of lunar material. Not only glasses, but objects such as crystals and other smooth surfaces of sufficient specular character are worthy of study. In particular, light could be thrown on erosion processes.

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Genetic Polymorphism of Basic Proteins from Parotid Saliva

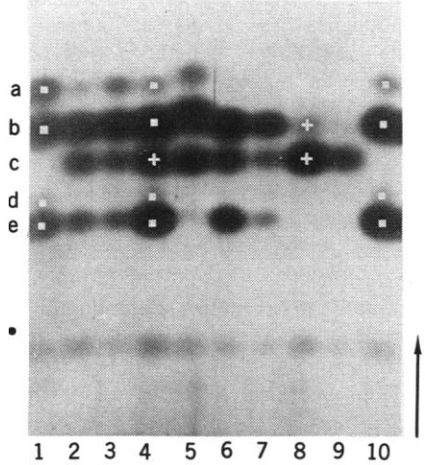
Abstract. *In a study of 90 randomly chosen parotid salivas from Blacks three phenotypes were observed during acid-urea starch-gel electrophoresis. Inheritance was controlled by two codominant alleles at an autosomal locus. Of 101 Caucasians, one had a heterozygous phenotype indistinguishable electrophoretically from that in Blacks. Gene frequencies were: for Blacks, parotid basic protein* $(Pb^1) = 0.84$, $(Pb^2) = 0.16$; *for Caucasians,* $(Pb^1) \sim 0.995$, $(Pb^2) \sim 0.005$.

The proteins of human serum and red cells have been extensively studied by electrophoretic and immunological methods, and many genetic polymorphisms have been identified (*1*). Since parotid fluid is easy to obtain (*2*) and is known to be a rich mixture of proteins, especially basic components (*3*), I examined it for the presence of new genetic polymorphisms. Genetic variation of salivary amylase has been described (*4*), and much is known about the relations between secretor, Lewis, ABO, and H genetic systems to factors in the saliva (*5*). Electrophoresis of parotid proteins in alkaline systems has been studied (*3*), but there have been only a few reports of electrophoresis on acid gels (*6*). Therefore, I studied basic parotid fluid proteins, using starch-gel electrophoresis in acid-urea buffers and a sensitive stain for arginine-rich basic proteins (*7*). I now report that there is genetic polymorphism in the fastest migrating basic components of the parotid saliva of Blacks, and that a parotid basic protein variant, electrophoretically indistinguishable from that in Blacks, was found in one Caucasian family.

Electrophoresis of concentrated parotid fluid (*8*) yielded at least 20 bands that stained for protein. However, genetic polymorphism was observed only in the fastest migrating basic components, and in these the mobility was greater than that of lysozyme (Fig. 1). Relatively stable band patterns of the fastest migrating polymorphic basic proteins were obtained from samples of normals and variants regardless of the time of day (or proximity to meal) that the sample was collected. The locations of bands representing proteins [including amylase, immunoglobulin A (IgA), lysozyme, and albumin] known to occur in parotid fluid were identified in the electrophoretic pattern (*9*) and did not correspond to the fastest migrating basic proteins that showed polymorphism.

Samples of parotid fluid from different adult populations were collected. Among 90 samples from Blacks, three patterns were observed. The most common was a four-band pattern labeled bands a, b, d, and e (Fig. 1, channels 1 and 10). This pattern is postulated to represent the common homozygous type (1-1) determined by an allele at an autosomal locus for these parotid basic proteins that I have designated Pb^1 (parotid basic protein). The next most common type was a five-band pattern with an additional band c, along with bands a, b, d, and e. The darkness of band d appears to vary directly with that of band e, and if band e was not very heavy, band d was usually not visible. There is some variation between samples of this type (Fig. 1, channels 2 to 7). In particular, bands a and e sometimes were so faint in relation to bands b and c that further concentration of the samples was needed to see them. This second general pattern is postulated to be the heterozygous phenotype (1-2) determined by the two alleles Pb^1 and Pb^2. The third and least common pattern showed predominance of band c, some protein at position b with a trace of protein just behind position a in concentrated samples, and no bands at positions a, d, and e (Fig. 1, channels 8 and 9). This pattern may represent the homozygous type (2-2) for the variant allele.

