

# INHERITANCE OF A PAROTID SECRETORY PROTEIN IN MICE AND ITS USE IN DETERMINING SALIVARY AMYLASE QUANTITATIVE VARIANTS

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## ABSTRACT

Among inbred strains of mice, a major protein, PSP, produced and secreted by the parotid glands, shows variation in electrophoretic mobility and in the peptides produced by cyanogen bromide treatment. This variation is inherited as a single Mendelian factor with two alleles showing co-dominant expression. In genetic crosses, it segregates independently from the amylase complex and is closely linked to the agouti locus on chromosome 2. The protein ratios between amylase and PSP in saliva, obtained by scanning of electrophoretic gel separations, were found to reflect genetic differences in salivary amylase production in strains YBR/Cv and C3H/As.

MUTATIONS at structural loci or at regulatory loci (PAIGEN 1971; BULFIELD 1972) can cause quantitative variation of enzymes and can, therefore, be used to investigate the types of genetic control mechanisms that exist in mammals (PAIGEN *et al.* 1975). Amylases of the mouse (*Mus musculus*) may be good model systems for investigating these genetic control mechanisms because they are subject to both quantitative and qualitative variation. Two tissue-specific amylases, from the parotid glands and pancreas, are present in the mouse, and the electrophoretic variation in each of these define the *Amy-1* and *Amy-2* loci, respectively (SICK and NIELSEN 1964). The loci are closely linked; no confirmed recombinants have been found (NIELSEN and SICK 1975). This complex of amylase loci is located on chromosome 3 (TAYLOR 1976; EICHER 1979). The demonstration of structural heterogeneity of pancreatic amylase in homozygous animals and genetic variation in the amounts of various forms of this amylase indicate a genetic determination by multiple structural genes for these enzymes (NIELSEN 1974; HJORTH, LUSIS and NIELSEN 1980). Salivary amylase shows no structural heterogeneity in homozygous animals, and the observed genetic variation in relative quantities of the electrophoretic forms in heterozygotes may reflect variation in genetic regulatory elements linked to the structural variation (HJORTH 1979; HJORTH, LUSIS and NIELSEN 1980).

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Thus, the genetic variation in amylase from each of the two tissues indicates a complex genetic determination of the enzymes.

Amylase levels are quite variable among individual rodents due to physiological changes in the stored secretory material. For example, in YBR/Cv, salivary amylase concentrations vary from 20 to 100 amylase units per gland tissue, so that mean values from 5 to 10 animals have to be used to demonstrate a two- to three-fold difference between strains (HJORTH 1979). This prompted the use of heterozygote ratios of allelic amylase isoenzymes. However, the sole use of heterozygotes limits the types of genetic variation that can be detected. It also puts serious constraints on attempts to find variants. In the present study, therefore, we explore the possibility of using a salivary protein as an internal standard for the quantification of salivary amylase in homozygous animals. This article describes the biochemical and genetic characterization of a parotid secretory protein (PSP) in the mouse. PSP shows genetic variation in structure, which is determined by a single locus linked to the agouti locus in chromosome 2; this locus and the amylase complex segregate independently. We have found that the quantitative difference of salivary amylase production between the strains YBR/Cv and C3H/As, previously described (HJORTH 1979), is reflected by the protein ratio of amylase to PSP in whole saliva. Application of the AMY/PSP ratio in screening for quantitative variants is discussed.

#### MATERIALS AND METHODS

*Materials:* Radioactive isotopes were obtained from Amersham, cyanogen bromide (CNBr) from Sigma, formic acid from Merck, Lipoluma and Lumasolve from Lumac Systems and acrylamide from Eastman Kodak. All other materials were of reagent grade.

*Animals and homogenates:* The C3H/As strain originated at the Statens Serum Institute, Copenhagen, and has been further inbred in this laboratory for 18 years. The CE/J, DE/Cv and YBR/Cv strains were kindly provided by V. M. CHAPMAN, Roswell Park Memorial Institute, Buffalo, and AKR/Bom, BALB/cBOM, CBA/JBom, C57BL/6JBom, C57BL/10JBom, DBA/2Bom, NZB/Bom and SJL/JBom were purchased from Gl. Bomholtgård Ltd., Denmark. The congenic strains C3H.Amy<sup>CE</sup>, C3H.Amy<sup>YBE</sup>, C3H.Amy<sup>w1</sup>, C3H.Amy<sup>w3</sup>, C3H.Amy<sup>w9</sup>, C3H.Amy<sup>w10</sup>, C3H.Amy<sup>w11</sup>, C3H.Amy<sup>w13</sup>, C3H.Amy<sup>w14</sup> and C3H.Amy<sup>w15</sup> were constructed by J. T. NIELSEN (Institute of Ecology and Genetics, University of Aarhus). This was accomplished through allelic transfer of the amylase complex from either a strain (for instance CE/J) or trapped wild mice into a C3H/As background by backcrossing for 10 generations using the electrophoretic difference of salivary amylase as a marker.

