adiabatic one. The slight downward trend of n with decreasing humidity (increasing expansion) could be caused by increased turbulence producing an inhomogeneous distribution of the nuclei and increased losses to the wall during expansion.

Some conditions suitable for kinetic studies have been established. Sulfuric acid condensation nuclei readily form in a humid SO_2 atmosphere irradiated by ultraviolet light or ionizing radiations. Immediately after formation, the condensation nuclei possess a broad size distribution characterized by single, structureless scattering and light attenuation curves. However, the condensation nuclei soon coalesce and form larger particles with a much narrower size distribution, and reliable measurements of n and r_c can be made by the methods described above. The number concentration n is proportional to the time of irradiation up to particle concentrations of 4×10^5 cm^{-3}. This work was carried out with a 1-mc ^{90}Sr-^{90}Y beta-ray source, 10 torr of water vapor, an SO_2 concentration of 0.78 part per million (ppm), and 740 torr of filtered tank air. Under these conditions a large response is obtained and measurements can be made at the first absorbance level of Fig. 2.

Some exploratory studies have also been carried out to determine the usefulness of our instrument in reaction kinetics. Carbon monoxide and methane appreciably diminish the number of nuclei formed in beta-irradiated air containing 2.53 ppm of SO_2. At this stage we theorize that these organic compounds interfere with the oxidation of SO_2 by the OH radicals formed by the action of the beta rays on the air–water vapor system (*13*). This competition may be represented simply as:

$$SO_2 + OH \rightarrow \text{condensation nuclei}$$
$$CO + OH \rightarrow \text{no condensation nuclei}$$
$$CH_4 + OH \rightarrow \text{no condensation nuclei}$$

Thus, by measuring the inhibition of nucleus formation by CO and CH_4 separately, we can deduce the rate constant ratio $k(CO + OH)/k(CH_4 + OH)$ (*14*). At 25°C we obtain a ratio of 22.9, a result that compares favorably with Greiner's value of 16.8 (*15*), obtained by kinetic spectroscopy at 300°K. Thus a new method for measuring relative rate constants for reactions of OH radicals may be developed with the Argonne condensation nucleus discriminator. Similar laboratory studies could be carried out with other radicals such as H, HO_2, and O as well as with ozone and singlet O_2.

Condensation nucleus counters are widely employed in atmospheric research (*3, 4, 10*), and because of their high sensitivity in detecting particles with radii in the range 10 to 200 Å they are being used to study the minute chemical changes involved in gas-to-particle conversion reactions (*16*). With the improved size discrimination and absolute counting features of our instrument, we believe that it will find general application in physical and chemical studies on condensation nuclei.

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References and Notes

1. V. J. Shaefer, testimony before the Senate Subcommittee on Public Works, 91st Congress, 2nd Session, 16 to 18 March 1970; published in *Third Annual Report* (Atmospheric Sciences Research Center, State University of New York, Albany, 1970), p. 1.
2. L. D. Carver, *Ind. Res.* **13**, 41 (August 1971); A. Hogan, in *Third Annual Report* (Atmospheric Sciences Research Center, State University of New York, Albany, 1970), p. 36; L. W. Pollak and T. Murphy, *Geofis. Pura Appl.* **25**, 44 (1953).
3. G. F. Skala, *Anal. Chem.* **35**, 702 (1963).
4. K. T. Whitby, B. Y. H. Lin, R. B. Husar, N. J. Barsic, *J. Colloid Interface Sci.* **39**, 136 (1972).
5. F. T. Gucker, C. T. O'Konski, H. B. Packard, J. N. Pitts, Jr., *J. Amer. Chem. Soc.* **69**, 2422 (1947).
6. G. Mie, *Ann. Phys. Leipzig* **25**, 377 (1908).
7. National Bureau of Standards, *Tables of Scattering Functions for Spherical Particles* (Government Printing Office, Washington, D.C., 1949), p. ix; R. O. Gumprecht and C. M. Sliepcevich, *Tables for Light-Scattering Functions for Spherical Particles* (Univ. of Michigan Press, Ann Arbor, 1951), p. x; *Tables of Riccati-Bessel Functions for Large Arguments and Orders* (Univ. of Michigan Press, Ann Arbor, 1951), p. xii.
8. B. J. Mason, *Discuss. Faraday Soc.* **30**, 20 (1960).
9. T. A. Rich, *Geofis. Pura Appl.* **31**, 60 (1955).
10. The critical radius depends on the nature of the particles. Equation 3 applies to homogeneous nucleation and to water-insoluble particles. In water-soluble particles the activity of the solution must also be taken in account (*11*). In the absence of these data and, indeed, in the case of air, with no knowledge of the nucleating species, we use Eq. 3 without correction for particle activity.
11. K. G. Vohra and P. V. N. Nair, *J. Atmos. Sci.* **28**, 280 (1971).
12. Gardner Associates, Schnectady, New York.
13. Beta-ray energy absorbed by O_2 and N_2 in moist air produces OH radicals within 10^{-8} second even at H_2O pressures of 0.2 torr. (S. Gordon and W. A. Mulac, unpublished results.
14. E. J. Hart and K. H. Schmidt, *Radiat. Res.*, in press.
15. N. R. Greiner, *J. Chem. Phys.* **46**, 2795 (1967).
16. K. G. Vohra, K. N. Vasudevan, P. V. N. Nair, *J. Geophys. Res.* **75**, 2951 (1970).
17. Work performed under the auspices of the U.S. Atomic Energy Commission.
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Genetic Polymorphism of Proline-Rich Human Salivary Proteins

