*Am J Hum Genet* 27:292–303, 1975

# Genetic Studies of Human Acidic Salivary Protein (Pa)

ROBERT D. FRIEDMAN,[1] A. DONALD MERRITT,[2] AND MARIAN L. RIVAS[2, 3]

## INTRODUCTION

Human saliva contains numerous proteins, some of which constitute genetic polymorphisms. Salivary amylase ($Amy_1$) [1], salivary basic (Pb) [2, 3] and acidic (Pa) [4–6] proteins, proline-rich salivary proteins (Pr) [7, 8], and double-band salivary proteins (Db) [8] are all autosomal polymorphisms showing racial variation. The present studies further define the phenotypic expression of the Pa locus by means of starch and acrylamide gel electrophoresis of parotid, submandibular, and whole saliva, including comparisons with the products of the loci Pb, Pr, and Db described by Azen [2, 7, 8].

## MATERIALS AND METHODS

Samples of saliva, blood, and urine were collected randomly from Americans of Caucasian, African, and Oriental descent. Whole saliva was stimulated by chewing paraffin gum, parotid saliva was collected with a Carlson-Crittenden type cup, and submandibular fluid was obtained with either an individually fabricated collector or the silicone rubber molded device previously described [9]. (Whole saliva was satisfactory for typing in only half of the samples even if frozen at the time of collection.) Samples were frozen, lyophilized, and stored at −20°C for later analysis. Members of families in which Pa variants segregated were genotyped by standard procedures for the following genetic marker systems: ABO, secretor (Sec), Rh, MNS, Kell (K), Duffy (Fy), Kidd (Jk), P, red cell phosphoglucomutase-1 ($PGM_1$), haptoglobin (Hp), red cell acid phosphatase (AcP), salivary amylase ($Amy_1$), pancreatic amylase ($Amy_2$), salivary basic proteins (Pb), proline-rich salivary proteins (Pr), and double-band salivary proteins (Db).

Electrophoresis was performed in aluminum lactate, pH 2.4, on 4 M urea starch gels similar to those described by Sung and Smithies [10] and Azen [2]. The contents of the starch gel were: starch (Connaught, Toronto, Canada, lot 257-1), 79 g; urea, 120 g; commercial aluminum lactate, 3.53 g; and lactic acid, 11.4 ml in 400 ml distilled water. The bridge of 0.03 M aluminum lactate buffer was adjusted to pH 2.4 with lactic acid.

Received September 23, 1974; revised December 6, 1974.

Publication no. 74-01 from the Department of Medical Genetics. This work was supported in part by U.S. Public Health Service grants P01 GM 21054 and 19178 and a Rutgers University Research Council Summer Faculty Fellowship Grant.

[1] Department of Biochemistry, Temple University School of Dentistry, Philadelphia, Pennsylvania 19140.

[2] Department of Medical Genetics, Indiana University Medical Center, Indianapolis, Indiana 46202.

[3] Department of Biological Sciences, Douglass College, Rutgers University, New Brunswick, New Jersey 08903.

All lyophilized salivary samples were resuspended at five times the original concentration in gel buffer with 8 M urea, applied 12 cm from the cathodal end of a 12 × 27 cm gel, sealed with petrolatum, and covered with plastic film. Vertical electrophoresis was performed at a constant current of 15–20 mA/gel and terminated at 20 hr. Prior to staining, the 6-mm thick gels were sliced into 3-mm gels. Both types of gels were stained using either 1% Amido Black 10B in 2% acetic acid or by the method developed by Sung and Smithies [10] for arginine-rich proteins.

Comparative electrophoretic studies of Pr and Pa were performed in the acid-urea starch gel system and the anionic acrylamide gel described by Azen and Oppenheim [7]. Parotid saliva from known Pr 1–2 individuals of Pa(+) and Pa(−) phenotypes was subjected to electrophoresis in a wide origin slot. After cutting a narrow longitudinal strip from the gel and staining for the Pr bands to identify their electrophoretic position, slices perpendicular to the direction of migration were cut from the gel through areas designated as pre-Pr, Pr 1, Pr 2, between Pr 2 and 3, Pr 3, Pr 4, and post-Pr. The horizontal gel strips were crushed and eluted with gel buffer, centrifuged, lyophilized, and then subjected to electrophoresis in the acid-urea starch gel system. After determining the electrophoretic position of the Pa band, its mobility relative to Pr 1 and 2 and the cathodal band of the Db system described by Azen and Denniston [8] was evaluated in 5%, 6%, and 7% acrylamide gels.

