility at pH 8.6. The present study presents electrophoretic patterns of 6 specimens of parotid saliva from 5 individuals.

Methods. Six samples of parotid saliva of 60 ml each were collected from 5 individuals by means of a modified* Lashley cup (3). They were concentrated by ultrafiltration(4) at 4°C, dialyzed against 0.1 ionic strength veronal at pH 8.6, filtered, made up to 11 ml, and subjected to boundary electrophoresis in the standard cell of the Aminco Model B Electrophoresis Apparatus. Duplicate specimens of 60 ml of parotid saliva were concentrated(4), made up to 20 ml and samples were taken before and after ultrafiltration for protein analysis by the biuret reaction(5). Mobilities were determined according to standard procedures(6) and the relative concentrations were calculated from

the Rayleigh fringes(7) shown in Fig. 1 with the customary assumption that the refractive index increment of all the components was the same. The minor components II and IV (Fig. 1) were included with component III in the calculation of the relative concentrations.

Results. As shown in Table I, the extent of concentration of protein after ultrafiltration (5.4 fold) closely paralleled concentration in volume (5.5 fold, or 60 ml to 11 ml), indicating that essentially no protein was lost by ultrafiltration. Before ultrafiltration the parotid salivas contained 18.7-58.9 mg %

TABLE I. Protein Content of Parotid Saliva before and after Ultrafiltration (mg %).

Subject	Before	After	Degree of conc.	
			Volume	Protein
A	41.0	243.5	5.5	5.9
B	52.0	269.2	"	5.2
C	18.7	98.3	"	5.3
D	51.5	259.4	"	5.0
E	58.9	323.8	"	5.5
		Mean	5.5	5.4

* Prepared in Instrument Shop, N.I.H.

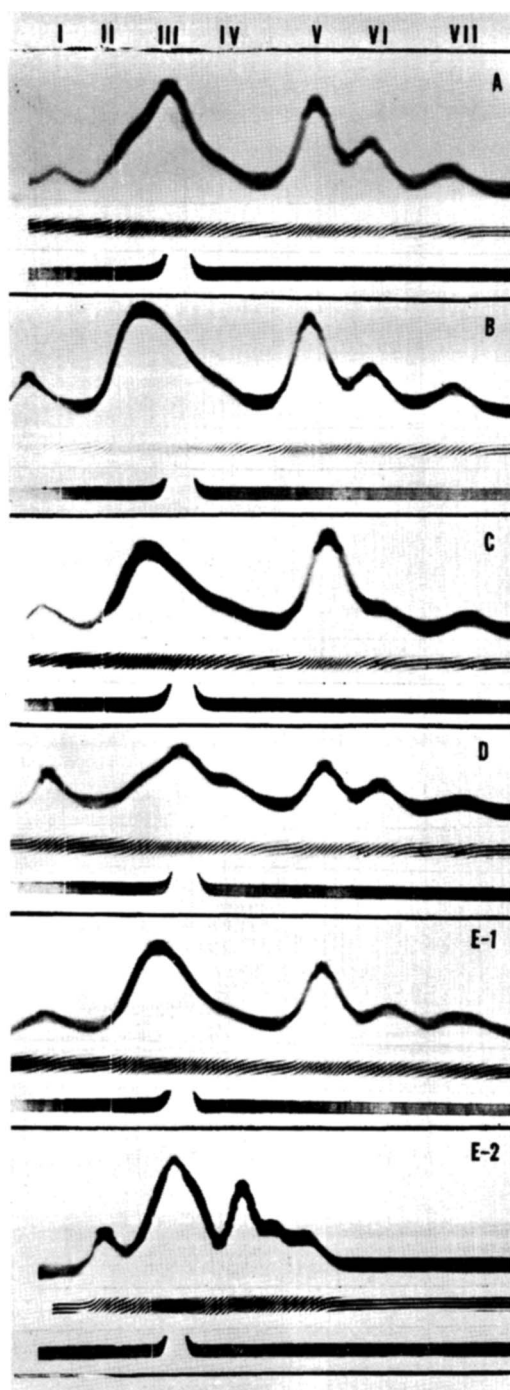


FIG. 1. Electrophoretic patterns of 6 samples of concentrated ($\times 5.4$) human parotid saliva from 5 individuals. Time of run 7200 sec. for samples A to E-1; for samples E-2, 3600 sec. Potential quotient 6.25 volts/cm. Starting boundary and Rayleigh fringes are included with each schlieren electrophoretic pattern.

protein.[†] The concentrated parotid saliva submitted for electrophoresis contained 98.3 – 323.8 mg % protein, and both positively and negatively charged components were observed.