Parotid glands were labelled by intraperitoneal injection of either 100  $\mu$ Ci L-(4,5-<sup>3</sup>H)-leucine (105 Ci/mmole), 25  $\mu$ Ci L-(U-<sup>14</sup>C)-leucine (300 mCi/mmole), or 500  $\mu$ Ci <sup>35</sup>S-methionine (500 Ci/mmole) for an hour pulse. Animals were sacrificed and parotid homogenates were prepared as previously described (HJORTH 1979).

*Cyanogen bromide treatment:* Cyanogen bromide (CNBr) cleavage of parotid homogenates was carried out using 10-fold excess (w/w) of CNBr over protein in 70% formic acid for 48 hr in the dark at room temperature. Samples were subsequently diluted 10-fold with water, lyophilized and stored at -20°. In one experiment, amylase was first selectively removed from the homogenate by an affinity chromatography procedure (SILVANNOVICH and HILL 1976). The run-through elutant was lyophilized and treated with CNBr.

*Polyacrylamide gel electrophoresis:* Discontinuous electrophoresis in sodium dodecyl sulfate was performed using the method of LAEMMLIC (1970). Small peptides were resolved by a modification of the method of LAEMMLIC in which the separating gel contained 13% acrylamide, 0.35%

N,N'-methylene bis acrylamide and 8M urea. A constant fraction of the parotid glands from the animals to be compared was applied to each gel slot. After electrophoresis, the gels were stained with Coomassie blue R-250. This localized the molecular weight standards, albumin (68,000), ovalbumin (43,000), chymotrypsinogen (26,000), myoglobin (17,000) and CNBr fragments of myoglobin (14,900, 8,300, 6,400 and 2,600).

The amount of  $^3\text{H}$ -leucine in protein was determined on individual slices of polyacrylamide gels after eluting in Lipoluma:Lumasolve:Water (9:1:0.1) at 50° for 48 hr. By this procedure, essentially 100% elution is achieved. The percent degradation of PSP by CNBr was then calculated from the amount of radioactivity in the 8,000 molecular-weight slice divided by the sum of the radioactivity in the 8,000 and 20,000 molecular-weight slices and multiplying this ratio by 100. The  $^{35}\text{S}$ - or  $^{14}\text{C}$ -labelled proteins were visualized by autoradiography of the dried gel, using Kodak X-OMAT R film.

*Isoelectric focusing:* Isoelectric focusing was performed as described by HJORTH, LUSIS and NIELSEN (1980). PSP was identified by autoradiography of the dried gel.

*Carbohydrate staining:* Carbohydrate was stained by the periodic acid Schiff procedure (GLOSSMAN and NEVILLE 1971).

*Electrophoretic separation and quantification of salivary proteins:* Isoproterenol-induced saliva was collected and prepared for electrophoresis as previously described (HJORTH 1979), except that samples were collected at 5, 10 and 15 min time points after injection of 0.1 ml of 0.1% isoproterenol sulfate. Native-gel electrophoresis was performed in vertical slab gels using a Tris-glycine, pH 8.1 buffer (CLARKE 1964) with 6% acrylamide at 200 V for 1 hr and then 400 V for 3 hr. Tap-water cooling was used. Gels were fixed in methanol:water:acetic acid (5:5:1), and protein was visualized by staining for 15 to 20 hr with 0.3% Coomassie blue R-250 dissolved in the above fixer. Gels were destained in the methanol:water:acetic acid (5:5:1) solution and reswollen in 5% acetic acid to their original volume.

Degradation of PSP was detected on the protein-stained native gels by the partial or total disappearance of the PSP band and the appearance of distinct degradation bands. PSP was often found to be degraded in saliva from adult male animals and, occasionally, in saliva from female animals. New saliva samples were collected from animals whose original samples contained degraded PSP. No degradation was ever seen in samples from parotid gland homogenates. The degradation could, however, be produced *in vitro* by adding submaxillary gland extracts to parotid gland homogenates. Therefore, the degradation of PSP probably occurs in the mouth by the action of proteases secreted by glands other than the parotids. PSP from YBR/Cv is more sensitive to this degradation than PSP from C3H/As and the degradation products of the two different PSP types show different banding patterns when analyzed by native gel electrophoresis.

The protein ratio of AMY/PSP was obtained by scanning the wet gels at 550 nm using a Beckman CDS-100 Computing Densitometer System (Model R-112). Mean values for saliva from the 5, 10 and 15 minute collections were then calculated. To avoid PSP degradation, the AMY/PSP ratio was determined on saliva only from female animals.