Some variation in relative intensities and mobilities of the bands in the common homozygous and heterozygous phenotype has been observed. Tests by incubation of samples with and without inhibitors of proteolysis suggest that the observed phenotypes are partly determined by enzymatic changes oc-



Channel	Phenotype	
1 and 10	1-1, Black	
2	1-2, Black	
3	1-2, Caucasian	
4	1-2, Son	Black
5	1-2, Father	Black
6	1-2, Son	Black
7	1-2, Son	Black
8	2-2, Father	Black
9	2-2, Black	
10	1-1, Black	

Fig. 1. Photograph of part of a gel (10 to 22 cm from sample slots) used for electrophoresis of parotid basic protein variants. This acid-urea starch-gel was stained for arginine-rich proteins and the most rapidly migrating basic proteins are shown. More slowly migrating proteins (amylase, albumin, and IgA) are not shown. Each channel contains a different sample. White squares and white crosses indicate the proteins determined by Pb^1 and Pb^2, respectively, in band patterns from representative samples of the three phenotypes observed (1-1, 1-2, and 2-2). For ease of photography, parotid proteins were concentrated only 1.6 times, and some bands shown by squares or crosses in representative samples were either faint or absent in other samples on this gel. However, they were seen in more concentrated specimens, except occasionally for band d, which varied directly with the heavier band e, and thus was absent when band e was weak. The position of lysozyme is indicated by a black spot and appeared as a zone of negative staining in more concentrated specimens.

curring in the parotid gland. A band migrating just in front of band a is occasionally seen in freshly collected specimens. This is a degradation product of proteins determined by Pb^1 and can be produced by incubation. Thus, the multiple bands in the electrophoretic patterns are probably due to a degradative process acting on the primary gene products of Pb^1 and Pb^2. The patterns are stable for at least 24 hours when parotid fluid is stored at 0°C and for at least 90 hours at room temperature if one-tenth their volume of 10 percent acetic acid is added to the samples just before storage.

The distribution of types in the randomly collected samples was consistent with the general hypothesis in that their occurrences were those expected from the Hardy-Weinberg rule. Of 90 randomly selected samples from American Blacks, 64 were of the common type (1-1), 23 were heterozygotes (1-2), and 3 were uncommon homozygous types (2-2). The Hardy-Weinberg equation shows that, at equilibrium, the expected numbers in the three classes would be 63.4, 24.3, and 2.3. The observed and expected values do not differ significantly ($P = .89$). From these data, approximate gene frequencies of Pb^1 and Pb^2 for Blacks were 0.84 and 0.16, respectively.

Among 101 samples from Caucasians, one gave a pattern indistinguishable electrophoretically from the heterozygous pattern (1-2) found in Blacks (Fig. 1, channel 3), whereas the rest gave a homozygous pattern (1-1) identical to that shown in Fig. 1, channels 1 and 10. The single heterozygous pattern found in a Caucasian was not demonstrably different from that found in Blacks. Nineteen samples from Orientals (17 Chinese, 1 Japanese, 1 Vietnamese) gave homozygous patterns (1-1) identical to that shown in Fig. 1 (channels 1 and 10). Thus, in Caucasians, the frequency of Pb^1 is ~ 0.995 and of Pb^2 is ~ 0.005; for Orientals, no estimate can be given since the sample size was quite small, but the frequency of Pb^2 is likely to be low.

Family studies were done to test the genetic hypothesis. In two Black families (Fig. 2, A and B) parents with the two putative homozygous phenotypes for the basic parotid proteins (1-1 × 2-2) had nine children, all with the expected heterozygous phenotype. In two families (Fig. 2), one Black (C) and the other Caucasian (D), parents with the common homozygous and heterozygous phenotypes (1-1 × 1-2) had four children, two with each parental phenotype. In two other branches of family D, both parents possessed the common homozygous phenotype (1-1) and all their five children were of the same common type. A single Caucasian family (D) in which the variant phenotype was observed was of Scotch-English-Welsh background, and its members were unaware of any Black ancestors. These data are consistent with a simple pattern of inheritance with two alleles (Pb^1 and Pb^2) at a single autosomal locus.