Saliva may be fractionated at 4°C by the dropwise addition of trichloroacetic acid (TCA) to a concentration of 8%. The Pa material precipitates, while similarly migrating glycoproteins remain in the supernatant after washing with 8% TCA and final centrifugation at 10,000 *g*. Electrophoresis of serum proteins and purified fractions of albumin, IgA, IgG, partially purified $\alpha$ and $\beta$ globulins, salivary and pancreatic amylase, and the partially purified Pa proteins described by Friedman and Merritt [5, 6] was performed in these gels for evaluation of comparative migrations.

## RESULTS

### *Electrophoretic Studies*

The Pa phenotypes in parotid or submandibular saliva are easily determined in acid-urea starch gels by the presence (+) or absence (—) of a darkly staining anodal protein (fig. 1). While whole saliva that is immediately frozen and lyophilized may allow Pa and Pb typing, it frequently produces a poorly resolved gel pattern. This variability has not been explained. It does not seem to be related to a whole-saliva factor per se, since short incubation of whole saliva which could not be typed for Pa and Pb with equal aliquots of parotid or submandibular saliva from the same individual was without apparent effect on the well defined gel patterns of the pure samples.

Figure 2 demonstrates the decrease in glycoproteins and other components migrating close to the Pa band when parotid saliva is precipitated with 8% TCA, another technique occasionally useful for typing. Figure 3 is a diagrammatic representation of the relative electrophoretic migration of proteins from saliva and blood and is consistent with those reported by Azen [3]. The Pb and $Amy_1$ gene products are clearly distinguished in the gel system. Though immunoglobulins appear to migrate in a position similar to the Pa band, the Pa protein stains poorly with Amido Black 10B, while immunoglobulins stain poorly with the Sung and Smithies stain [10].