## RESULTS

*Characterization of PSP:* Pulse labelling of the mouse parotid glands by injection of  $^{14}\text{C}$ -leucine and separation of the proteins by SDS-polyacrylamide gel electrophoresis, reveals a major protein of approximately 20,000 molecular weight (Figure 1). After an hour pulse, the mouse parotid glands incorporate 65 to 85% of the acid-insoluble, radio-labelled leucine into this single protein (the actual percentage depends on the strain being tested). It is a tissue-specific protein not found in other salivary glands, such as the submaxillary or sublingual glands, or in the pancreas. It is a glycoprotein, as judged by periodic acid Schiff staining; by isoelectric focusing the protein shows an isoelectric point of approxi-

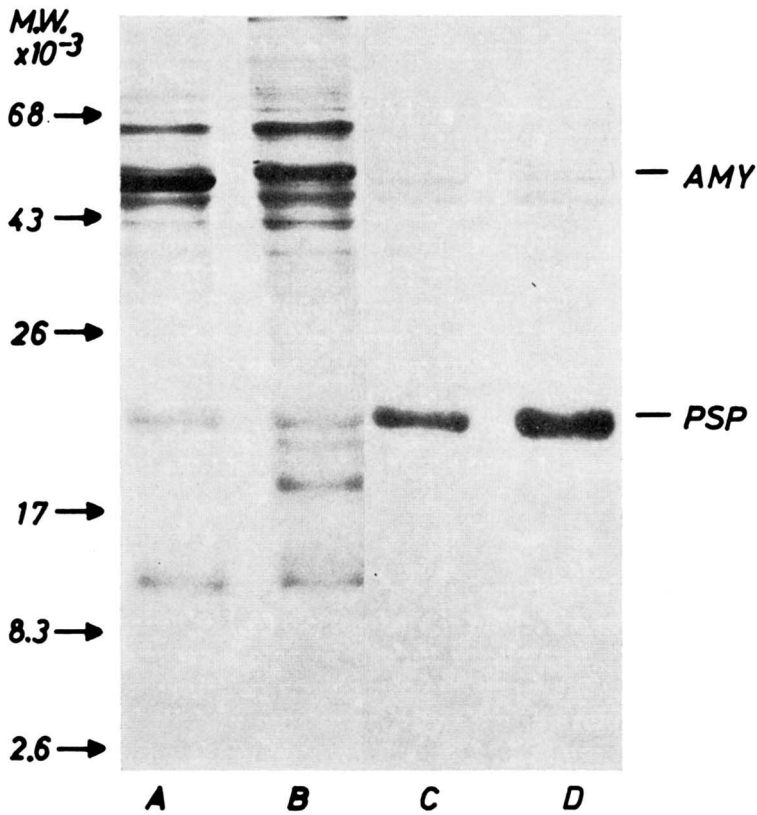


FIGURE 1.—Separation of parotid gland homogenates by SDS-polyacrylamide gel electrophoresis using 13% acrylamide and 8M urea. Channels (A) YBR and (B) C3H are stained for protein with Coomassie blue R-250. Channels (C) YBR and (D) C3H are autoradiograms of  $^{14}\text{C}$ -leucine-labelled homogenates.

mately 5.7. Since it appears in parotid gland homogenates and in whole saliva, we have named this protein PSP (Parotid Secretory Protein).

Pulse labelling of the mouse parotid glands by injection of  $^{35}\text{S}$ -methionine into animals of the inbred strains YBR/Cv and C3H/As and separation of the proteins by SDS-polyacrylamide gel electrophoresis reveals that PSP is labelled in the YBR strain, but not in the C3H strain (Figure 2). This result led us to predict that PSP(YBR) should be cleaved by treatment with cyanogen bromide (CNBr), a reagent that cleaves polypeptide chains after methionine residues, while PSP(C3H) should remain intact. After CNBr treatment for 48 hr, approximately 90% of PSP(YBR) is cleaved into two peptides of about 8,000 molecular weight (Figure 3, channel A). Because the total amount of  $^3\text{H}$ -leucine in PSP(YBR) can be accounted for after CNBr treatment (10% undegraded plus 90% in the 8,000 molecular-weight region), the most probable explanation is that only one methionine is present in PSP(YBR), located near the midpoint of the polypeptide chain. However, the estimated size of low molecular-weight

