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# Human Pancreatic α-Amylase: Phenotypic Codominance and New Electrophoretic Variants

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## INTRODUCTION

Human salivary ($AMY_1$) and pancreatic ($AMY_2$) amylase (α-1, 4-glucan 4-glucanohydrolase, E.C.3.2.1.1) are closely linked loci on chromosome 1 [1]. Multiple alleles have been revealed for both loci [2] by electrophoresis in Tris HCl buffered polyacrylamide sheet gels. With the exception of the recessive $AMY_1$ R phenotypes, those for salivary amylase have been classified as codominant [2], and the two pancreatic amylase alleles ($AMY_2{}^B$ and $AMY_2{}^C$) as autosomal dominant phenotypes [3]. We report here data obtained with a new electrophoretic system that shows these pancreatic amylase variants behaving as autosomal codominant allelic expressions. Three new pancreatic amylase variants are also reported.

## METHODS

### *Specimens*

Most urine samples were stored at 5°–10°C and analyzed within 2 weeks of collection. Storage of urine samples at −20°C for later analysis resulted in variable losses of enzyme activity. The analysis of urines in families where the mating type was $AMY_2$ B × $AMY_2$ A with the asparagine buffered polyacrylamide sheet gel electrophoretic system (see below) was attempted with frozen urine samples. Repeated analysis was often needed to assign a phenotype to these samples. New urine specimens were collected from family members whose stored samples either were unavailable or were not analyzable. Sera were either refrigerated and analyzed within a week or frozen at −20°C for later analysis. Occasionally serum samples shifted to a more anodal isozyme pattern after freezing.

### *Asparagine Buffered Sheet Gels*

The pH 7.90–8.15 gel, contained acrylamide, 28.0 g; *N*, *N'*-methylenebisacrylamide, 0.74 g; *N*,*N*,*N'*, *N'*-tetramethylethylenediamine, 0.34 ml; L-asparagine, 1.32 g; 480 ml water; and ammonium persulfate, 3.5% in water, 20 ml. The solution was polymerized in an eight-slot (Otto Hiller, Madison, Wisc.) starch gel electrophoresis mold. The reservoir buffer for the discontinuous buffer system contained 0.05 M Tris and 0.375 M glycine, pH 8.3. Vertical

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electrophoresis was carried out at room temperature at a constant current of 21 mA. The gel voltage increased from 1.4 V/cm to 8.9 V/cm during the 14 hr electrophoresis. The gel was sliced and stained for amylase by the starch-iodine procedure [4].

*Thin Layer Polyacrylamide Gel Isoelectric Focusing*

The gels contained acrylamide, 3.8%; *N*, *N'*-methylenebisacrylamide, 0.2%; glycerin, 20%; riboflavin, 0.0001%; and a total of 1% Ampholines (LKB Instruments, Rockville, Md.) and were prepared by the technique of Thomas et al. [5]. Pancreatic amylase variant samples were focused in a pH gradient of 5.0–9.5 containing a total of 1% Ampholines. The Ampholine combination was 0.1% pH 5–7, 0.2% pH 6–8, 0.2% pH 7–9, 0.3% pH 8–9.5, and 0.2% pH 5–8. Focusing was performed in a Desaga/Brinkman TLE Double Chamber with strip electrodes. Wicks of absorbent strips (Gelman, cat. no. 51291, Ann Arbor, Mich.) were placed under the electrodes. The wick was wetted with 1 M $H_3PO_4$ in 20% glycerin at the anode and with 1 M NaOH in 20% glycerin at the cathode. A constant LKB 2103 D.C. power supply was used. For the pH 5.0–9.5 gradient, constant power was maintained with an initial voltage of 600 V for 12 hr. The final voltage was constant at 2000 V. A 1% mixture of cytochrome c, hemoglobin, myoglobin, bovine serum albumin with bromphenol blue, ferritin, and ribonuclease was applied along one edge as a standard. Samples were applied with pieces of water-saturated S and S 470AC filter paper.

After focusing the proteins, pH measurements were taken at 0.5 cm intervals with Desaga/Brinkman flat membrane micro-electrodes. Gels were stained for amylase, according to the procedure of Ward et al. [4] without slicing the 1 mm thick, soft/gels.

