host animal is well known in chronic protozoal infections (*13*). However, the only prior example of viral antigenic shift within a persistently infected host animal has been in equine infectious anemia (EIA) virus infections of horses (*14*). In this disease, recurrences of viremia are associated with hemolysis and fever. The disease remits with the development of neutralizing antibody and relapses when antigenically altered virus appears in the plasma. The EIA virus is also a retravirus (*15*), but lysogenic infection of horses has yet to be demonstrated. The chronic demyelinating disease in sheep caused by visna virus does not appear to be a remitting and relapsing disease, but the apparent progressive neurological disease may represent a cumulative effect of clinically inapparent attacks induced by intermittent release of antigenically altered virus. Our studies of visna suggest that lysogeny and antigenic shift of a virus may combine to provide a unique mechanism for remitting and relapsing disease. Such mechanisms of pathogenesis might be considered in multiple sclerosis or relapsing nonneurological diseases of suspected viral etiology.

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New Genetic Marker in Human Parotid Saliva (Pm)

Abstract. *In 195 parotid saliva samples collected at random from a Japanese population, two phenotypes were observed by electrophoresis in acid-urea starch gels. The protein showing polymorphisms was detected in the middle zone between Pa and Pb, and was tentatively designated Pm. Inheritance was controlled by a dominant allele at an autosomal locus. The frequencies of the genes determining these phenotypes were, for the Japanese population studied,* $Pm^+ = .38 \pm .03$, $Pm^- = .62 \pm .03$.

A new genetic polymorphism from parotid salivary proteins is described herein. By means of acid-urea starch gel electrophoresis, Azen (*1*, *2*) detected a polymorphism in parotid basic proteins (Pb) of American blacks and, using a similar system, Friedman *et al.* (*3*) detected a polymorphism in an acidic salivary protein (Pa) present in whole, parotid, and submandibular saliva of certain individuals. By a modification of these methods, we have detected another genetic polymorphism in a Japanese population. This polymorphic protein is tentatively designated Pm (salivary parotid middle-band protein), and separates electrophoretically in the zone between Pa and Pb.

Determination of Pm phenotypes was also possible by the method described by Azen (*1*), and we used the following conditions for accurate typing. Samples of parotid saliva (whole saliva that was immediately frozen and lyophilized) were collected in glass test tubes with a double-chamber cup of the Curby type; the samples were rapidly frozen, lyophilized, and stored at −20°C for later analysis. Just before being subjected to electrophoresis, the dried samples were dissolved in gel buffer solution containing 8*M* urea in order to concentrate the proteins by approximately ten times. This treatment facilitated separation of the Pm band. The starch gel contained: starch (Connaught, Toronto), 79 g; urea, 120 g; commercial aluminum lactate, 3.53 g; and lactic acid, 11.4 ml in 400 ml

Table 1. Distribution of Pm phenotypes among Pa(+) and Pa(−) individuals. All 145 samples were of Pb (1-1) type. Observed and expected values were compared: 2 by 2 contingency, $\chi^2 = 1.066$; $.30 < P < .50$.

Individual	Pm phenotype				
	Pm(+)	Expected value	Pm(−)	Expected value	Total
Pa(+)	31	34	27	24	58
Pa(−)	54	51	33	36	87
Total	85		60		145

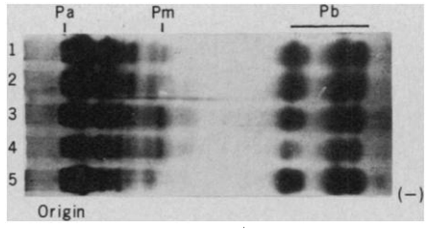

Fig. 1. Photograph of a gel on which parotid saliva was subjected to electrophoresis according to the method described by Azen (*1*). Channels 1 to 3 show the results of confirmation tests: mixtures (equal volumes) of Pm(+) and Pm(−) samples in channel 1; Pm(−) and Pm(−) samples in channel 2; and Pm(+) and Pm(+) samples in channel 3. Channel 4, Pm(+) from individual A; channel 5, Pm(−) from individual B.

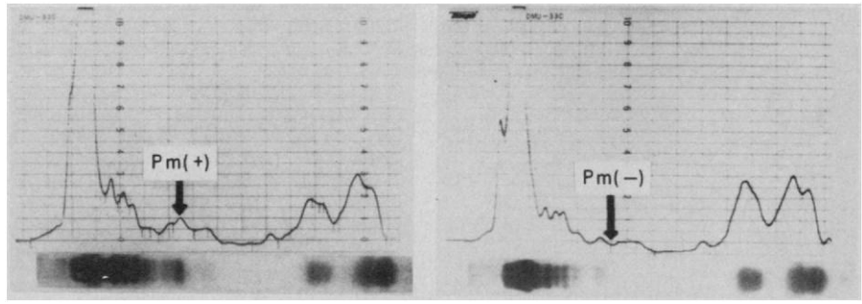

Fig. 2. Densitometric tracing patterns of Pm(+) and Pm(−) parotid saliva samples after electrophoresis. The Pm(+) sample has a peak in the zone indicated by the arrow, while the Pm(−) sample lacks this peak.

of distilled water. The bridge of 0.03*M* aluminum lactate buffer was adjusted to *p*H 2.4 with lactic acid. Two sheets of filter-paper strips were inserted 4 cm from the anodal end of the gel (12 by 16 by 0.6 cm), and 30-μl samples were applied. Horizontal electrophoresis was performed at a constant voltage of 150 volts for 12 to 13 hours. The gel surface was covered with Saran wrap to prevent evaporation, and a box containing ice was placed on the gel. During electrophoresis the gel current intensity was approximately 45 ma. Prior to being stained, the 6-mm thick gel was cut into 2-mm slices; the upper slice was discarded and the lower two slices were stained by the method developed by Sung and Smithies (*4*) for arginine-rich proteins.