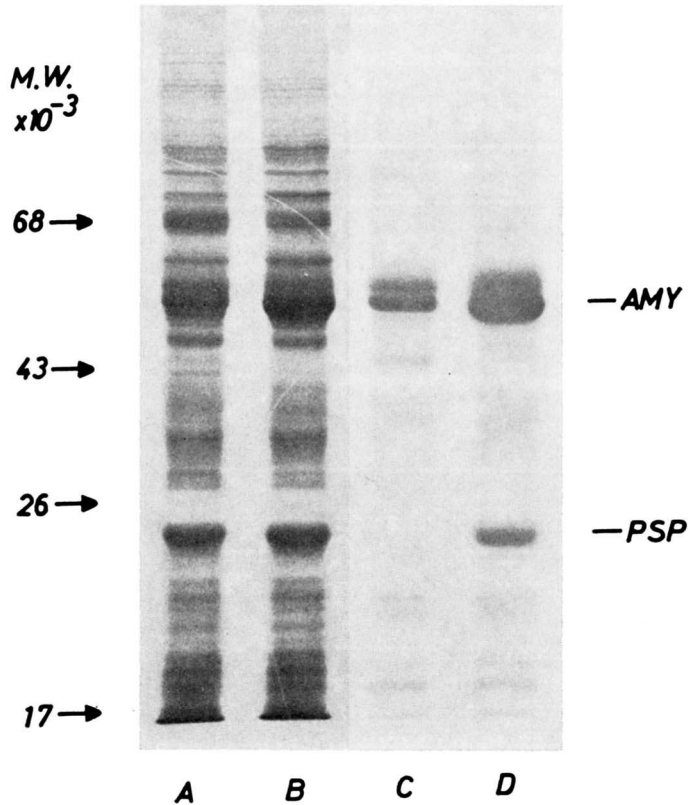


FIGURE 2.—Separation of parotid gland homogenates by SDS-polyacrylamide gel electrophoresis, using 10% acrylamide. Channels (A) C3H and (B) YBR are stained for protein with Coomassie blue R-250. Channels (C) C3H and (D) YBR are autoradiograms of <sup>35</sup>S-methionine labelled homogenates.

peptides obtained from electrophoresis in dodecyl sulfate may be inaccurate by as much as 20% (SWANK and MUNKRES 1971). An alternative explanation is that PSP(YBR) has two methionines, so that three peptides of approximately 7,000 molecular weight are produced by CNBr treatment. Other minor radioactive bands seen in Figure 3 are peptides of salivary amylase produced by the CNBr treatment. These are removed when amylase is selectively removed from the homogenate by affinity chromatography before the treatment with CNBr. PSP(C3H) remains undegraded after treatment with CNBr (Figure 3, channel B). This result, together with the absence of <sup>35</sup>S-methionine label in PSP(C3H), demonstrates that PSP(C3H) contains no methionine residues.

*Genetics of PSP:* Heterozygotes of YBR/Cv and C3H/As had nearly 50% of PSP sensitive to CNBr. Among backcross animals, two PSP, CNBr-sensitivity classes were found: the hybrid class and the homozygous parental class (Figure 3, channels C–G; Figure 4). This demonstrates that PSP is determined by a single locus, *Psp*, and that the alleles defined by CNBr sensitivity are co-domi-

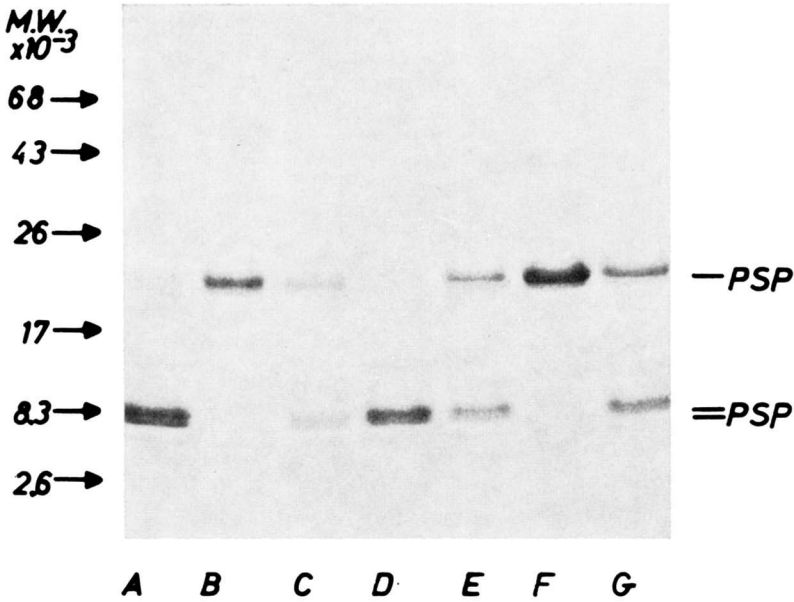


FIGURE 3.—Autoradiograms of  $^{14}\text{C}$ -leucine-labelled homogenates treated by CNBr for 48 hr and analyzed by SDS-polyacrylamide gel electrophoresis, using 13% acrylamide and 8M urea. Channels are (A) YBR, (B) C3H, (C)  $F_1$ , (D and E)  $F_1 \times \text{YBR}$  and (F and G)  $F_1 \times \text{C3H}$ .