Abstract. *In randomly collected saliva samples from 120 Caucasians, 79 Blacks, and 40 Chinese, three phenotypes were observed by electrophoresis in alkaline slab polyacrylamide gels. The proteins showing polymorphism were identical with four previously characterized proline-rich proteins. Inheritance is controlled by two autosomal codominant alleles. The gene frequencies were for Caucasians,* $Pr^1 = 0.73$, $Pr^2 = 0.27$; *for Blacks,* $Pr^1 = 0.80$, $Pr^2 = 0.20$; *for Chinese,* $Pr^1 = 0.84$, $Pr^2 = 0.16$.

A new genetic polymorphism among a set of four proline-rich proteins from parotid saliva is described. These proteins, designated as proteins I, II, III, and IV, have been isolated and partially characterized (*1*). They display chemical features which in certain respects resemble collagen, and in other ways have a similarity to enamel protein. These proteins are notably rich in proline, glycine, glutamine, and asparagine (*1*) and have a marked affinity for hydroxyapatite (*2*). Our finding of a common genetic polymorphism among proline-rich salivary proteins should stimulate the search for a possible relation between genetic variation of these proteins and oral disease (*3*). The high frequency of this polymorphism among all the populations studied indicates that it may be useful for other types of genetic research, especially linkage studies.

Electrophoresis of concentrated parotid saliva in alkaline slab polyacrylamide gels and staining with a 3,3′-dimethoxybenzidine–hydrogen peroxide solution revealed a series of rapidly migrating negative-staining bands on an opaque or brown background (*4, 5*).

These proteins exhibit unusual characteristics when stained with amido black and Coomassie blue (*1*). In a collection of random samples from different racial groups, three patterns were recognized among four of these proteins (Fig. 1: I, II, III, and IV). Proteins I and II in region A and proteins III and IV in region B show parallel changes in the different samples. Negative-staining proteins other than I, II, III, and IV (Fig. 1) were

not apparently related to this polymorphism. These unrelated proteins were concentrated in region A, and one of them occasionally overlapped protein I. Typing of the patterns by inspection of the proteins in region B is consequently more reliable.

The electrophoretic patterns of the proteins I, II, III, and IV are probably determined by two autosomal codominant alleles at a locus which we designate *Pr* (proline-rich), for reasons considered below. The Pr^1 was postulated as the determinant of proteins I and III; and Pr^2, as the determinant for proteins II and IV. The pattern showing a single slow protein (protein I) in region A and a single slow protein (protein III) in region B (with an occasional trace of protein at the positions of proteins II and IV) was postulated to represent the common homozygous (1-1) type determined by allele Pr^1 (Fig. 1, channels 3 and 4). The pattern with two closely spaced proteins (I and II) in region A and two closely spaced proteins (III and IV) in region B was postulated to represent the heterozygous (1-2) type determined by alleles Pr^1 and Pr^2 (Fig. 1, channels 2 and 6). The pattern with the single fast protein II in region A and the single fast protein IV in region B (with an occasional trace of protein seen just ahead of proteins II and IV) was postulated to represent the uncommon homozygous 2-2 type determined by Pr^2 (Fig. 1, channels 1 and 5).