*Segregation Analysis*

Segregation analysis was performed on 55 families of mating type $AMY_2$ BA × $AMY_2$ AA. The 133 children were classified as either $AMY_2$ BA or $AMY_2$ AA on discontinuous asparagine buffered polyacrylamide sheet gels by the maximum likelihood method of Morton [6, 7], utilizing the computer program SEGRAN.

## RESULTS

### *Codominant Classification of $AMY_2$ Phenotypes*

The discontinuous asparagine buffered polyacrylamide sheet gel electrophoretic system allowed the identification of pancreatic amylase B homozygotes. Figure 1 shows a comparison of urines from individuals with the genotypes $AMY_2^A/AMY_2^A$, $AMY_2^B/AMY_2^A$, and $AMY_2^B/AMY_2^B$ on Tris HCl [3] and asparagine buffered polyacrylamide sheet gels. The multiple isozymes seen for both normal ($AMY_2$ A) and variant ($AMY_2$ B, C, D, E, and F) pancreatic amylase phenotypes are numbered from the cathode as previously described [3]. Only the even-numbered isozymes (2, 4, 6 . . .) are shown because the odd-numbered isozymes are seen in pancreatic extract and pancreatic cyst fluid but not in urine [2]. These gels show that in the Tris HCl gel, although $AMY_2$ B4 and B6 were slightly slower in electrophoretic mobility than $AMY_2$ A2 and A4, respectively, the separate isozymes in each band (A4 and B2, or A6 and B4) could not be separated in the heterozygote, $AMY_2$ BA. In addition, $AMY_1$ A2 contributes to the intensity of the $AMY_2$ A4 isozyme region in the $AMY_2$ BA individual shown in figure 1. In contrast, the asparagine buffered gel (fig. 1, right side) clearly separated $AMY_2$ B4 and B6 from $AMY_2$ A2 and $AMY_2$ A4, respectively, and the heterozygote $AMY_2$ BA, could be distinguished from either homozygote, $AMY_2$ AA or $AMY_2$ BB.

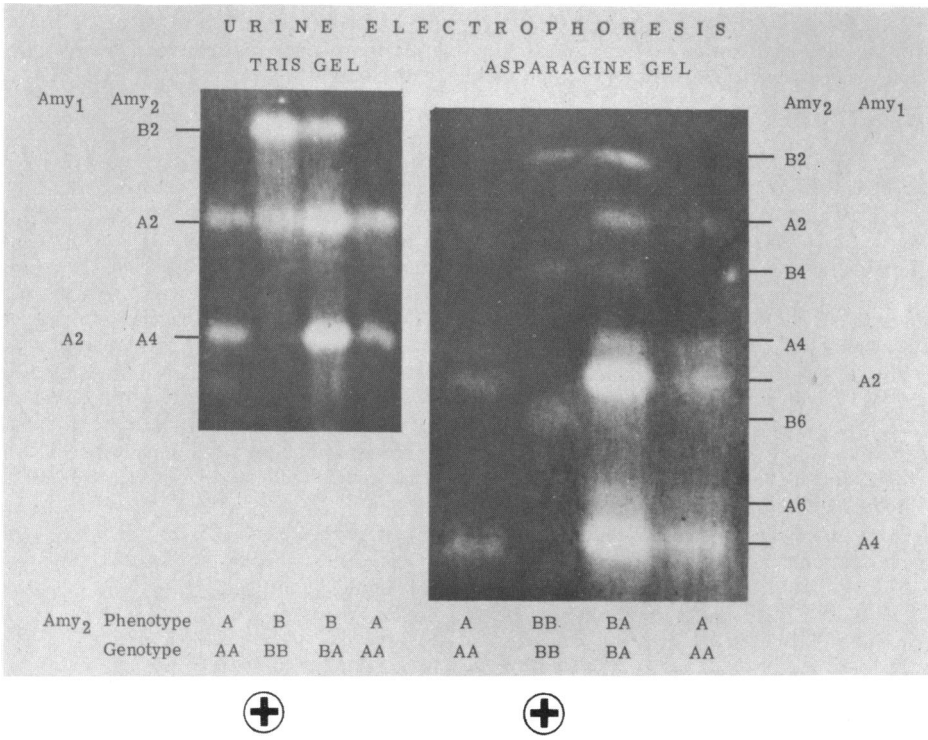


FIG. 1.—Electrophoretic separation of variant pancreatic amylase heterozygotes and homozygotes ($AMY_2$ BA and $AMY_2$ BB). Comparison of $AMY_2$ AA, $AMY_2$ BA, and $AMY_2$ BB phenotypes on Tris HCl buffered polyacrylamide gels *(left)* and asparagine buffered polyacrylamide gels *(right)*.