nant. Since the PSP from the two alleles differ in primary structure, *Psp* must be the structural gene for the protein. The YBR allele was designated *Psp<sup>a</sup>* and the C3H allele, *Psp<sup>b</sup>*. Among inbred strains, DBA/2Bom and NZB/Bom have the *Psp<sup>a</sup>* allele, while AKR/Bom, BALB/cBom, CBA/JBom, CE/J, C57BL/6JBom, C57BL/10JBom, DE/Cv and SJL/JBom have the *psp<sup>b</sup>* allele.

Examination of whole saliva by native-gel electrophoresis and staining for protein provides an additional and more rapid method for phenotyping PSP (Figure 5). The position of PSP on native gels was originally defined using  $^{14}\text{C}$ -leucine-labelled parotid homogenates (picture not shown). The CNBr-sensitive PSP, from YBR, is fast in native-gel electrophoresis, whereas the CNBr-insensitive PSP, from C3H, is slow; heterozygotes have both bands. In 39 backcross animals to either YBR or C3H (Figure 4), there was 100% concordance between CNBr-sensitivity phenotype and the native-gel phenotype. This was also found for the eight inbred strains mentioned above. The phenotypic differences seen by the two techniques thus may arise from variation at determinants within the same structural locus. The genetic data obtained by native electrophoresis of saliva are shown in Table 1 and include the 39 backcross and eight  $F_1$  animals from Figure 4. These data substantiate the results obtained by the CNBr method, indicating single-gene, co-dominant inheritance of PSP. PSP typing by this native-gel technique is, however, slightly less reliable than that by CNBr treatment because PSP in saliva from males is often degraded, as noted above.

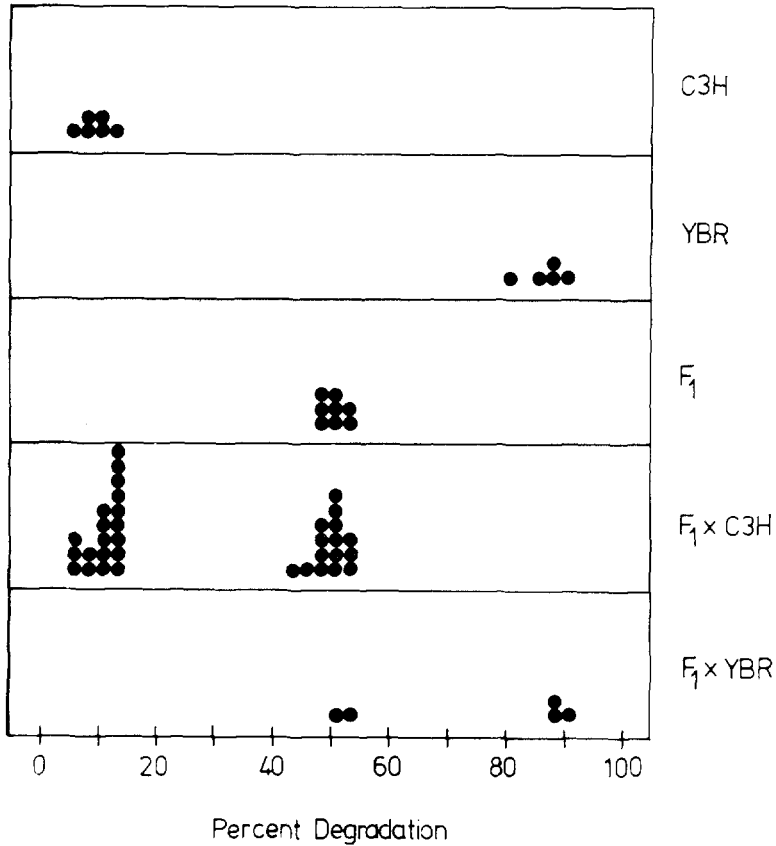


FIGURE 4.—Percent degradation of PSP by CNBr using parotid gland homogenates of individual mice injected with  $^3\text{H}$ -leucine. The percent degradation of PSP by CNBr was calculated as described in MATERIALS AND METHODS.

