Paper Electrophoresis of Rat Salivary Secretions.* (29100)

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Paper electrophoresis of salivary secretions appears to provide a useful tool in physiologic and pathologic investigation on small animals(1,2), as well as on man(3-5) where it has more frequently been employed. In early electrophoretic studies on small animals, *e.g.*, rat, secretory fluid was collected from the oral cavity rather than directly from salivary ducts; hence, the identity of the source of the collected fluid and the degree of contamination by oral debris were uncertain. Recent improvement(6) in technique for collection of salivary secretions directly from ducts, in small animals, has made it feasible to investigate, more precisely, the protein components of the salivary secretions. In this investigation, electrophoretic patterns of the secretions from individual parotid, submaxillary and sublingual glands of rat have been examined and conditions required for such analysis have been further defined. A highly inbred strain of rat, as well as another strain, randomly-bred, have been used to examine the role of genetic constancy as a condition for reproducible electrophoretic analyses. The results indicate that conditions for satisfactory electrophoretic analysis of rat salivary secretions are readily attained, especially in the case of parotid saliva.

Materials and methods. Four-month-old rats of the randomly-bred Long-Evans strain and the highly inbred (82 generations of brother $\times$ sister matings) Fischer/344 strain were maintained on standard laboratory diet and tap water, given *ad libitum*. The rats were fasted (water continued *ad lib*) for 24 hours prior to collection of saliva samples. Immediately prior to collection of samples, animals were anesthetized by injection (i.p.) of secobarbital sodium (5 mg/100 g body weight). Salivary ducts were dissected free and cut. Animals were then administered a single supermaximal dose (1 mg/100 g body weight) of pilocarpine HCl and salivas were simultaneously collected in separate tared vessels, from cut ends of right and left gland ducts(6) for a period of 5 minutes. Blood was obtained by jugular puncture and allowed to clot, whereupon serum was separated. Serum and saliva samples were stored at 4°C until analysis.

Determinations of total protein were made using Folin-Ciocalteu phenol reagent(7). Protein was expressed as grams of crystalline bovine albumin.

Submaxillary secretions were introduced into small cellophane casings and dialyzed against distilled water at 4°C for 24 hours. Sublingual saliva and dialysates of submaxillary saliva were concentrated under vacuum for about 48 hours at 4°C. Samples were then made to a concentration of 2 to 4$\times$ and 10 to 20$\times$ the original, in the case of sublingual and submaxillary salivas, respectively, by addition of distilled water.

Electrophoretic separation of saliva and serum proteins was made by the method of Block, Durrum and Zweig(8), employing a Spinco model R paper electrophoresis system with a series D Durrum-type cell, model RD-2 Duostat and model RB Analytrol. Veronal buffer, (pH 8.6 ionic strength 0.075) and Schleicher and Schuell 2043 A mgl paper were used. Samples of saliva or serum were applied to the paper strips and separation was made at 4°C for 16 hours at a constant current of 10 ma per cell. After drying (30 minutes at 120-130°C), the strips were stained with Amido Black 10B, as described by Sweeney *et al*(1). Parotid components were consecutively numbered starting with the most anodal component.

An analysis of variance was performed for comparison of values from right and left glands of individual rats as well as for comparison of values between rats of the same or different strains. A transformation factor ($\sqrt{}$ value + 0.5) was applied to the per-

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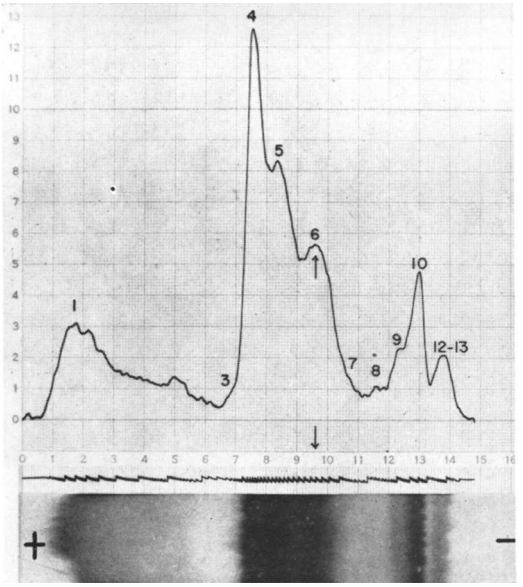

FIG. 1. Electrophoretic pattern and Analytrol tracing of parotid saliva. Components are numbered in the tracing.

centage values to give a more normal distribution.