Segregation analysis on families of mating type $AMY_2$ BA × $AMY_2$ AA showed that all $AMY_2$ AB individuals were correctly classified. In 55 families with a total of 133 children, 70 were AA and 63 AB. The probability of a segregating family was defined as $(1 - h)(1 - q^s)$, while the probability of a nonsegregating family (no $AMY_2$ A offspring) was $h + (1 - h)q^s$, where $h$ is the probability that an $AMY_2$ B parent was genotypically $AMY_2{}^B/AMY_2{}^B$, and $q$ is the probability of an $AMY_2$ BA child, where $p = 1 - q$ equals the probability of an $AMY_2$ AA child, while $s$ is the sibship size [6]. The null hypothesis was $p = .5$ and $h = 0$. $U_p(-4.11)$ and $U_h$ $(-3.00)$ are the maximum likelihood scores for $p$ and $h$, respectively, and $K_{pp}(258.87)$ and $K_{hh}(583)$ are the variances of these scores. To test the null hypothesis, $p = .5$ and $h = 0$, $\chi^2{}_{2\,df} = U_p{}^2 K^{pp} + U_h{}^2 K^{hh} + 2U_pU_h K^{ph}$, where $K^{pp}$, $K^{hh}$, and $K^{ph}$ are the elements in the inverse of the variance-covariance matrix, was utilized. This was not significant ($P > .1$); thus the null hypothesis was accepted. This analysis was consistent with all variant parents being genotypically $AMY_2{}^B/AMY_2{}^A$ and $P = .5$.

*New Pancreatic Amylase Phenotypes*

All $AMY_2$ BA individuals studied had an $AMY_2$ A2 band as intense as the $AMY_2$ B2 band on asparagine buffered polyacrylamide sheet gels (fig. 1). Individual II-I (family

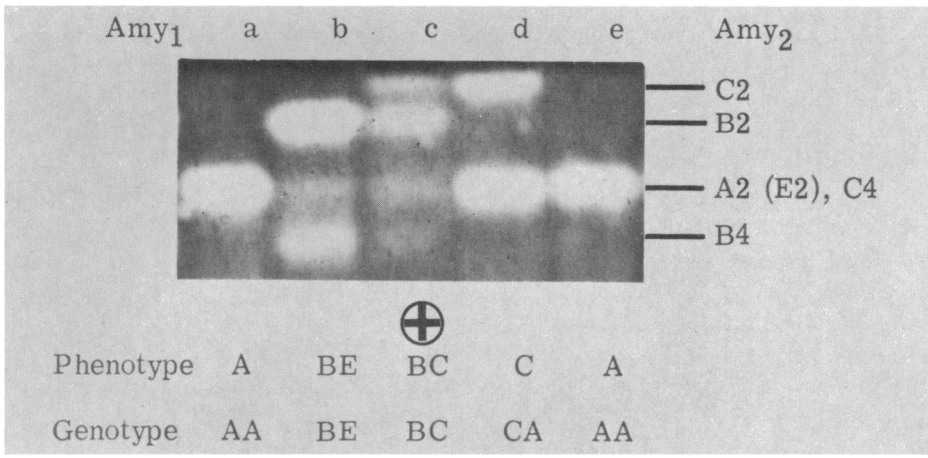


FIG. 2.—Asparagine buffered polyacrylamide gel electrophoresis comparing pancreatic amylase phenotypes. *Channel a:* $AMY^2$ A; *channel b:* $AMY^2$ BE; *channel c:* $AMY^2$ BC; *channel d:* $AMY^2$ C; and *channel e:* $AMY_2$ A.