Figure 1 shows the Pm band located between bands Pa and Pb. The Pm phenotypes are determined by the presence (+) or absence (−) of this middle-band protein. Figure 2 shows that the densitometric tracing pattern of the Pm(−) sample lacks the peak typical of Pm(+) samples.

In Fig. 3, which shows a gel used for the electrophoresis of parotid saliva samples from five individuals, the Pb proteins have migrated out from the gel frame. By this method, the migration distance of the Pm band was increased, and the separation of the Pm zone was improved. The band that migrates after the Pm band can be recognized in both Pm(+) and Pm(−) samples and simplifies Pm typing. Saliva collected from Pm(−) individuals at different times of the day and on different days never revealed the Pm protein. The Pm protein is also observed in whole saliva and seems to be fairly stable compared with other salivary proteins.

The Pm(+) phenotype has been observed in both of the two racial populations studied, Japanese and Chinese. Among 195 samples from the Japanese population, 120 (61.5 percent) were of the Pm(+) type and 75 (38.5 percent) were of the Pm(−) type. Among 20 samples from the Chinese population, ten were Pm(+) and the other ten were Pm(−).

Family studies including 28 families and 59 offspring were done to test the genetic hypothesis. Of ten matings where both parents had the Pm(+) phenotype, there were 24 Pm(+) and five Pm(−) offspring. Of eight matings where both parents were Pm(−), all 11 offspring had the expected Pm(−) phenotype. Of ten matings where one parent was Pm(+) and the other Pm(−), there were 14 Pm(+) and five Pm(−) offspring. These data are consistent with the hypothesis that the Pm(+) phenotype is an autosomal dominant trait. From this hypothesis, estimates of the frequencies of the genes that determine these phenotypes in Japanese were, for Pm^+, .38 ± .03, and for Pm^-, .62 ± .03.

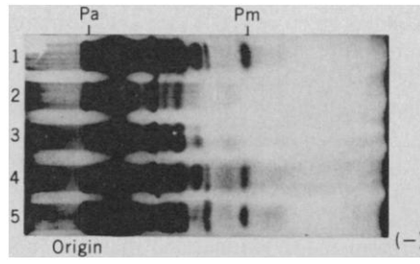

Fig. 3. Electrophoresis of parotid saliva from five individuals by our modified method. For the accurate typing of Pm phenotypes, saliva samples were concentrated approximately ten times and horizontal electrophoresis was performed at a constant voltage of 150 volts (45 ma) for 12 to 13 hours. The Pb proteins have migrated out from the gel. The broad band indicated by a black spot migrates after the Pm band and is recognized in both Pm(+) and Pm(−) samples. Each channel contains a sample from a different individual, and the Pm and Pa phenotypes of each sample are, respectively, channel 1, Pm(+) and Pa(+); channel 2, Pm(−) and Pa(+); channel 3, Pm(−) and Pa(−); channel 4, Pm(+) and Pa(−); and channel 5, Pm(+) and Pa(+).

Table 1 shows the distribution of Pm and Pa phenotypes in 145 unrelated Japanese typed for both marker systems. Estimates of the frequencies of the genes determining Pa^+ and Pa^- individuals in this sample were .23 ± .03 and .77 ± .03, respectively. The observed distribution of 58 Pa(+) (59.0 expected) and 87 Pa(−) (86.0 expected) is consistent with Hardy-Weinberg equilibrium (χ^2 = 0.029; $.80 < P < .90$). When this sample was tested for equilibrium with respect to the two loci, the observed distribution of Pm and Pa phenotypes is compatible with expected equilibrium frequencies (χ^2 = 1.006; $.30 < P < .50$). In addition, all 145 samples were of the Pb (1-1) type (*1*). From these results we concluded that Pm is not related to Pa or Pb.

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In vitro Growth of *Mycobacterium lepraemurium*, an Obligate Intracellular Microbe

Abstract. *By using an ultrasensitive technique to measure adenosine triphosphate in terms of functional biomass, we have confirmed that* Mycobacterium lepraemurium *(the agent of rat leprosy and a classical obligate intracellular microbe) grows in vitro in the Nakamura system. By using a sulfhydryl-containing medium that occupies 65 to 75 percent of the culture tube volume, together with the five supplements recommended by Nakamura, we have obtained growth rates some eight times above the original. The new physicochemical environment and the use of adenosine triphosphate as an index of energy status in the presence and absence of growth provide a basis for investigating the physiology and growth of other noncultivated microbes.*

For 103 years (1873 to 1976) the inability to cultivate *Mycobacterium leprae* in vitro has been a major bottleneck in leprosy research. For 73 years (1903 to 1976) a second "obligate intracellular" mycobacterium, *M. lepraemurium* (the agent of rat leprosy), has been used by many investigators as an interim model because (i) its growth potential is higher than that of *M. leprae* and (ii) it is transmissible in rats and mice. The decades of attempts to cultivate these bacteria in vitro suggest that success will require more than the testing of different combinations of nutrients and will depend in part upon the application of new principles or conditions.

Using firefly bioluminescence, we have developed (*1*) an ultrasensitive method for measuring adenosine triphosphate (ATP) in terms of functional biomass (the percentage of ATP in the suspension or culture) in unwashed host-grown bacteria and in bacteria grown in