It seems clear that most of the Pr material may be separated from Pa by gel

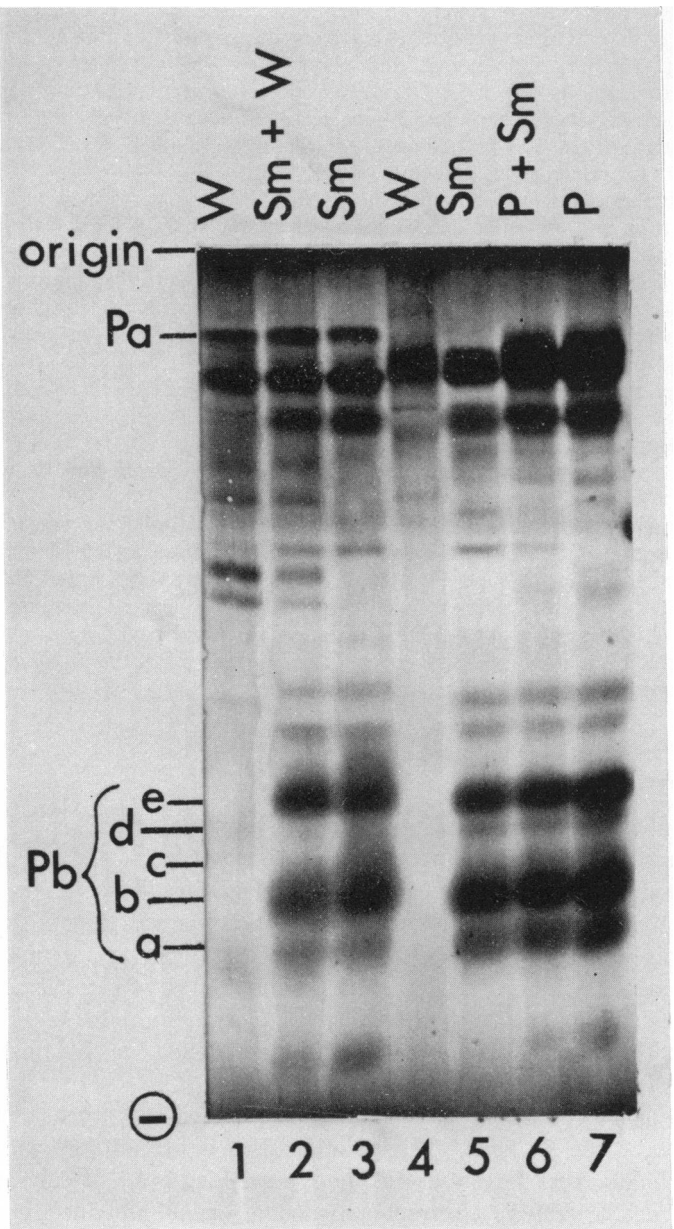


FIG. 1.—Pa(+) and Pa(—) phenotypes in parotid (P), whole (W), submandibular (Sm), and mixed saliva as expressed in an acid-urea starch gel (pH 2.4) stained by the method of Sung and Smithies [10]. *Channels 1–3,* Pa(+), individual A; *channels 4–7,* Pa(—), individual B. Position of five components of Pb system noted on left; all samples are Pb 1-1 and lack band C. Note relatively poor salivary protein patterns in whole saliva samples.

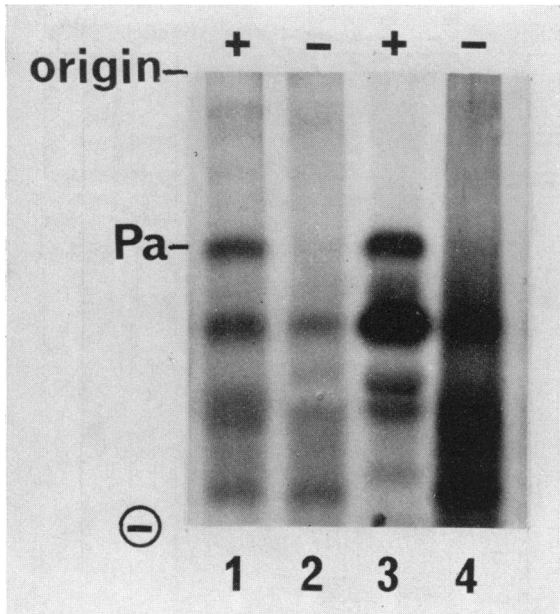


FIG. 2.—Electrophoresis of parotid saliva from four individuals precipitated in 8% TCA in acid-urea starch gel (pH 2.4); only proximal one-third of gel shown. Pa(+) phenotypes in channels 1 and 3. Note decrease in glycoproteins and other components compared to fig. 1.

filtration and DEAE chromatography or by prior electrophoresis. The Pr bands 1, 2, 3, and 4 migrate in the complex mixture of proteins cathodal to the Pa band, forming a poorly resolved broad third band on electrophoresis in acid-urea starch gels (figs. 1 and 3).

The position of the Pa band, as determined by chemical separation or by elution from a Pr gel, was between Pr 1 and Pr 2 in a 5% acrylamide gel at pH 8.3. Acrylamide concentrations up to 7.5% retarded Pa migration more than that of the four Pr bands, making Pa typing possible in the acrylamide gel system. The Pa and Pr proteins stain similarly by the technique of Azen and Oppenheim [7] or Sung and Smithies [10].

*Population Studies*

The Pa(+) phenotype has been observed in each of the three racial groups studied. The distribution of Pa phenotypes in a random sample of 458 unrelated individuals is summarized in table 1. Among 330 Caucasian Americans, 38.2% were Pa(+), giving gene frequency estimates for $Pa^+$ and $Pa^-$ of .21 ± .02 and .79 ± .02, respectively; 25.4% of 122 black Americans were Pa(+), giving gene frequency estimates of .14 ± .02 for $Pa^+$ and .86 ± .02 for $Pa^-$. Four of six Orientals were Pa(+), suggesting a high frequency of $Pa^+$ in this racial group.