no. 20466; fig. 2, channel b) was suspected of being $AMY_2$ BB, but her mother (fig. 3, individual I-2; family no. 20466) was $AMY_2$ A. Individual II-I (family no. 20466; fig. 2, channel b) has an intense $AMY_2$ B2 band and a very weak $AMY_2$ "A2" band. This weak $AMY_2$ "A2" isozyme has been designated $AMY_2$ E2 in the $AMY_2$ BE phenotype. Several other individuals with a similar phenotype have also been designated as $AMY_2$ BE. Individual I-2 (family no. 20466; fig. 3) has been designated as $AMY_2$ AE, although this phenotype cannot be identified electrophoretically.

*Isozyme $AMY_2$ C*

Asparagine buffered polyacrylamide gel permitted the identification of another common pancreatic amylase variant ($AMY_2^C$, fig. 2, channel d). The electrophoretic migration rates (fig. 2) of the $AMY_2^B$ and $AMY_2^C$ patterns were the reverse of their rates in the Tris HCl buffered gel [3]. $AMY_2$ C4 could not be distinguished from $AMY_2$ A2 isozymes in either gel system.

Several doubly variant heterozygotes for pancreatic amylase ($AMY_2$ BC) were identified (fig. 2, channel c). These individuals made it possible to determine the electrophoretic position of the $AMY_2$ C4 isozyme. The doubly variant individual is shown in the pedigree in figure 3 (individual I-2, family no. 50437). Each of her tested children was heterozygous at the $AMY_2$ locus; each expressed the $AMY_2^A$ allele and one of the variant pancreatic amylase alleles ($AMY_2^B$ or $AMY_2^C$), but never both.

*Amylase Isozyme $AMY_2$ D*

Two new pancreatic amylase phenotypes have been identified, each in a single white family. $AMY_2$ D was identified in a mother-daughter pair while another sib had the normal pancreatic amylase phenotype, $AMY_2$ A. $AMY_2$ D had essentially the same electrophoretic mobility as $AMY_2$ B in the Tris HCl buffered gel but was slower than $AMY_2$ C in the asparagine buffered gel (fig. 4). $AMY_2$ D4 could not be separated from

$AMY_2$ A2 in either electrophoretic system. This variant deteriorated rapidly at room temperature and on refrigeration so that it could not be detected following electrophoretic separation of the amylase in either gel system after several days.

*Amylase Isozyme $AMY_2$ F*

Asparagine buffered gels and isoelectric focusing aided in the identification of the other new pancreatic amylase variant, $AMY_2^F$ (fig. 5, channel c and fig. 4). This heterozygote, $AMY_2$ FA, had the normal $AMY_2$ A components on isoelectric focusing when compared to the individual in channel a, figure 5, who was $AMY_2$ A. Channels d, b, and c in figure 5 are heterozygous for the $AMY_2^B$, $AMY_2^C$, and $AMY_2^F$ alleles, and each also has the $AMY_2^A$ allele. An $AMY_2$ BB, previously identified in the asparagine buffered system, is shown in figure 5, channel e. This individual has only $AMY_2$ B isozymes.

## DISCUSSION

The complex electrophoretic phenotypes expressed by the multiple alleles at the salivary and pancreatic amylase loci have been difficult to explain. The primary enzymatic products for both $AMY_1$ and $AMY_2$, have been shown to be modified by two post-translational modifications, glycosidation and deamidation [2, 8, 9].