The localization of *Psp* on the genetic map of the mouse was found through further analysis of animals from the crosses between YBR/Cv and C3H/As. Besides differing for *Psp*, these strains differ at the salivary amylase locus *Amy-1*, the agouti locus (*a*) on chromosome 2 and the brown locus (*b*) on chromosome 4 (HJORTH 1979; STAATS 1976). C3H/As has AMY-A and the dominant coat-color alleles *A* and *B*, whereas YBR/Cv has AMY-B and the recessive coat color alleles *a* and *b*. Of the backcross progeny between F<sub>1</sub> (YBR/Cv × C3H/As) females and YBR/Cv males, 61 were scored visually for their coat-color types and by native-gel electrophoresis for their AMY and PSP types. The alleles at each locus showed Mendelian segregation. The alleles of the *Psp* and *a* loci co-segregate in their parental combinations, and no recombinant types were found (Table 2). This indicates that *Psp* is linked to *a* at a distance that, at the 95% confidence level, does not exceed 5 cM. The data indicate that *Psp-Amy-1*, *Psp-b* and *b-Amy-1* loci are not linked since 31, 31 and 32 animals with recombinant types for these pairs, respectively, were found.

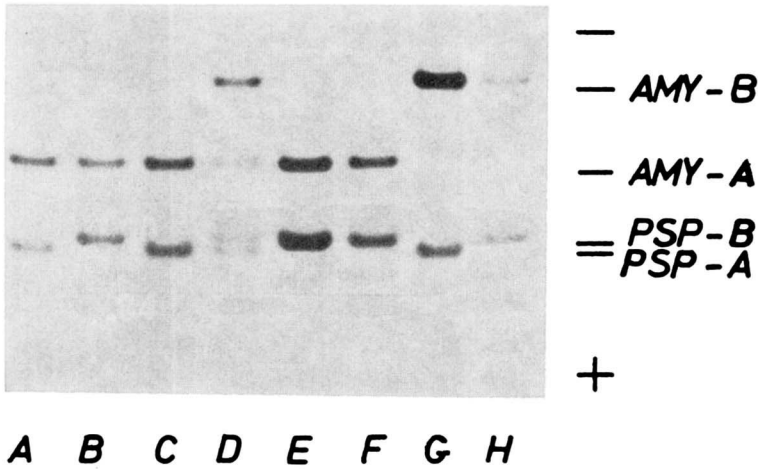


FIGURE 5.—Separation of whole saliva by native-gel electrophoresis. The gels were stained with Coomassie blue R-250. Channels are (A) DBA/2Bom, (B) C3H/As, (C) NZB/Bom, (D) YBR/Cv and C3H/As mixture, (E) AKR/Bom, (F) BALB/cBom, (G) YBR/Cv, and (H) CE/J.

TABLE 1

*Segregation of salivary PSP types revealed by native gel electrophoresis*

Parents	PSP-A ( $Psp^a/Psp^a$ )	Offspring PSP-AB ( $Psp^a/Psp^b$ )	PSP-B ( $Psp^b/Psp^b$ )	Total
PSP-A × PSP-B ( $Psp^a/Psp^a \times Psp^b/Psp^b$ )	0	10	0	10
PSP-AB × PSP-A ( $Psp^a/Psp^b \times Psp^a/Psp^a$ )	24	22	0	46
PSP-AB × PSP-B ( $Psp^a/Psp^b \times Psp^b/Psp^b$ )	0	23	31	54
PSP-AB × PSP-AB ( $Psp^a/Psp^b \times Psp^a/Psp^b$ )	10	16	9	35

The deduced genotypes are given in brackets.

TABLE 2

*Segregation of agouti types and native electrophoretic salivary PSP types among 61 offspring of the backcross  $F_1$  (YBR/Cv × C3H/As) × YBR/Cv*

Cross: ♀ $\frac{a Psp^a}{A Psp^b} \times \delta \delta \frac{a Psp^a}{a Psp^a}$		
Parental types:	Offspring types	Number
	agouti, PSP-AB ( $A Psp^b/a Psp^a$ )	28
	nonagouti, PSP-A ( $a Psp^a/a Psp^a$ )	33

The deduced genotypes are given in brackets. No recombinant types were found.



The observation that *Psp* and *Amy-1* segregate independently is supported by the presence of the C3H/As allele, *Psp<sup>b</sup>*, in the congenic strain C3H. *Amy<sup>YBR</sup>*. The way this strain was constructed (see MATERIALS AND METHODS) indicates that it has the C3H/As genome, except for the amylase complex and a surrounding segment of DNA derived from YBR/Cv. The *Psp* locus is, thus, not included in this transferred DNA segment.