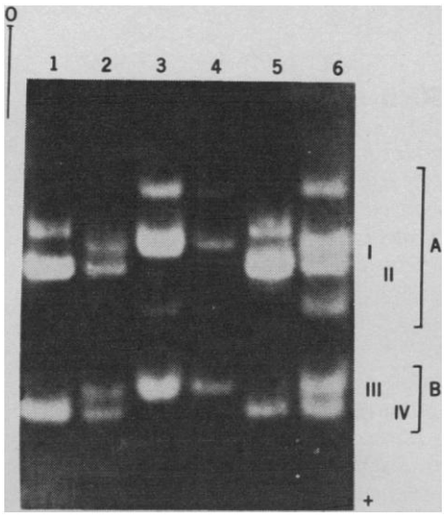

Fig. 1. Proline-rich protein phenotypes. Parotid saliva samples were concentrated ten times, subjected to electrophoresis in alkaline polyacrylamide-gel slabs, and stained with a solution of 3,3′-dimethoxybenzidine and hydrogen peroxide. Only the distal two-thirds of the gel from the origin (*O*) is shown. Serum albumin (not shown) migrates in a position between proteins II and III. Channels 1 to 6 contain samples from different Caucasians. Proteins I and III represent the products of Pr^1, and proteins II and IV represent the products of Pr^2. (Channels 3 and 4) Homozygous Pr 1-1 type (proteins I and III); (channels 2 and 6) heterozygous Pr 1-2 type (proteins I, II, III, and IV); (channels 1 and 5) homozygous Pr 2-2 type (proteins II and IV).

Randomly collected parotid saliva samples were studied for the frequencies of presumed phenotypes, and application of the Hardy-Weinberg rule gave good agreement between observed and expected values indicating excellent fit by the χ^2 test ($P = .89$), supporting the genetic hypothesis. Among samples from 120 Caucasians, 66 were common 1-1 homozygotes (63.9 expected), 44 were 1-2 heterozygotes (47.3 expected), and 10 were uncommon 2-2 homozygotes (8.8 expected). Among samples from 79 Blacks there were 52 of the 1-1 type (50.5 expected), 22 of the 1-2 type (25.3 expected), and 5 of the 2-2 type (3.2 expected). Samples from 40 Chinese showed 28 of the 1-1 type (28.1 expected), 11 of the 1-2 type (10.9 expected), and 1 of the 2-2 type (1.0 expected). From these data gene frequencies were: for Caucasians, Pr^1 is 0.73, Pr^2 is 0.27; for Blacks, Pr^1 is 0.80, Pr^2 is 0.20; for Chinese, Pr^1 is 0.84, Pr^2 is 0.16. Family data including 24 families and 57 children are in complete agreement with our genetic hypothesis. Of eight matings where both parents were 1-2 heterozygotes, there were five 2-2 homozygotes, nine 1-2 heterozygotes, and one 1-1 homozygote. Of nine matings where both parents were 1-1 homozygotes, all 24 children were 1-1 homozygotes. Of seven matings where one parent was a 1-2 heterozygote and the other a 1-1 homozygote, there were nine 1-2 heterozygotes and nine 1-1 homozygotes.

These proteins (I, II, III, and IV) showing polymorphism were suspected (on the basis of several lines of evidence) to be identical with the closely related set of four, small, proline-rich proteins already isolated and characterized from pooled parotid saliva (*1*). First, polymorphic proteins I, II, III, and IV and the proline-rich salivary proteins show the unusual property of being difficult to stain with amido black and Coomassie blue. Second, after slab polyacrylamide-gel electrophoresis, there was marked resemblance in the pattern of the four polymorphic proteins with the previously reported disc-gel electrophoretic patterns of the four proline-rich proteins isolated from pooled parotid saliva (*1*). Third, if it is assumed that the most common genetic type in the pool contributes proteins I and III, one would expect that the recovery of proteins I and III from pooled parotid saliva should exceed that of II and IV, and this is what was observed. Thus, in a typical experiment (*1*), 21 mg, 8 mg, 22 mg, and 10 mg of proteins I, II, III, and IV, respectively, were recovered as final