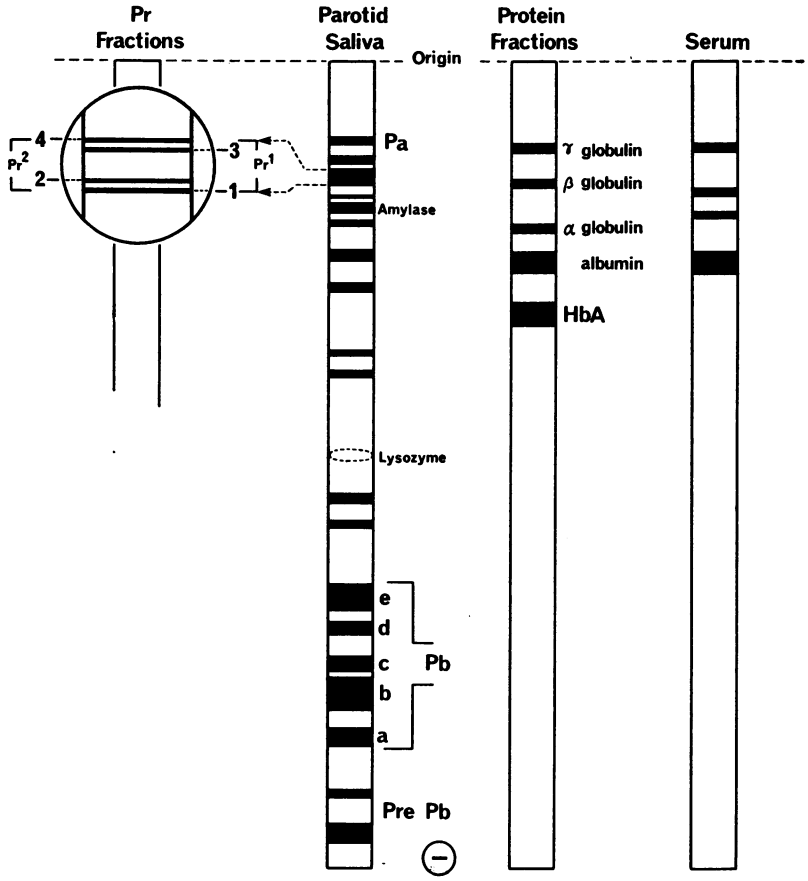


FIG. 3.—Diagram of relative electrophoretic migration of proteins from parotid saliva and serum. Positions of purified fractions of serum proteins and Hb A in acid-urea starch gel system (pH 2.7) given for comparison of relative migrations. Pb and $Amy_1$ gene products easily distinguished in gel; Pr proteins (1, 2, 3, and 4) separated from Pa by gel filtration and DEAE chromatography or by prior electrophoresis migrate to region of third major cathodal band as indicated on left. Unlabeled salivary protein components have not been identified.

*Genetic Studies*

Genetic transmission was studied in families of Pa(+) individuals ascertained in our random survey. Thus far 50 Pa(+) × Pa(—), 25 Pa(+) × Pa(+), and 29 Pa(—) × Pa(—) Caucasian matings with tested offspring have been ascertained. Table 2 summarizes the distribution of Pa phenotypes among offspring of these matings and the results of segregation analysis. In most of the Pa(+) × Pa(—) and Pa(+) × Pa(+) families, presence of Pa(—) offspring is evidence that the Pa(+) parent(s) is, in fact, heterozygous. In others, the possibility remains that the parent(s) could have been homozygous for the $Pa^+$ allele. For each of the 47 potentially homozygous offspring of Pa(+) × Pa(+) matings,

TABLE 1

POPULATION DISTRIBUTION OF Pa PHENOTYPES

| ETHNIC GROUP | NO. TESTED | PHENOTYPES | | GENE FREQUENCY | |
|---|---|---|---|---|---|
| | | Pa(+) | Pa(−) | $Pa^+$ | $Pa^-$ |
| Caucasian American ..... | 330 | 126 (38.2) | 204 (61.8) | .214 ± .017 | .786 ± .017 |
| Black American ..... | 122 | 31 (25.4) | 91 (74.6) | .136 ± .023 | .864 ± .023 |
| Oriental American ..... | 6 | 4 (66.7) | 2 (33.3) | .423 ± .167 | .577 ± .167 |

NOTE.—Percentage appears in parentheses.

the Pa band is electrophoretically indistinguishable from that found in known heterozygotes. Clear demonstration of autosomal dominant inheritance of the Pa(+) phenotype was seen in a number of 3-generation families. Male-to-male transmission of the Pa(+) phenotype was observed, thereby excluding X-linked inheritance.

