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Polyacrylamide Gel Electrophoresis of Twin and Nontwin Parotid Salivary Proteins[1] (39252)

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Early separations of parotid salivary proteins in starch–urea–formate gels at pH 3.7 led to the conclusion that the relative concentration of many proteins in parotid saliva is under strong genetic control (1). Subsequent studies demonstrated polymorphism in selected salivary proteins including amylase (2–4), basic proteins (5), and proline-rich proteins (6). We developed a technique for electrophoresis in polyacrylamide gel slabs that gives reproducible separation of salivary proteins from an individual (average 25 bands per subject from parotid saliva) and that demonstrates marked variability among individuals in salivary protein composition (7, 8). In this paper complete parotid salivary protein patterns obtained by our procedure are compared among monozygotic (MZ) twins, dizygotic (DZ) twins, nontwin sibs, and unrelated male and female subjects of the same age range as the twins.

Methods. Ten to fifteen milliliters of stimulated parotid saliva (sour candy) was collected using a Teflon suction cup device (9) from each subject in the following groups (first milliliter of each sample was rejected): (1) 12 pairs of MZ twins; (2) 19 pairs of same sex DZ twins; (3) 10 pairs of nontwin sibs; (4) 15 pairs of unrelated females; (5) 7 pairs of unrelated males; and (6) 15 pairs of unrelated males and females. All specimens were centrifuged for 10 min at approximately 1000*g* at 4°. The supernatants were dialyzed against distilled water at 4° for 48 hr and lyophilized. Most subjects were 18–24 years of age. Nontwin individuals were of the same age range as the twin pairs. Zygosity of the twins was determined by 20 different blood group tests.

Polyacrylamide gels 15-cm wide, 12-cm high, and 2-mm thick were formed at pH 9.0 between glass plates separated by appropriate gaskets; 7.5 cm of 10% gel was overlaid with 3.0 cm of 6% gel. After polymerization of the above gels, 25 sample slots, 3-mm wide, were formed in 8% gel immediately above the 6% gel by means of a Lucite well former. All gels were prepared from standard formulas based on the proposals of Davis (10). The lyophilized salivary samples were reconstituted to 10 mg/ml with 0.9% NaCl. Duplicate 30-μl samples of the solutions were transferred to sample wells. Gels were subjected to electrophoresis (electrophoresis vessel fabricated at the University of Minnesota) at 40 mA constant current, pH 9.0, until bromphenol blue marker dye reached the bottom of the gel (approximately 3.5 hr). The proteins in the gels were fixed by immersing the gels in 50% trichloroacetic acid for 30 min, and were stained for 30 min with 0.1% Coomassie brilliant blue in 50% trichloroacetic acid (the preparations were continually agitated during both fixing and staining). Excess stain was removed by slow shaking of the gels with several batches of 10% acetic acid. Band positions were recorded by photographing the gels under standardized conditions. The reproducibility of the above technique has been verified and discussed in previous publications (7, 8).

Comparison of banding patterns between subject pairs was made by first scoring the staining of each of the 36 different protein bands detected by the described technique as absent, light, medium, or heavy. A number was then assigned to each band as follows: absent, 0; light, 2; medium, 4; or heavy, 6. Numerical scores were then compared between subject pairs for each of the 36 bands. Any differences between the numerical scores were recorded. The total numerical difference between the 36 protein band scores was tabulated for each subject pair. The numerical differences between

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each band and the total numerical differences for the 36 bands were then subjected to statistical analyses. All band comparisons and scoring were performed by a single individual.

Results. Figure 1 illustrates that typical electrophoretic patterns obtained from separation of parotid salivary proteins of MZ twins (left panel) are both qualitatively and quantitatively similar, but that those from DZ twins (right panel) are distinctly different. Figure 2 records the usual variability of parotid proteins found among unrelated subjects.