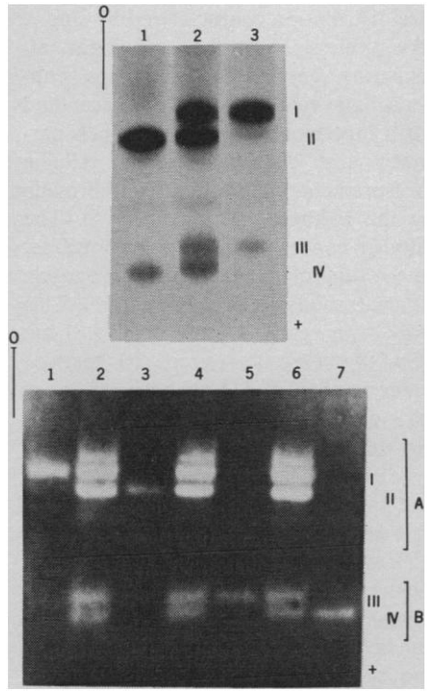

Fig. 2. (Top) Purified proline-rich proteins from individual parotid saliva samples of different genetic types after disc-gel electrophoresis. The gels were stained with amido black, and only the distal portions from the origin (*O*) are shown. The faint band between proteins II and III is serum albumin, used as a marker to align the three gels. A trace of an additional protein is migrating slightly anodally to proteins I and II. (Gel 1) Proline-rich proteins from a Pr 2-2 homozygote; (gel 2) proline-rich proteins from a Pr 1-2 heterozygote; (gel 3) proline-rich proteins from a Pr 1-1 homozygote. (Bottom) Unpurified parotid saliva proteins showing genetic polymorphism compared with the four purified proline-rich proteins after slab polyacrylamide-gel electrophoresis. The gel was stained with a solution of 3,3′-dimethoxybenzidine and hydrogen peroxide, and only the distal half of the gel from the origin (*O*) is shown. The protein migrating slightly cathodally to protein I (channels 2, 4, and 6) is unrelated to the genetic polymorphism. (Channel 1) Proline-rich protein I; (channel 3) proline-rich protein II; (channel 5) proline-rich protein III; (channel 7) proline-rich protein IV; (channels 2, 4, and 6) unpurified parotid saliva proteins showing genetic polymorphism from a Pr 1-2 heterozygote.

yields from pooled parotid saliva. Fourth, parotid saliva samples of the three phenotypes, as determined by the 3,3′-dimethoxybenzidine stain on polyacrylamide gel, were subjected to fractionation on a Sephadex G-50 column. The individual fractions were monitored by disc-gel electrophoresis. In the elution profile of the two homozygous samples, only proline-rich proteins I and III or II and IV could be detected, whereas, in the case of the heterozygous sample, all four proline-rich proteins were detected (Fig. 2, top). Finally, the electrophoretic mobility of the four purified, proline-rich proteins was identical with the unpurified salivary proteins exhibiting genetic polymorphism on slab polyacrylamide-gel electrophoresis (Fig. 2, bottom). These data make it highly likely that the proteins showing polymorphism are identical to the proline-rich proteins previously purified and characterized (*1*).

These proline-rich proteins were also found in submaxillary fluid from adults as well as in the parotid saliva of newborns and premature infants. Interconversion, or disappearance, of the proline-rich proteins was not seen after incubation of the saliva at 37°C for up to 2 hours. Furthermore, patterns of proline-rich proteins were easy to type and were stable after storage of samples for at least 9 months at −70°C. Initial data (*6*) from linkage studies indicate that there is no correspondence between the proline-rich protein phenotypes and the phenotypes observed in the following genetic systems: ABO, Rh, MNSs, Kell, Kidd, Lutheran, Duffy, Lewis, secretor, haptoglobin, transferrin, group-specific components, parotid basic protein, and hemoglobin (beta chain determinant).

The parotid saliva contains many interesting proteins, only a few of which have been isolated and characterized. It is also an easily obtained source of secretory proteins and has proved useful in the search for new genetic polymorphisms, as indicated by the proline-rich (Pr) protein polymorphism herein described and the recently reported parotid basic (Pb) protein polymorphism in man (*5*).