Using the gene frequency estimate of .79 for the $Pa^-$ allele in Caucasians and assuming that the population is panmictic with respect to the Pa locus, the expected proportion (Snyder's ratio) of Pa(−) offspring from Pa(+) × Pa(−) matings is $q/(1+q)$ or .44, and that of Pa(−) offspring from Pa(+) × Pa(+) matings is $q^2/(1+q)^2$ or .194. Among the 117 offspring of Pa(+) × Pa(−) matings, 56 Pa(−) offspring were observed (51.5 expected, $\chi^2_1 = 0.70$). Among 56 offspring of Pa(+) × Pa(+) matings, nine were Pa(−) (10.9 expected, $\chi^2_1 = 0.41$). Of the 66 offspring of Pa(−) × Pa(−) parents, all showed the ex-

TABLE 2

FAMILIAL SEGREGATION OF Pa PHENOTYPES

| MATING (♂ × ♀) | NO. SIBSHIPS | OFFSPRING | | | | |
|---|---|---|---|---|---|---|
| | | Male | | Female | | |
| | | Pa(+) | Pa(−) | Pa(+) | Pa(−) | TOTAL |
| Pa(+) × Pa(−) ..... | 28 | 23 | 21 | 17 | 13 | 74 |
| Pa(−) × Pa(+) ..... | 22 | 12 | 14 | 9 | 8 | 43 |
| Total* ..... | 50 | 35 | 35 | 26 | 21 | 117 |
| Pa(+) × Pa(+)† ..... | 25 | 26 | 6 | 21 | 3 | 56 |
| Pa(−) × Pa(−) ..... | 29 | 0 | 33 | 0 | 33 | 66 |

* $\chi^2_1 = 0.70$, $.30 < P < .50$. Expected Pa(−) offspring based on Snyder's ratio = 51.5.
† $\chi^2_1 = 0.41$, $.50 < P < .60$. Expected Pa(−) offspring based on Snyder's ratio = 10.9.

pected Pa(—) phenotype. These results are consistent with the hypothesis that the Pa(+) phenotype is an autosomal dominant trait.

The Hardy-Weinberg relationship predicts that in a sample of randomly selected parental pairs, the Pa(+) × Pa(—), Pa(+) × Pa(+), and Pa(—) × Pa(—) mating types should occur at frequencies of .47, .15, and .38, respectively. Our sample of 149 random matings consisted of 70 Pa(+) × Pa(—) (70.0 expected), 29 Pa(+) × Pa(+) (22.4 expected), and 50 Pa(—) × Pa(—) (56.6 expected); $\chi^2_2 = 2.71$, $.20 < P < .30$.

*Relationship of Pa to Pr and Db Phenotypes*

Table 3 shows the distribution of Pa and Pr phenotypes in 139 unrelated Caucasians typed for both marker systems. The gene frequencies of $Pr^1$ and $Pr^2$ es-

TABLE 3

DISTRIBUTION OF Pr PHENOTYPES AMONG Pa(+) AND Pa(—) INDIVIDUALS

| | PHENOTYPE* | | | |
|---|---|---|---|---|
| | 1-1 | 1-2 | 2-2 | TOTAL |
| Pa(+) ..... | 1 (25.0) | 48 (29.2) | 9 (3.8) | 58 |
| Pa(—) ..... | 59 (35.0) | 22 (40.8) | 0 (5.2) | 81 |
| Total ..... | 60 | 70 | 9 | 139 |

NOTE.—Expected values in parentheses; $2 \times 3$ contingency $\chi^2_2 = 72.91$, $P < .001$.

* Two-allele system.

timated from this sample, .68 ± .03 and .32 ± .03, respectively, are not significantly different from those reported by Azen and Oppenheim [7] for a two-allele Pr locus ($Pr^1 = .73 \pm .03$). The observed distribution of 60 Pr 1-1 (64.3 expected), 70 Pr 1-2 (60.5 expected), and nine Pr 2-2 (14.2 expected) is consistent with Hardy-Weinberg equilibrium ($\chi^2_1 = 3.68$, $.05 < P < .10$). However, when this sample is tested for equilibrium with respect to the two loci, a highly significant deviation from that expected is observed ($\chi^2_2 = 72.9$, $P < .001$).

Table 4 summarizes the results of a similar analysis performed on data obtained from 136 Caucasians typed for both Pa and Db. The gene frequency of $Db^-$ in this sample is .84 ± .02, which is not significantly different from the estimate of .88 ± .02 reported by Azen and Denniston [8]. As in the case of Pa and Pr, the observed distribution of Pa and Db phenotypes is not compatible with expected equilibrium frequencies ($\chi^2_1 = 12.9$, $P < .001$).