As shown in Table II and Fig. 1, the mobilities and the relative concentration of the components were essentially similar for all individuals. To date, no electrophoretic patterns on parotid saliva have been presented in the literature although a preliminary report has appeared stating that 8 components have been observed with a major fraction showing a very low mobility at pH 8.6(2). Reports have also appeared on the electrophoresis of whole human saliva(9,10).

In the present study, 5 distinct peaks (I, III, V, VI, VII) were seen in every case, and 2 additional peaks (II, IV) appeared as “shoulders” in some individuals. Two peaks (III, V) accounted for 75% of the components with the major peak comprising 50% of the fractions and showing a mean mobility of $+0.3 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1} \text{ volt}^{-1}$. This finding confirms the report of a peak of similar mobility. The fastest moving component had a mean mobility of $-4.18 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1} \text{ volt}^{-1}$. Although identification of the various components awaits further investigation, none has the mobility of albumins and some may perhaps be globulins in view of their low mobility(4,6). Since no runs were made at pH values other than 8.6, the extent of the contribution of the salt anomaly to peaks II, III, and IV was not evaluated.

Summary. Six samples of parotid saliva from 5 individuals were subjected to boundary electrophoresis at pH 8.6 in 0.1 ionic strength veronal buffer. Seven peaks were obtained with 2 peaks accounting for 75% of the components. The major peak was essentially stationary with mean mobility of $+0.3 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1} \text{ volt}^{-1}$, and comprised 50% of the components. The fastest moving constituent had a mean mobility of -4.18×10^{-5}

[†] These findings do not agree with those of Bramkamp who reported concentrations varying from 10-90 mg % protein nitrogen(8) or approximately 60-560 mg % protein. Variance is probably due to differences in analytical procedures.

TABLE II. Fractions of Concentrated Parotid Saliva Separated by Boundary Electrophoresis.*

Component	A	B	C	D	E-1†	E-2†	Mean $\pm$ S.E.
Mobility $\times 10^{-5}$ cm ² sec. ⁻¹ volt ⁻¹							
I	+1.90	+2.24	+2.11	+2.28	+2.14	+2.15	+2.14 $\pm$.08
II	+ .85	+ .60	+ .86				+ .77 $\pm$.08
III	+ .17	+ .24	+ .57	+ .50	+ .39	+ .04	+ .32 $\pm$.08
IV	- .74	- .60	- .83	- .81	- .71	- .93	- .77 $\pm$.08
V	-2.11	-1.94	-2.22	-1.99	-2.22	-2.02	-2.08 $\pm$.05
VI	-3.02	-2.84	-3.07	-2.91	-3.31	-2.95	-3.02 $\pm$.07
VII	-4.25	-4.05	-4.20	-4.21	-4.49	-3.86	-4.18 $\pm$.09
Relative concentration (%)							
I	6.6	13.2	8.0	7.4	8.0	5.6	8.1 $\pm$.93
II, III, IV	50.8	52.8	50.0	53.3	52.0	51.4	51.7 $\pm$.51
V	21.3	20.8	32.0	20.5	22.7	23.6	23.5 $\pm$ 1.77
VI	16.4	7.5	6.0	14.7	8.0	9.7	10.4 $\pm$ 1.72
VII	4.9	5.7	4.0	4.1	9.3	9.7	6.3 $\pm$ 1.05

* Taken from descending boundary.

† Same individual run 3 wk apart.

cm² sec.⁻¹ volt⁻¹.

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Paper Chromatographic Method for Estimation of Phenylalanine.* (23124)

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In phenylketonuria serum phenylalanine levels are high(1). Removal of phenylalanine from the diet results in a marked decrease in blood phenylalanine to near normal levels(2,3). The methods for determination of phenylalanine are largely based on modifications of the Kapeller-Adler procedure(4) in which phenylalanine is oxidized to benzoic acid and subsequently nitrated to the 3,4-dinitro derivative(3,5). The enzymatic decarboxylation of phenylalanine to phenylethylamine and determination of the resulting base as described by Udenfriend and Cooper

has also served as a method for obtaining phenylalanine values in serum(6). When children are to be treated with a phenylalanine-low diet, there is need for a simple and rapid method for determination of serum phenylalanine. We have used a paper chromatographic method requiring a very small volume of blood in order to obtain a clear picture of the individual response to a low phenylalanine diet.

Methods. Phenylalanine in blood serum. A small sample of blood such as can be obtained from skin puncture is sufficient for the estimation of the phenylalanine content of serum. Protein is removed by adding 2 ml

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