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References and Notes

1. F. G. Oppenheim, D. I. Hay, C. Franzblau, *Biochemistry* **10**, 4233 (1971).
2. D. I. Hay, *J. Dent. Res.* **48**, 806 (1969).
3. R. J. Gorlin, R. E. Stallard, B. L. Shapiro, *J. Periodontol.* **38**, 5 (1967); P. N. Baer, L. B. Crittenden, G. E. Jay, J. E. Lieberman, *J. Dent. Res.* **40**, 23 (1961); C. J. Witkop, unpublished data: W. J. Schull, J. V. Neel and many Japanese collaborators, *The Effects of Inbreeding on Japanese Children* (Harper & Row, New York, 1965), p. 228; C. S. Chung, D. W. Runck, J. D. Niswander, S. E. Bilben, M. C. W. Kau, *J. Dent. Res.* **49**, 1374 (1970); C. J. Witkop, Ed., *Genetics and Dental Health* (McGraw-Hill, New York, 1961).
4. Parotid fluid samples were collected from adults, children, and infants with the use of an acrylic capsule and hard lemon candy stimulation (*5*): Samples were routinely stored at −70°C. A standard alkaline polyacrylamide-gel slab was prepared by mixing 200 ml of tris borate–boric acid buffer, *p*H 8.6 to 8.9 (0.088*M* in tris), 11.0 g of Cyanogum (E-C Apparatus Corp., Philadelphia), 0.2 ml of *N, N, N′,N′*-tetramethylethylenediamine, and 2.0 ml of 10 percent ammonium persulfate. Gels were cast in a vertical electrophoresis apparatus with the use of a special Teflon slot former (E-C Apparatus Corp.). The bridge solution was of the same composition as the gel buffer. Prior to electrophoresis, frozen samples were lyophilized and dissolved in tris borate–boric acid gel buffer to concentrate the samples about five to ten times. Sucrose (15 mg/0.1 ml) and bromphenol blue as a marker were also added. The samples were centrifuged at 35,000*g* for 15 minutes and were electrophoresed in the polyacrylamide gels for approximately 3½ hours at constant voltage (250 volts) with tap water (12° to 15°C) cooling, after a preliminary run of about 2 hours. The gels were then stained at room temperature for about 30 to 60 minutes with a solution containing 400 to 600 mg of 3,3′-dimethoxybenzidine (Eastman) and 5.0 ml of glacial acetic acid per 100 ml. The resulting patterns could be seen as translucent gray-blue bands against a gray, somewhat opaque background when viewed by reflected light against a black background. The patterns could be typed and photographed, but the background could be advantageously darkened by addition of hydrogen peroxide. The staining solution was then poured off and fresh staining solution was added to which 0.1 to 0.3 ml of hydrogen peroxide per 100 ml of solution was added. The solution turned brown, and after about 30 to 60 minutes at room temperature, a pattern of white bands could be seen against a brown background by transmitted light. Depending on the lot used, the concentration of 3,3′-dimethoxybenzidine and hydrogen peroxide may need to be varied to produce the proper brown color for rapid staining. To remove a superficial brown deposit on the gel after staining, the surface was rinsed with water and gently rubbed by a hand covered with a latex glove. The phenotypes were easy to visualize, although they were more difficult to photograph. When the patterns could not be seen because of inadvertent overstaining, the gel could be partially destained with visualization of the patterns within several hours after washing in 2 percent ascorbic acid. Although the reason for negative staining reaction of the proline-rich proteins is not entirely clear, it appears that when the gel is initially exposed to acidified 3,3′-dimethoxybenzidine, the 3,3′-dimethoxybenzidine precipitates in the gel, but this precipitation is inhibited at the positions of the proline-rich proteins, which appear as translucent bands against an opaque background. In the next step, after addition of fresh stain solution containing hydrogen peroxide, the opaque background takes a brown stain and the proline-rich proteins appear as white bands by transmitted light.
5. E. A. Azen, *Science* **176**, 673 (1972); *Biochem. Genet.*, in press.
6. W. Bias and E. A. Azen, unpublished results.
7. Supported by NIH grants AMO 9555-07, DEO 3658-08, and DEO 3433-01. We thank Mrs. H. Lin for technical assistance, Dr. W. Bias for doing genetic typing and providing saliva samples, and Dr. O. Smithies for helpful discussions.

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Virus-Induced Transformation without Cell Division

Abstract. *Interference with the cell cycle by vinblastine sulfate immediately after cells were infected with Rous sarcoma virus had little effect on the development of two metabolic changes that occur in transformed cells. These results, along with an earlier demonstration of morphological changes developing in infected nondividing cells, demonstrate that the phenotypic development of the malignant state can occur without the intervention of cell divisions after infection by Rous sarcoma virus.*

Transformation of cells to malignant forms commonly is observed several cell generations after the initial carcinogenic event. The assessment of transformation in cell culture is based on morphological changes in the cells (such as increased rounding or refractility), on altered associations between cells (for instance, more random orientation or increased cell density at growth saturation), or on altered growth potential (such as increased growth in suspension). Although the malignant change can occur in the single cell, the identification of malignant transformation usually has required an accumulation of transformed cells several cell generations after induction. Transformation by Rous sarcoma virus (RSV), particularly the Bryan "high titer" strain, has the unique advantage that single transformed cells can be identified by their characteristic morphology. The ability to identify individual transformed cells allowed us to show earlier that a single round of DNA synthesis was sufficient for successful transformation by RSV (*1*), and that morphological transformation can occur in RSV-infected cells that have not divided (*2*).

Morphological changes may be considered an insufficient criterion for malignant transformation, and are difficult to assess quantitatively. However, biochemical changes often accompany malignant transformation, and these can be used as a measure of transformation. Cells transformed by RSV have an increased capacity for hexose