*Linkage Relationships of Pa*

No definite recombinants between Pa and Pr have been observed among 15 offspring of seven informative double backcross matings. Two sibships were in-

TABLE 4

DISTRIBUTION OF Db PHENOTYPES AMONG Pa(+) AND Pa(−) INDIVIDUALS

| | PHENOTYPE | | |
|---|---|---|---|
| | Db+ | Db− | TOTAL |
| Pa(+) ..... | 7 (16.3) | 50 (40.7) | 57 |
| Pa(−) ..... | 32 (22.7) | 47 (56.3) | 79 |
| Total ..... | 39 | 97 | 136 |

NOTE.—Expected values in parentheses; $2 \times 2$ contingency $\chi^2_1 = 12.90$, $P < .001$.

formative for Pa and Db; there were no certain recombinants among the six scorable offspring. The maximum likelihood estimate of the recombination fraction ($\hat{\theta}$) for each of the Pa:Pr and Pa:Db intervals is, therefore, $\hat{\theta} = 0.00$. Preliminary linkage analyses show no significant positive lod scores between Pa and ABO, Sec, Rh, MNS, Kell, Fy, Jk, Hp, P, $PGM_1$, AcP, and $Amy_2$.

## DISCUSSION

These data extend our earlier reports of a human salivary protein polymorphism [4–6]. Since this salivary protein is acidic compared to the basic salivary protein polymorphism Pb described by Azen [2], we have designated the locus Pa. In acid-urea starch gel, the presence or absence of the Pa band (fig. 1) determines the two phenotypes at the Pa locus. Though Pa and Pb phenotypes are easily typed in submandibular and parotid saliva, care must be taken in interpreting results from whole saliva. Even when whole saliva is frozen immediately upon collection for lyophilization and electrophoresis, we find that only half the samples prove suitable for Pa and Pb classification.

The Pa phenotype in acid-urea starch gels is even clearer when parotid saliva is precipitated with 8% TCA. Since the glycoproteins, just cathodal to Pa, are soluble, the precipitated Pa(+) material is a well defined band (fig. 2). That Pa is not a serum immunoglobulin or other serum fraction is clear from differential staining of these proteins. Immunoglobulins stain well with Amido Black 10B but very faintly with the arginine staining method of Sung and Smithies [10]; the converse is true for the Pa protein.

Biochemical studies show that the Pa protein has a high proportion of proline [5, 6] and is therefore similar to, but not identical with, four proline-rich salivary proteins (Pr) described by Oppenheim et al. [11] and later evaluated genetically by Azen and Oppenheim [7]. The locus, designated Pr, was first postulated to have two alleles, $Pr^1$ and $Pr^2$, constituting an autosomal codominant polymorphism. The products of the $Pr^1$ and $Pr^2$ alleles are, respectively, the pairs of salivary proteins now designated Pr 1 and 3 and Pr 2 and 4 [8]. The Pa protein has

233 proline residues per 1,000 amino acid residues [5, 6], whereas Pr proteins 1, 2, 3, and 4 have 271, 261, 212, and 220 proline residues, respectively. Additional similarities between the Pa and Pr proteins are noted in the proportion of other amino acids. Aspartic acid, however, is far less for Pa: 21 compared to 76, 77, 107, and 103 residues per 1,000 for the Pr proteins.

Comparative electrophoretic studies of Pa and these Pr proteins in the same gel system have allowed us to exclude their electrophoretic identity. The migrations of individual Pr proteins are close together and cathodal to Pa in the acid-urea starch gel utilized for the detection of Pa and Pb (fig. 3). In a Tris-borate acrylamide system, the Pa band migrates between Pr 1 and Pr 2 when the concentration of acrylamide is 5% and migrates cathodal to Pr 1 at higher (6.5%–7.5%) acrylamide concentrations. Similar results are described in detail elsewhere [12].

Segregation and population genetic analyses of the Pa phenotypes strongly suggest that the Pa(+) phenotype is a heritable trait with an autosomal dominant pattern of transmission (table 2). Two alleles, $Pa^+$ and $Pa^-$, are postulated for the Pa locus, with $Pa^+$ frequencies of .21 and .14 in Caucasian and black Americans, respectively (table 1). The finding of four Pa(+) individuals among six tested Orientals indicates a high frequency of the $Pa^+$ allele in this population. The close agreement of the observed distribution of Pa(+) and Pa(—) offspring from the three possible mating types, Pa(+) × Pa(—), Pa(+) × Pa(+), and Pa(—) × Pa(—), and the observed distribution of the latter with that expected under Hardy-Weinberg equilibrium conditions further support our genetic interpretation of the data.