Results. Electrophoresis of rat parotid saliva, without prior concentration or dialysis, yielded patterns showing 10-12 clearly discernible bands (Fig. 1). Dialysis of the parotid samples, before electrophoresis, further improved somewhat the separation of the components which migrated most rapidly toward the cathode, but was not generally necessary in routine separations. Concentrating the parotid saliva, with or without prior dialysis, did not qualitatively change the electrophoretic patterns, but did increase the density of each band. This increase in density detrimentally affected the clarity of the separation in most cases. Chemical estimation of the concentration of total protein of rat parotid saliva (Table I) yielded values which usually were at least half to three-

TABLE I. Total Protein of Rat Serum and Parotid Saliva.

Strain	Serum (g %)	Parotid saliva (g %)
Fischer	(3) 7.38 ±.07*	(9) 5.62 ±.13
Long-Evans	(3) 7.59 ±.07	(12) 4.10 ±.18

* Standard error. No. of rats shown in parentheses.

quarters those usually found with serum. Hence, it was generally unnecessary to pretreat the parotid saliva, either by dialysis or by concentrating, to obtain satisfactory electrophoretic separation of protein components.

Submaxillary and sublingual salivas, by contrast, contained little protein (about 10% of parotid value, by chemical determination) and gave barely discernible bands on electrophoresis, unless subjected to prior concentration. However, prior concentration alone, did not usually give satisfactory separation of bands on electrophoresis (Fig. 2). With sub-

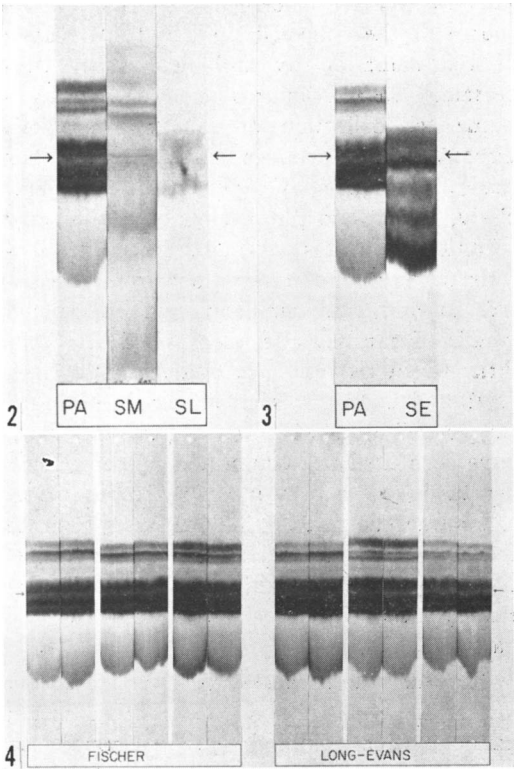

FIG. 2. Electrophoretic patterns of undialyzed parotid, submaxillary and sublingual saliva. Submaxillary saliva was concentrated 20× and sublingual 2.5×. Parotid saliva was not concentrated.

FIG. 3. Electrophoretic patterns of parotid saliva and serum. Each pattern contains equivalent amounts of protein.

FIG. 4. Electrophoretic patterns of parotid saliva from Fischer (inbred) and Long-Evans (randomly-bred) rats. Each pair of patterns represents secretions from a gland pair.

maxillary saliva, dialysis and concentration before electrophoresis did result in improved separations and increased the number of dis-

TABLE II. Paper Electrophoretic Components and Total Protein of Rat Parotid Saliva.

Strain	% total protein — Component — 1	3	4	5	6	7	8	9	10	12-13	Total protein (g %)
Fischer (9)	21.7 *	1.7	21.5	21.9	14.3	2.7	2.6	2.8	6.5	4.3	5.62
	7.66†	.17	10.29	11.18	3.75	1.09	.42	.70	1.67	10.77	.36
	4.05‡	.12	4.25	1.27	1.73	.17	.68	.24	.53	1.39	.21
	1.99§	1.44	2.47	8.77	2.05	6.89	.61	2.92	3.05	8.96	1.68
Long-Evans (12)	17.7	2.3	30.1	20.2	13.4	2.5	3.3	3.6	3.4	3.5	4.10
	20.78	.31	8.00	15.34	22.80	.36	3.59	2.29	1.60	15.91	1.34
	1.72	.14	3.95	3.23	2.86	.14	.97	.53	.77	.77	.19
	11.40	2.18	2.05	5.16	8.02	2.70	2.89	4.06	1.75	18.06	7.20

No. of rats shown in parentheses.