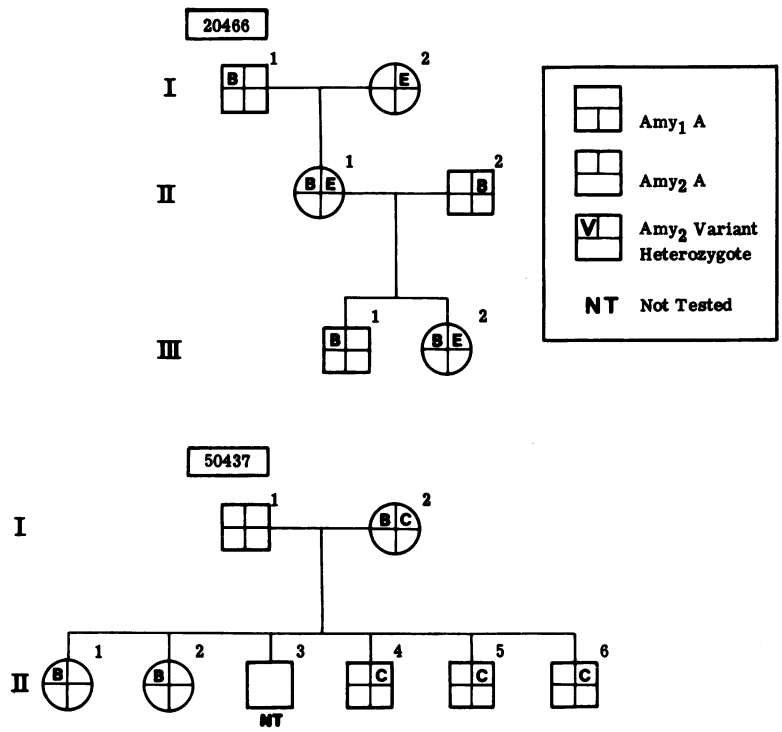


FIG. 3.—Families with pancreatic amylase ($AMY_2$) variants.

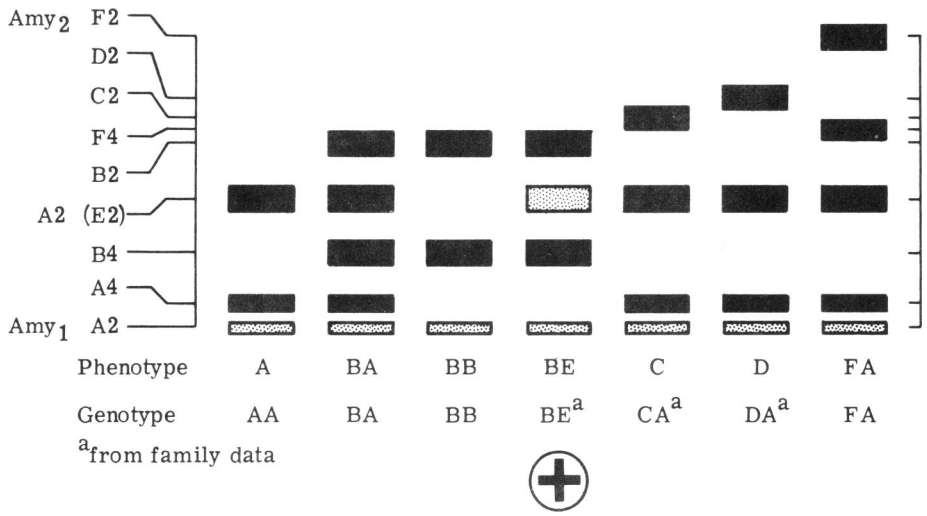


FIG. 4.—Diagrammatic representation of pancreatic amylase ($AMY_2$) phenotypes determined by electrophoretic separation on asparagine buffered polyacrylamide sheet gels. Genotypes for the pancreatic amylase phenotypes $AMY_2$ BE, $AMY_2$ C, and $AMY_2$ D determined from family data.

The multiple alleles for both amylases add to the complex isozyme patterns seen on polyacrylamide gel electrophoresis separations. Except for the $AMY_1{}^R$ variants inherited as autosomal recessive traits, all other salivary ($AMY_1$) and pancreatic ($AMY_2$) amylase expressions had been classified as dominant. The salivary amylase alleles, except for $AMY_1{}^R$, have been reclassified as codominants, since complete variant isozyme patterns similar to the $AMY_1$ A phenotype have been demonstrated in obligate heterozygotes [10].

The discontinuous asparagine buffered polyacrylamide sheet gel allowed the classification of the previously identified pancreatic amylase phenotypes as codominants ($AMY_2$ A, B, and C). All isozymes in pancreatic amylase BA heterozygotes and BB homozygotes can be identified in this electrophoretic system (fig. 1). Although it has not been possible to separate $AMY_2$ C4 from $AMY_2$ A2 in obligate $AMY_2$ CA heterozygotes, the pancreatic amylase C4 isozyme has been identified in an $AMY_2$ BC individual (fig. 2, channel c [2]). In $AMY_2$ BC individuals, all pancreatic amylase B and C isozymes were identifiable (fig. 2).