In tests of genetic independence of Pa of each of the presently defined salivary protein polymorphic systems summarized in table 5, a state of disequilibrium was noted between Pa and Pr ($\chi^2_2 = 72.9$, $P < .001$) (table 3) and between Pa and Db ($\chi^2_1 = 12.9$, $P < .001$) (table 4). When Pr phenotypes were grouped into Pr 1-1 and non-Pr 1-1 (Pr 1-2 and Pr 2-2) and tested for independence from the Pa(+) and Pa(—) phenotypes, a strong association between Pa(+) and genotypes having the $Pr^2$ allele was noted ($\chi^2_1 = 69.7$, $P < .001$). Because of the rare occurrence in Caucasians of variants at the Pb and $Amy_1$ loci, it was not possible to disassociate Pa from either $Amy_1$ or Pb.

Azen and Denniston [8] have recently expanded the Pr system to include cathodal proteins at position X, showing strong association with the products of the $Pr^1$ (Pr 1 and 3) or $Pr^2$ allele (Pr 2 and 4). The presence of proteins at this position has been interpreted as distinguishing two additional alleles at the Pr locus, $Pr^{1'}$ and $Pr^{2'}$ (see table 5). The reported family and population genetic studies were, in general, supportive of one locus with four Pr alleles. The genetics of another pair of salivary proteins, Db (for double band), inherited as an autosomal dominant trait, and its relationship to Pr are also discussed [8].

We have shown that Pa and Pr proteins are similar in their amino acid composition [6]. Furthermore, Db, like the Pr and Pa proteins, has a negative staining reaction with 3,3′-dimethoxybenzidine and hydrogen peroxide. It is clear that

TABLE 5

ALLELIC FREQUENCIES OF SALIVARY PROTEIN POLYMORPHISMS

| Locus and Allele | Caucasian American | Black American | Oriental American | Reference |
|---|---|---|---|---|
| Pa: | | | | |
| *Pa*+ | .21 ± .02 | .14 ± .02 | .42 ± .17 | Present study |
| *Pa*− | .79 ± .02 | .86 ± .02 | .58 ± .17 | |
| | (330) | (122) | (6) | |
| Pr:* | | | | |
| $Pr^1$ | .73 ± .03 | .80 ± .03 | .84 ± .04 | [7] |
| $Pr^2$ | .27 ± .03 | .20 ± .03 | .16 ± .04 | |
| | (120) | (79) | (40) | |
| Pr:† | | | | |
| $Pr^{1'}$ | .005 ± .005 | .050 ± .015 | .000 ± .000 | [8] |
| $Pr^1$ | .640 ± .034 | .700 ± .032 | .770 ± .040 | |
| $Pr^{2'}$ | .275 ± .032 | .170 ± .026 | .230 ± .040 | |
| $Pr^2$ | .080 ± .019 | .080 ± .019 | .000 ± .000 | |
| | (100) | (100) | (54) | |
| Pb: | | | | |
| $Pb^1$ | .995 ± .005 | .840 ± .027 | | [2] |
| $Pb^2$ | .005 ± .005 | .160 ± .027 | | |
| | (101) | (90) | | |
| Db: | | | | |
| *Db*+ | .12 ± .02 | .56 ± .04 | .07 ± .02 | [8] |
| *Db*− | .88 ± .02 | .44 ± .04 | .93 ± .02 | |
| | (100) | (100) | (54) | |
| $Amy_1$: | | | | |
| $Amy_1{}^A$ | .995 ± .0002 | .961 ± .010 | | [1] |
| $Amy_1{}^V$‡ | .005 ± .0002 | .039 ± .010 | | |
| | (961) | (208) | | |

NOTE.—Number of subjects shown in parentheses.
* Two-allele system.
† Four-allele system.
‡ All $Amy_1$ variant alleles combined.

these proteins are not independent of one another (tables 3 and 4). The semantic relationship of Pa and proteins at position X has not been easy to resolve. Furthermore, the question of whether the complex multibanded electrophoretic phenotypes represent the products of one or more loci is difficult.

Recent evidence based on electrophoretic comparisons indicates that the Pa protein may correspond to a protein in the X position. Azen and Denniston [8] report gene frequencies in Caucasians of .005 and .080 for $Pr^{1'}$ and $Pr^2$, respectively, which could easily account for the few Pr 1-1, Pa(+) and Pr 2-2, Pa(−) individuals in the present study (table 3). One might also expect the sum of the frequencies of $Pr^{1'}$ and $Pr^{2'}$ alleles (.28) to approximate that of $Pa^+$ (.21). Moreover, 1′-1 individuals should correspond to our Pr 1-1, Pa(+) classification. (The one individual in this class is phenotypically 1′-1 in the acrylamide system.) Similarly, Pr 1-2′ should be equivalent to Pr 1-2, Pa(+), and so on. The proposed relationships are summarized in table 6. The contingency $\chi^2_3$ value of 2.21 is not significant, suggesting that these data are compatible with either interpretation.