Results from detailed analysis of the protein patterns are summarized in Table I. Percentage concordance (percentage of the bands present or absent at equivalent positions on the gels from both members of the subject pairs) showed only an occasional difference between MZ twins, more differences between DZ twins and between nontwin sibs, and even more differences between unrelated male or female subject pairs. No differences were evident between the parotid protein patterns of male and female subjects. The total numerical difference scores for the 36 protein bands provide both a qualitative and semiquantitative comparison among the experimental groups. This comparison also gave a clear separation among groups (MZ twins < DZ twins and nontwin sibs < unrelated subjects). Mean concordance and total numerical difference scores from 16 pairs of samples collected from the same subjects at differing times were 98.8% and 8.38, respectively. These values are not significantly dif-

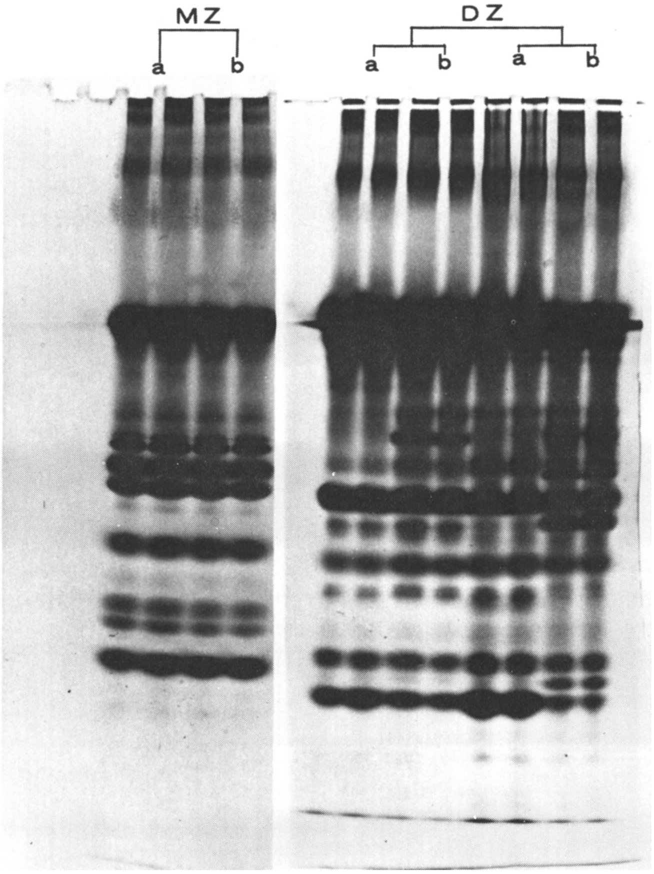

FIG. 1. Polyacrylamide gel slab patterns of parotid salivary proteins stained with Coomassie blue from twin subjects. Left panel, monozygotic twins; right panel, two pairs of dizygotic twins. Each sample was analyzed in duplicate on adjacent channels.

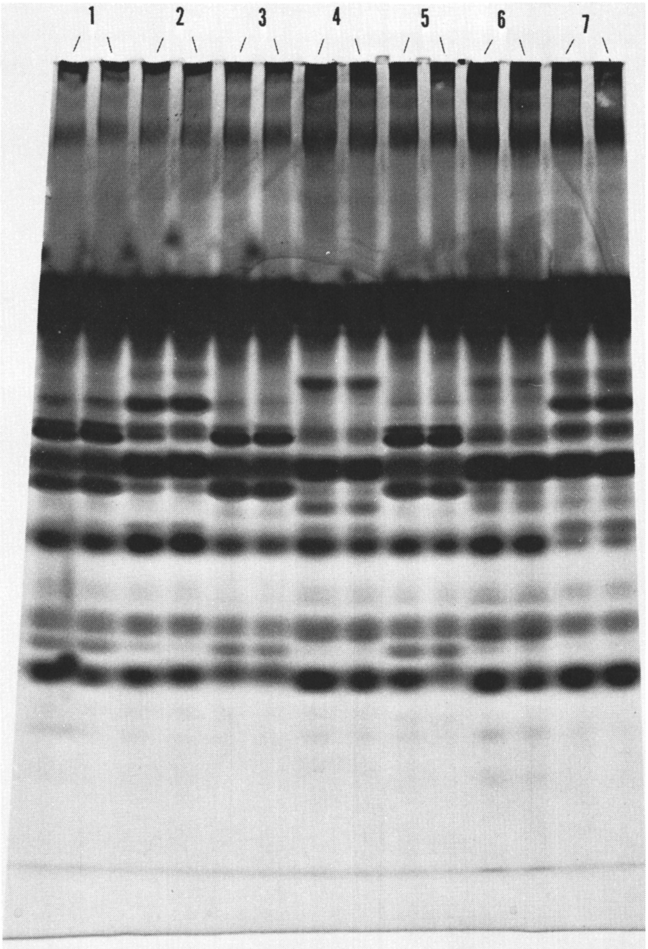

FIG. 2. Polyacrylamide gel slab patterns of parotid salivary proteins stained with Coomassie blue from seven unrelated subjects. Each sample was analyzed in duplicate on adjacent channels.

ferent from those of MZ twins. Variances of the protein patterns were calculated for the several experimental groups as the sum of the variances of each of the 36 bands. The variances separated the groups in the same order as obtained by the other calculations.