* Mean.

† Mean variance between rats computed without transformation factor (V_1).

‡ Mean variance within rats computed without transformation factor (V_2).

§ $\frac{V_1}{V_2}$ (F) when V_1 and V_2 computed with a transformation factor for percentage values.

For Fischer Strain, F (.05) = 3.23 and F (.01) = 5.47.

For Long-Evans Strain, F (.05) = 2.72 and F (.01) = 4.22.

cernible components from 8 to 11. Comparison of the mobility of components in parotid fluid and dialyzed, concentrated submaxillary saliva indicated that many of the components of parotid saliva possibly were present also in submaxillary saliva, but in greatly reduced concentration. With sublingual saliva, sufficient volume to test the effect of dialysis was difficult to obtain, and this fluid had the further disadvantage of extreme viscosity, making it difficult to apply samples evenly to the paper. Analyses which were obtained indicated that sublingual saliva was separable into only 2 poorly delineated components. It was apparent then, that parotid saliva was the salivary secretion of choice for electrophoretic analysis, and ensuing experiments were limited to that fluid.

Parotid salivas were obtained from inbred Fischer and randomly-bred Long-Evans male rats and were analyzed, chemically, for total protein and, electrophoretically, for protein components. Serum samples from each strain of rat were similarly analyzed for comparison with saliva. Parotid saliva of Fischer rats contained total protein to the extent, on the average of 76% of total protein of serum from the same strain; while in Long-Evans rats, total protein of parotid saliva, in relation to that of homologous serum, was a little lower (average, 54%). Data are shown in Table I. Untreated parotid saliva from each strain could be separated into at least 10 components (Fig. 1) and occasionally into as many as 12. Components were designated by consecutive numbering, with the most anodal component as 1 and the most cathodal component as 12-13 (Fig. 1).

Components 1 and 4 to 6 accounted for approximately 80% of the total protein of parotid saliva (Table II); all 4 of these components migrated to the anode, with the exception of a portion of component 6, which migrated to the cathode (Fig. 1). Component 2 was only occasionally demonstrable. Of the 4 most cathodal components (representing about 15% of the total protein), the last cathodal component (12-13) also could not be separated in all of the samples, and therefore it was referred to as a single band (Fig. 1). Dialysis of parotid saliva resulted in enhanced separation of component 12-13 into discrete bands, and also in appearance of band 11.

Components of parotid saliva and homologous serum were not easily matched in the electrophoretic patterns, even when volumes of saliva and serum samples were adjusted to contain similar amounts of protein (Fig. 3). None of the 5 components of serum had an exact counterpart, on the basis of mobility, to a component of parotid saliva, although there were components in parotid saliva which matched components of serum fairly

closely in mobility. It is possible that serum proteins are present in parotid saliva and that protein interaction(9) alters component-mobility in the secretion, but these data offer no evidence on this point. It seems clear, however, that none of the components of serum corresponded to any of the 4 most cathodal components of parotid saliva, since with serum, all components except a portion of the γ-globulin component migrated toward the anode.

Saliva samples collected simultaneously, but separately, from parotid glands of a single animal, showed little difference (measured as variance) in concentration of total protein, analyzed chemically, or individual protein components, analyzed electrophoretically, in Long-Evans and inbred Fischer strains (Table II; Fig. 4). Moreover, the mean variance between rats of the highly inbred Fischer strain was not significantly different in salivary concentration of total protein and most individual components, from the mean variance found between glands of a pair; only in the case of components 5, 7, 12-13, did the variance between rats exceed that within individuals. With randomly-bred Long-Evans rats, by contrast, there was, in general, significantly greater mean variance between parotid salivas of individual rats, with regard to concentration of total protein and individual components, than between parotid salivas from glands of a pair. Only components 3, 4, 7, and possibly 10 did not differ significantly between randomly bred Long-Evans rats (Table II). (A significant difference is found for component 10 if one aberrant value is ignored.) Finally, the mean variance between gland pairs of the Fischer strain was not significantly (.05) different from the mean variance between gland pairs of the Long-Evans strain, except possibly in the case of component 10.