Three new pancreatic amylase variants were identified with the aid of the asparagine buffered polyacrylamide sheet gel electrophoretic system and, in one case, also thin layer polyacrylamide isoelectric focusing. Two of these new variants, $AMY_2{}^D$ and $AMY_2{}^F$, have an additional more slowly migrating isozyme band, similar to the previously identified pancreatic amylase variants, $AMY_2{}^B$ and $AMY_2{}^C$ (figs. 2, 4, and 5). $AMY_2$ F is a codominant phenotype as normal $AMY_2$ A isozymes were detected with isoelectric focusing along with the $AMY_2$ F isozymes. However, $AMY_2$ D has been classified as a dominant phenotype since we could not distinguish the $AMY_2$ D4

isozyme from the $AMY_2$ A2 isozyme in an obligate heterozygote ($AMY_2$ DA).

While the majority of variants for salivary and pancreatic amylase are classified as codominant traits, recessive phenotypes for salivary amylase ($AMY_1$ R) have been identified, and now one has been identified ($AMY_2$ E) for pancreatic amylase. The new pancreatic amylase variant, $AMY_2^E$, has been more difficult to establish than the other variants. Currently, it has been identified only in heterozygotes who are $AMY_2$ BE. The electrophoretic position of this weak band was the same as the $AMY_2$ A2 band and originally suggested that the individual might be an $AMY_2$ B homozygote (fig. 2). However, individual II-I (family no. 20466, fig. 3) cannot be an $AMY_2$ B homozygote as her mother (I-2, family no. 20466) was $AMY_2$ A. It has not been possible to separate this allele electrophoretically from the $AMY_2^A$ allele in any of our electrophoretic systems nor by isoelectric focusing in thin layer polyacrylamide gels. We suggest that this new phenotype ($AMY_2$ E) may be a quantitative variant possibly due to one of the following mechanisms: (1) decreased synthesis of the enzyme; (2) altered kinetics; (3) variable, within locus duplications; or (4) alterations due to a modifier locus.