Discussion. Previous work from our laboratory with the same electrophoresis procedure as applied to the present study demonstrated marked differences among Caucasian subjects (7, 8). In the present study, environmental contributions to the protein differences were not evident since variability was similar between samples from the same subject and from MZ twins. Analyses (11) suggest that genetic factors contribute at least most of the previously detected variability. Evidence for the significance of genetic contributions to the protein differences among individuals is given by the equal variability between samples from DZ twins and from nontwin sibs. In addition, the differences between the latter two groups were significantly greater than those of MZ twins, but were significantly less than those of unrelated subjects.

A single procedure will not completely separate a mixture as complex as the salivary proteins. Thus, genetic or environmental influence on certain proteins may not be detected by the procedures of the present study. For example, salivary amylase occurs in three bands present in all subjects. Thus, polymorphisms of salivary amylase, such as those previously reported (2–4), were not evident. In addition, the experimental con-

TABLE I. PERCENTAGE CONCORDANCE, NUMERICAL DIFFERENCE SCORES, AND VARIABILITY AMONG PAROTID SALIVARY PATTERNS OF TWIN AND NONTWIN SUBJECTS.

Experimental group	Number of pairs	Concordance[a] (%)	Numerical difference score[a]	Variance[b]
MZ twins	12	98.6 ± 1.00 (88.8-100)	4.3 ± 2.01 (0-22)	6.8
DZ twins	19	93.8 ± 1.63 (77.7-100)[c]	18.3 ± 3.59 (0-54)[d]	30.0[d]
Nontwin sibs	10	93.0 ± 1.29 (88.8 - 100)[d]	21.4 ± 3.81 (6-40)[d]	33.8[d]
Female	15	80.3 ± 1.75 (72.2-88.8)[d,f]	41.7 ± 2.57 (24-58)[d,f]	71.1[d,e]
Male	7	83.3 ± 3.21 (69.4-97.2)[d,f]	41.1 ± 7.22 (6-62)[d,f]	75.9[d,e]
Female/male	15	80.3 ± 1.69 (72.2-91.7)[d,f]	42.0 ± 2.68 (26-58)[d,f]	70.2[d,e]

[a] Results expressed as mean ± SEM. Range of values given in parentheses. Significance of differences determined by Student's *t* test.

[b] Significance of differences determined by *F* test.

[c] Significantly different from MZ twins, $P < 0.05$.

[d] Significantly different from MZ twins, $P < 0.01$.

[e] Significantly different from DZ twins, $P < 0.05$.

[f] Significantly different from DZ twins, $P < 0.01$.

ditions of the present study do not permit entrance of proteins with an isoelectric pH greater than nine into the gel. The structural and functional characteristics of the proteins in most of the electrophoretic bands are unknown. Nevertheless, it is apparent from the results of this investigation that genetic factors are the major contributor to variability of parotid salivary proteins among subjects. It is likely that environmental factors would contribute more to differences of mixed salivary proteins among individuals where the fluid is exposed to highly variable conditions within the oral cavity.

The data summarized in this report gave clear separation among experimental groups. However, under the conditions used here, identification of subjects as MZ or DZ twins is not possible from the protein patterns. Less than 100% concordance was observed in 2 of 12 MZ twin pairs, and 100% concordance was noted in 6 of 19 DZ twins. Furthermore, the total numerical difference scores of MZ and DZ twins gave overlapping values.

Summary. Parotid salivary proteins from monozygotic twins, dizygotic twins, nontwin sibs, and unrelated male and female subjects of the same age range as the twins were separated by polyacrylamide gel slab electrophoresis at pH 9.0. Variability of the stained protein patterns increased in the order: monozygotic twins; dizygotic twins and nontwin sibs; unrelated subjects. It is concluded that genetic factors are the major contributors to variability of parotid salivary proteins among subjects.

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