Consistent with this interpretation of the backcross data is the phenotypic distribution among F<sub>2</sub> mice from the intercross of F<sub>1</sub> (YBR/Cv × C3H/As) animals. The results for the *a* and *Psp* loci are shown in Table 3. The data deviate significantly from that expected if *a* and *Psp* segregate independently. The distribution, however, is close to that expected if *a* and *Psp* were completely linked. Only one apparent recombinant animal was found. This animal died before it produced any offspring, so that genetic transmission of the presumed recombinant chromosome could not be investigated. Using the method of ROBINSON (1971), the distance between *a* and *Psp* was estimated to 3 ± 3cM, which is in keeping with the estimate from the backcross. We conclude, therefore, that *Psp* is located on chromosome 2, close to *a*, and that it is not linked to the *Amy* complex.

*The protein ratio AMY/PSP:* Since amylase concentrations are variable in homozygous animals, as noted above, it was our rationale to find an internal standard whose concentration varied coordinately with that of amylase and to use the ratio of amylase to the internal standard for detecting quantitative amylase variants. To find out if PSP is such a suitable internal standard, the variation in the protein ratios of amylase to PSP, derived from protein scanning of native gels of saliva, was determined in 10 inbred and nine congenic strains (Figure 6a). Five females (10 from C3H/As) were analyzed from each strain. In any one experiment, one female from each of five to 10 strains were analyzed

TABLE 3

*Segregation of agouti types and native electrophoretic salivary PSP types among 34 offspring of the intercross between F<sub>1</sub> (YBR/Cv × C3H/As) animals*

	Cross: ♀♀ $\frac{a Psp^a}{A Psp^b} \times \delta\delta \frac{a Psp^a}{A Psp^b}$			Total
	PSP-A ( <i>Psp<sup>a</sup>/Psp<sup>a</sup></i> )	PSP-AB ( <i>Psp<sup>a</sup>/Psp<sup>b</sup></i> )	PS-B ( <i>Psp<sup>b</sup>/Psp<sup>b</sup></i> )	
Agouti ( <i>A/A</i> and <i>A/a</i> )	0 <i>0*(6.4)</i>	15 <i>17.0(12.8)</i>	9 <i>8.5(6.4)</i>	24 25.5
nonagouti ( <i>a/a</i> )	9 <i>8.5(2.1)</i>	1 <i>0(4.2)</i>	0 <i>0(2.1)</i>	10 8.5
Total	9 8.5	16 17.0	9 8.5	34

The deduced genotypes are given in parentheses.

\* Numbers in italics are those expected if *a* and *Psp* were completely linked; whereas, those in parentheses are expected if the loci segregated independently.

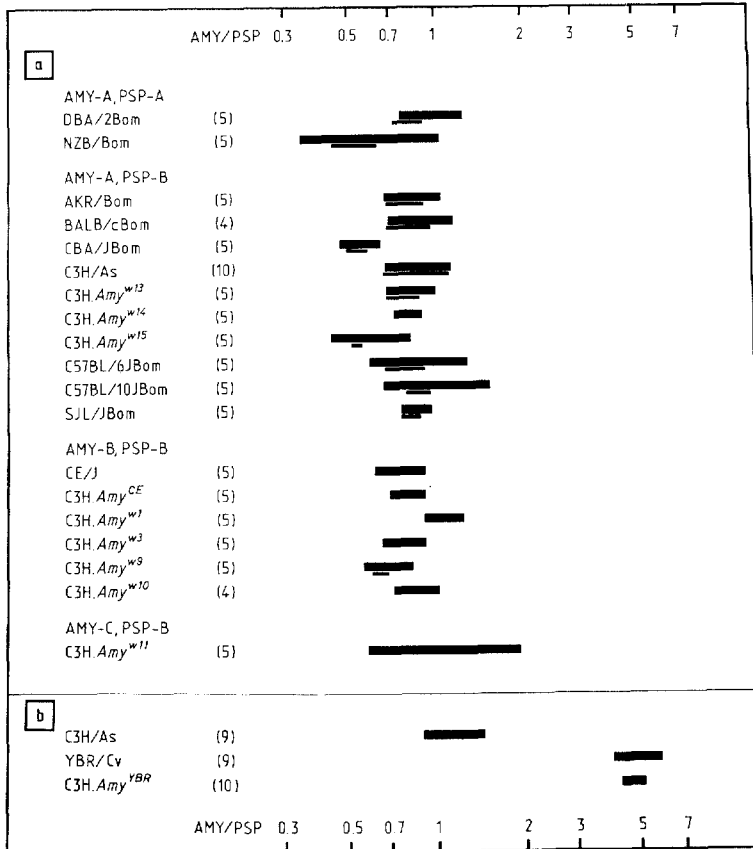


FIGURE 6.—AMY/PSP protein ratios obtained by scanning native-gel separations of whole saliva proteins. All saliva samples were obtained from female animals. In 6a, each individual from a given strain was analyzed in a separate experiment. The number of animals from each strain is given in brackets. The bars represent the range of values as the mean  $\pm$  the standard deviation. Thick bars give total data. Thinner bars indicate that the unusually high values from one experiment were omitted (see text for details). The individuals of the three strains in 6b were analyzed together in one experiment.