TABLE 6

DISTRIBUTION OF Pa AND Pr PHENOTYPES CLASSIFIED AS EXPRESSIONS OF ONE OR TWO LOCI

| ONE LOCUS* | | TWO LOCI† | |
|---|---|---|---|
| Phenotype | No. Observed | Phenotype | No. Observed |
| Pr 1′-1 | 1 | Pa(+), Pr 1-1 | 1 |
| Pr 1-1 | 39 | Pa(−), Pr 1-1 | 59 |
| Pr 1-2′ | 37 | Pa(+), Pr 1-2 | 49 |
| Pr 1-2 | 12 | Pa(−), Pr 1-2 | 22 |
| Pr 2-2′; 2′-2′ | 11 | Pa(+), Pr 2-2 | 9 |
| Pr 2-2 | 0 | Pa(−), Pr 2-2 | 0 |
| Pr 1′-1′‡ | 0 | Pa(+), Pr ?-? | 0 |
| Pr 1′-2; Pr 1′-2′§ | 0 | Pa(+), Pr 2-? | 0 |
| Total | 100 | Total | 139 |

NOTE.—$2 \times 4$ contingency $\chi^2_3 = 2.21$, $.50 < P < .60$; 1′-1 class combined with 1-1 due to small observed numbers.

* Four Pr alleles. Observed numbers taken from [8].

† Two Pa and two Pr alleles. Observed numbers from present study.

‡ Electrophoretic pattern postulated to show only X and Pr 3 bands.

§ Electrophoretic pattern for both phenotypes consists of X and Pr 2, 3, and 4 bands.

While the distribution of classes reveals no inconsistencies, the possibility is not excluded that Pa and Pr are two distinct closely linked loci which have not yet reached an equilibrium state. Linkage disequilibrium, a not infrequent finding among tightly linked loci, could account for the apparent association of Pa and Pr phenotypes observed in our sampling. Family data presented by Azen and Denniston [8] in support of the four-allele Pr system are equally consistent with the hypothesis of two closely linked loci. Distinguishing between the two alternatives, namely, one Pr locus with multiple alleles and two loci, Pa and Pr, each with two alleles, is difficult with information presently available. In fact, the problems encountered are highly reminiscent of those at the Rh and MNS loci.

By treating Pa, Pr, and Db as three loci, we have not found recombinants among 21 offspring of appropriate backcrosses. Similarly, Azen and Denniston [8] reported no recombinants between Pr (four-allele system) and Db among 33 offspring of nine informative matings.

We do not disagree with the interpretations of Azen and Denniston [8] except insofar as the status of the loci is concerned. The association and/or linkage of these loci cannot be considered a closed issue. Since Pa, Pr, and Db have not been dissociated from each other, they cannot be claimed to have achieved the status of independent loci. At this point there may be one, two, or three loci. Nonetheless, treating them operationally as distinct loci may provide insight into their biochemical, functional, and genetic relationships and thereby increase their usefulness as genetic markers.

SUMMARY

The phenotypic expression of a dominantly inherited human salivary acidic protein (Pa) has been described in acid-urea starch and in Tris-borate acrylamide gel systems. Estimates of the $Pa^+$ allelic frequencies in American Caucasians, American blacks, and Orientals are .21, .14, and .42, respectively. The genetic and biochemical similarities to another series of proline-rich salivary proteins, Pr, and to a pair of similarly staining salivary proteins, Db (double band), are evaluated. It is concluded that either one locus or two (or three) tightly linked loci are viable explanations for this polymorphic system(s). It is suggested that the three factors, Pa, Pr, and Db, be treated as separate loci to allow clarification of their genetic relationships.

ACKNOWLEDGMENTS

We appreciate the helpful discussions with Dr. E. A. Azen. The technical assistance of R. Caldwell, M. J. Dobbs, C. Moore, R. Newell, J. Wilson, and N. Wilson and the field studies for sample collection by M. Karn, B. Molesworth, A. Pigg, and J. Stafford are gratefully acknowledged.

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