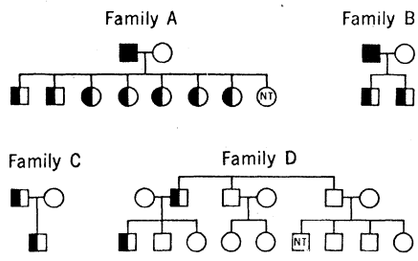


Fig. 2. Inheritance of parotid basic protein variants. Families were not randomly chosen but were selected because a member possessed a variant phenotype. Open, filled, and half-filled symbols represent phenotypes 1-1, 2-2, and 1-2, respectively. *NT* indicates saliva not tested. Families A, B, and C are Black; Family D is Caucasian.

The initial data on blood and saliva groups from families A, B, C, and D (Fig. 2) were provided by Dr. Wilma Bias (Immunogenetics Laboratory, Baltimore City Hospital, Baltimore, Md.). There was no obvious one-to-one correspondence between the basic parotid protein phenotypes and the phenotypes observed in the following genetic systems: ABO, Rh, MNS's, Kell, Kidd, Lutheran, Duffy, Lewis, secretor, haptoglobin, transferrin, group-specific components, and hemoglobin (beta-chain determinant). These data indicated nonidentity of the genes determining the above traits and those determining the parotid basic protein polymorphism.

This polymorphism of the parotid basic proteins should prove useful for genetic research, since the frequency of this polymorphism, which appears relatively restricted to Blacks, is high. Other genetic markers on red blood cells which are present in high frequency and are known to be virtually restricted to Blacks include Fy (a-b-), antigen V and Js^a (*5*).

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8. To collect parotid fluid, an acrylic plastic capsule (*2*) provided by O. Hiller, P.O. Box 1294, Madison, Wisconsin, was placed over Stensen's duct in the mouth, and the flow of saliva was stimulated by having the subject suck on a hard lemon candy. The parotid fluid was collected in a siliconized glass tube to prevent possible adsorption of basic proteins to the glass (personal communication from Dr. J. A. Mangos). Samples of parotid fluid (0.5 to 2.0 ml) were kept at 0°C usually for no more than several hours and frozen as shells in test tubes; they were then lyophilized and stored at —20°C. Just before electrophoresis, the dried samples were dissolved in gel buffer containing 8*M* urea to concentrate the proteins 1.5 to 10 times. Starch gels contained 400 ml of aluminum lactate–lactic acid buffer, *p*H 2.4 [prepared from activated aluminum foil (*7*)], 70 g of starch (lot 275-1, Connaught, Toronto), and urea 120 g (4*M*) (*10*). An aluminum lactate–lactic acid gel buffer (0.03*M* in aluminum, *p*H 2.4) may also be prepared from aluminum lactate powder (K. K. Laboratories), but staining of the gels with this buffer was not as reproducible. However, the buffer is satisfactory for the bridge solutions. After inserting the samples, vertical starch-gel electrophoresis was carried out at 2.4 volt/cm for approximately 15 to 18 hours with a power supply adapted for constant current output. The gels were sliced and the bottom slice was stained for arginine-rich proteins (*7*). The upper slice was stained with 1 percent Amido Black in 2.0 percent acetic acid. As compared to the standard Amido Black stain, the special stain was particularly sensitive in detecting many of the basic proteins, especially the fastest migrating components that showed polymorphism.
9. After electrophoresis of parotid fluid, the position of the lysozyme band (Fig. 1) was identified by overlaying the sliced gel with 0.06 percent suspension of *Micrococcus lysodeikticus* in 0.06*M* phosphate buffer, *p*H 6.2, containing 1.0 percent agarose. A zone of bacterial lysis was noted, and this corresponded electrophoretically to the lysozyme of human tears, which served as a useful marker. The positions of bands representing the slower migrating proteins, amylase, albumin, and IgA are not shown in Fig. 1. The parotid amylase band was identified as a zone of destroyed starch after electrophoresis, followed by dialysis of the acid-urea starch-gel slice in 0.02*M* phosphate buffer, *p*H 6.9, for 24 hours. Evidence for the location of albumin and IgA bands was obtained by electrophoresis of diluted serum and purified parotid IgA (courtesy of Dr. R. Hong) in channels adjacent to the proteins of whole parotid fluid.
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