together. PSP was found to be degraded in the samples from two out of 100 females analyzed; thus, their electrophoretograms were not scanned. The values from one experiment were considerably higher than from the rest, demonstrating an experimental source of error. When the values from this experiment were omitted, the variation within all the involved strains decreased (*cf.*, thick and thinner bars of Figure 6a). The large variation in strain C3H.Amy<sup>w11</sup> is caused by a single high value. When this is omitted, the mean and the standard deviation decreases to 0.93 and 0.18, respectively. New saliva samples were taken from the same five females of this strain and they were analyzed together. These AMY/PSP ratios had a mean of 0.79 and a standard deviation of 0.14. Thus, the variation in the AMY/PSP ratio within each strain is moderate and

considerably smaller than the variation in total gland amylase, suggesting coordinate variation of PSP and amylase.

The production and amount of salivary amylase in strain YBR/Cv is two to three times higher than in strain C3H/As (HJORTH 1979). The ratios of amylase to PSP in saliva are approximately four times greater in YBR/Cv and C3H.*Amy*<sup>YBR</sup> than in C3H/As (Figure 6b). This difference must reflect a difference in amylase production since C3H/As and C3H.*Amy*<sup>YBR</sup> have the same PSP allele by descent and differ genetically by only a small segment of DNA containing the amylase complex. The small differences in the protein ratios of amylase to PSP among the congenic lines (Figure 6a) may, thus, also represent differences in production of amylase. The observed lower value for C3H.*Amy*<sup>CE</sup> than for C3H.*Amy*<sup>wt</sup> is in keeping with this interpretation, since the salivary amylase allele from the CE/J strain contributes less than that from C3H.*Amy*<sup>wt</sup> in heterozygotes to C3H/As (HJORTH 1979).

#### DISCUSSION

The present study demonstrates that the Parotid Secretory Protein (PSP) in mouse shows a genetic variation in structure that is determined by a single locus linked to the agouti locus on chromosome 2. The *Psp* locus may prove valuable in understanding the molecular biology of eukaryotic genes because its protein product is present in a very high amount in a single specialized tissue. Coordinate variation in the amounts of amylase and PSP in saliva was found for an array of inbred mouse strains. The reason for this may be that the two proteins are packed together in, and during salivary secretions released from, zymogen granules in a ratio set by the capacity of the respective genes to direct production of the two proteins. This suggests that altered ratios of the two proteins in saliva may reflect altered production of either of the proteins and, thus, provide a simple way to identify quantitative variants for the proteins. The increased salivary AMY/PSP ratio demonstrated for those strains showing higher salivary amylase production as a result of the YBR/Cv-derived amylase complex, supports this suggestion.

The general use of the AMY/PSP ratio to detect quantitative variation of salivary amylase and PSP depends on the ability to distinguish between variation in amylase and variation in PSP when mice with altered ratios of the two are found. Since *Amy-1* and *Psp* are not linked, it is possible to construct tester stocks with different, but known, alleles of the two loci. First, consider a variant resulting from a genetic element that is *cis*-acting or that acts in an allele-specific way. It should then be possible, by crossing this variant to animals of a tester strain with the alternative electrophoretic form of both amylase and PSP, to determine directly from the saliva electrophoretogram of the offspring whether amylase or PSP is altered. By crossing variant offspring to animals of the previously used tester strain for an additional generation, the linkage of the variant gene to *Amy* and *Psp* can be studied. If a variant is due to a *trans*-acting gene, then two generations of backcrosses to an appropriate tester strain will also be

necessary. Second-generation offspring that show altered AMY/PSP ratios should be assayed for amylase concentration in the parotid glands. If for instance, this amylase content is normal, the variant gene must cause a change in the amount of PSP. It should be possible to use this procedure for dominantly and co-dominantly inherited variants. For recessive variants, the first-generation offspring from matings to tester stocks should be intercrossed and second generation offspring analyzed, as described above.

Using the ratio between amylase and PSP in saliva to detect quantitative variants of the two proteins in mouse has the advantage of being rapid and simple, and female animals screened need not be sacrificed. Besides the ability to detect *cis*-acting quantitative variants, it should be possible, by this method, to detect *trans*-acting variants. Such variants have been described in other systems (BOUBELIK *et al.* 1975; LUSIS and PAIGEN 1975; ABRAHAM and DOANE 1978; MACDONALD and AYALA 1978), and they constitute an important class of variants for the comprehension of gene expression in eukaryotes.

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