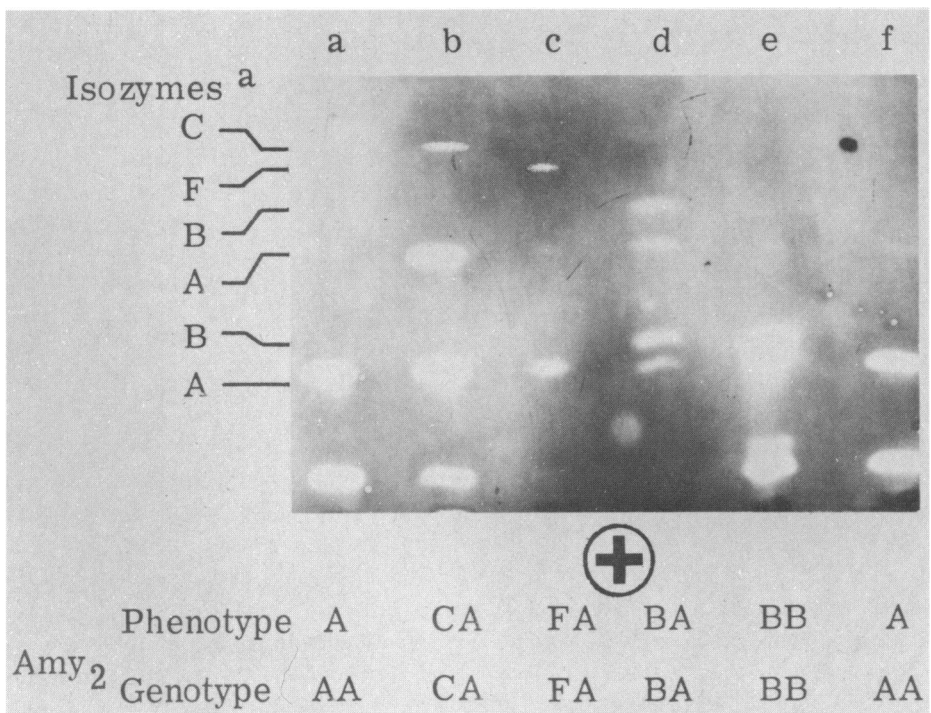


FIG. 5.—Thin layer polyacrylamide isoelectric focusing gel (pH gradient 5.0–9.5) of pancreatic amylase ($AMY_2$) phenotypes. The most cathodal isozymes which appear stippled in *channels b and c* required retouching in order to be visible after photographic reproduction. The most anodal isozyme bands are composed primarily of $AMY_1$ A isozymes which are variably expressed in urine in different individuals, as well as more anodal $AMY_2$ bands. The isozymes have not been numbered as their relationship to those seen in other electrophoretic systems has not been determined.

SUMMARY

The genetic heterogeneity of human pancreatic α-amylase (α-1,4-glucan 4-glucanohydrolase, E.C.3.2.1.1) has been better defined through the development of an asparagine buffered electrophoretic gel system. Three alleles had been identified for the pancreatic amylase locus, $AMY_2$, with two variant alleles as autosomal dominant traits on Tris HCl buffered sheet gels. The asparagine buffered sheet gel now allows the differentiation of the genotypes $AMY_2^B/AMY_2^B$, $AMY_2^B/AMY_2^A$, and $AMY_2^B/AMY_2^C$, thus classifying these three alleles as codominants. Asparagine buffered polyacrylamide gels and thin layer polyacrylamide isoelectric focusing aided in the identification of three new pancreatic amylase variants: $AMY_2^D$, $AMY_2^E$, and $AMY_2^F$. $AMY_2^E$ has been identified only in $AMY_2$ B and $AMY_2$ E individuals. This allele is proposed as a quantitative activity variant with essentially the same electrophoretic mobility as $AMY_2^A$. The other new autosomal variants have each been identified in single white families. $AMY_2^D$ is dominant and $AMY_2^F$ is a codominant trait as shown on thin layer polyacrylamide isoelectric focusing gels.

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REFERENCES

1. MERRITT AD, LOVRIEN EW, RIVAS ML, CONNEALLY PM: Human amylase loci: genetic linkage with the Duffy blood group and assignment to linkage group I. *Am J Hum Genet* 25:523–538, 1973
2. KARN RC, ROSENBLUM BB, WARD JC, MERRITT AD: Genetic and post-translational mechanisms determining human amylase isozyme heterogeneity, in *Isozymes: IV Genetics and Evolution*, edited by MARKERT CL, New York, Academic Press, 1975, pp 761–774
3. MERRITT AD, RIVAS ML, BIXLER D, NEWELL R: Salivary and pancreatic amylase: electrophoretic characterizations and genetic studies. *Am J Hum Genet* 25:510–522, 1973
4. WARD JC, MERRITT AD, BIXLER D: Human salivary amylase: genetics of electrophoretic variants. *Am J Hum Genet* 23:403–409, 1971
5. THOMAS JM, MERRITT AD, HODES ME: Analytical isoelectric focusing: improved methodology for the study of protein polymorphisms and genetic disease states. *Am J Hum Genet* 27:88A, 1975
6. MORTON NE: Genetic tests under incomplete ascertainment. *Am J Hum Genet* 11:1–16, 1959
7. MORTON NE: Segregation and linkage, in *Methodology in Human Genetics*, edited by BURDETTE W, San Francisco, Holden-Day, 1962, pp 76–91
8. KARN RC, ROSENBLUM BB, MERRITT AD: A biochemical explanation for the complex isozyme patterns of salivary amylase ($Amy_1$) and pancreatic amylase ($Amy_2$). *Am J Hum Genet* 25:39A, 1973
9. KARN RC, SHULKIN JD, MERRITT AD, NEWELL RC: Evidence for post-translational modification of human salivary amylase ($Amy_1$) isozymes. *Biochem Genet* 10:341–350, 1973
10. KARN RC, ROSENBLUM BB, WARD JC, MERRITT AD, SHULKIN JD: Immunological relationships and post-translational modifications of human salivary amylase ($Amy_1$) and pancreatic amylase ($Amy_2$) isozymes. *Biochem Genet* 12:485–499, 1974