Discussion. Parotid saliva appears to be the salivary secretion of choice for investigation of protein components of rat saliva by paper electrophoresis. This secretion is readily obtained, in adequate quantity for electrophoresis, directly from the duct of the gland, thus eliminating difficulties of identification of the secretion and contamination by oral debris. The protein content of rat parotid saliva is more than half that of serum, which eliminates need for concentrating the fluid before analysis. Dialysis is also unnecessary for satisfactory separation of the protein components. Parotid salivas from paired parotid glands of a single animal, with rare exception, provided similar electrophoretic patterns and concentrations of total protein, even with a non-inbred strain of rat (Long-Evans). Thus, in experiments which permit physiological alteration of only one gland, the contralateral gland serves as a satisfactory control, with inbred as well as in non-inbred animals. With an inbred strain, there was the further advantage of only slightly greater variation between patterns from parotid salivas of separate rats than between patterns from salivas of paired glands. The principal advantage of the inbred strain is thus to be found in experiments which do not permit selective alteration of only one gland of a pair.

The origin of the protein components, separated here from rat parotid saliva by paper electrophoresis, was not determined. The 4 most cathodal components from parotid saliva clearly were not present in serum in discernible quantities and very probably orginated in the gland cells. Thus, even if serum proteins are transferred to saliva, as has been implied(10), it appears that at least 15% of the protein in parotid saliva of rat is synthesized by gland cells.

Summary. Using paper electrophoresis, satisfactory separation of protein components in salivary secretions of the rat was most conveniently obtained with parotid saliva. This secretion was readily obtained in uncontaminated form from the cut surface of the exteriorized parotid duct and required neither concentration nor dialysis for satisfactory electrophoresis. Quantitative variation in total protein and electrophoretic patterns was small between secretions of the right and left glands of rats of an inbred strain and a randomly-bred strain. The variation between rats of an inbred strain, in contrast to a randomly-bred strain, was generally of the same order of magnitude as variation between right and left glands.

Paper electrophoresis of parotid saliva of the rat can provide patterns which can be of value in a semi-quantitative analysis of protein components.

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Site of Formation of the Group-Specific Component and Certain Other Serum Proteins.* (29101)

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Since the initial observations of Hirschfeld(1) on the group-specific component (Gc) considerable work has been performed to identify and characterize this a_2 globulin. The demonstrated polymorphism of this system has been shown to be under genetic control(2), and population studies have given additional information concerning the existence of uncommon genetic variants(3). Schultze(4) and Cleve and Bearn(5), have isolated and partially characterized, the group-specific components. Partial characterization of the two inherited components of this system was reported recently from this laboratory(6). However, there is still little information concerning the role of this protein in health or disease. In this study, an attempt has been made to determine the site of synthesis of the group-specific component. Using the techniques elaborated by Hochwald, Thorbecke, and Asofsky(7) various human tissues were cultured *in vitro* in the presence of radioactive amino acids. The incorporated label was studied in newly synthesized serum proteins by autoradiography following immunoelectrophoresis.

Materials and methods. Biopsy specimens of normal human liver, spleen, lymph node, stomach, jejunum, pancreas, skeletal muscle, were obtained at time of surgery and placed in the culture medium (T. C. No. 199 with added bicarbonate—Microbiological Associates). Fifty mg wet weight of each tissue was cut into small fragments under sterile conditions and placed in roller tubes containing 2 ml reinforced Eagle's culture medium deficient in either lysine or isoleucine(8). The pH of the medium had previously been adjusted following addition of 2-5 μc/ml of either C^{14} lysine or C^{14} isoleucine (Schwartz 1000 μc/mg) to the tube lacking that particular essential amino acid. The tubes were then placed either on a shaker platform or in a roller tube apparatus at 37°C for 24-48 hours, at which time the tubes were frozen quickly, thawed, and centrifuged at 4° to remove sediment. The supernatant was dialyzed against 0.9% NaCl at 4°C for 48 hours, using Visking tubing of 8/32 diameter

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