Am J Hum Genet 25:510–522, 1973

Salivary and Pancreatic Amylase: Electrophoretic Characterizations and Genetic Studies

A. DONALD MERRITT,[1] MARIAN L. RIVAS, [1,2] DAVID BIXLER,[1] AND RUTH NEWELL[1]

INTRODUCTION

The amylases are ubiquitous enzymes which occur in both plant and animal life. Mammalian amylases have been designated as α amylases (α-1,4-glucan 4-glucanohydrolase; E.C.3.2.1.1) and in the human are produced in the salivary glands (Amy_1) and pancreas (Amy_2). Genetic variation in human salivary amylases has been the subject of a recent review [1]. It is our purpose here to define further the extent of polymorphisms at the Amy_1 and Amy_2 loci by means of agar and sheet polyacrylamide electrophoresis of human pancreas, serum, urine, and saliva and to present population data from Caucasians and Afro-Americans as well as limited evaluations of black Africans and individuals of Oriental ancestry. These data will be compared with other studies [2–4] to clarify the interpretation of amylase isozyme variation in man.

MATERIALS AND METHODS

Urine, for the Amy_2 phenotype, and parotid or whole saliva, for the Amy_1 phenotype, were collected randomly for electrophoresis. When a variant pattern was noted, family studies were performed. Saliva samples were either tested immediately or frozen and stored at −20° C for later analysis; urine samples were kept cold and analyzed within 2 days because frozen samples usually proved unsatisfactory for testing. Human pancreatic homogenates from specimens obtained at autopsy, whether fresh or frozen, had high amylase activity. Serum and plasma (ACD) samples, either frozen or fresh, had detectable isozyme patterns.

The details of the discontinuous vertical sheet polyacrylamide electrophoretic system for parotid and whole saliva have been reported [1]. All samples were applied 12 cm from the cathodal end of a 12 × 27-cm gel, sealed with petrolatum, and covered with plastic film. Vertical electrophoresis was performed at a constant current of 20 ma per gel and terminated when the buffer front reached a distance 12 cm from the origin, approximately 15 hr. The 6-mm-thick gel was sliced into two 3-mm gels, one of which was incubated in 0.02 M phosphate buffer, *p*H 6.9, containing 0.0067 M NaCl and 1% solu-

Received December 19, 1972; revised March 22, 1973.

This investigation was supported in part by U.S. Public Health Service research grants GM-19178 and HL-15166, and a Rutgers University Research Council grant. D. Bixler is a career development awardee, U.S. Public Health Service grant DE 05945.

[1] Department of Medical Genetics, Indiana University Medical Center, Indianapolis, Indiana 46202.

[2] Department of Biological Sciences, Douglass College, Rutgers University, New Brunswick, New Jersey 08903.

© 1973 by the American Society of Human Genetics. All rights reserved.

ble starch. The incubation times for gels varied inversely with the amylase activity of the sample. Although saliva and pancreatic extracts required only 15-min incubation in hydrolyzed soluble starch followed by immediate staining with KI-iodine solution, serum and urine required 60-min incubation in soluble starch followed by a brief water rinse and incubation for up to 2 hr at 37° C prior to iodine staining. The isozyme patterns developed immediately and were documented with photographs. The comparable amylase concentrations in saliva, urine, and serum were approximately 200:10:1, respectively.

The other half of the gel was stained for protein with 0.5% amido black in methanol-water-acetic acid (5:5:1) and destained in 3% acetic acid when partially purified amylase was used for isozyme detection. These samples were prepared by cold 40% ethanol extraction, glycogen coupling, centrifugation, dilution with buffer, and digestion at room temperature [5].

Electrophoretic studies were also performed in agar following the method of Kamarýt [6]. In our hands, his barbital buffer system did not give as satisfactory a result as a Tris buffer system although all observed patterns were similar to, if not identical with, those described in Kamarýt's publications [2, 6–8]. When the latter was used, a stock buffer (1.0 M Tris-HCl, *p*H 8.2) was diluted 1:20 for a 1% agar gel and 1:10 for the electrode chambers. A variety of agars, listed in order of increasing sulfur content and electroendosmosis, were evaluated including Agarose (Fisher Scientific Co.), Ionagar No. 2 (Consolidated Laboratories, Inc.), and purified agar (Difco Laboratories). Moreover, various electrophoretic chambers gave comparable results including the Durrum cell, Wieme cell, and a Plexiglas box similar to the Wieme apparatus.

The Durrum cell was most convenient since it accommodated both 8.3 cm × 10.2 cm lantern slides and 2.5 cm × 7.5 cm microscope slides, which allowed direct comparison of multiple samples. Ten ml of 1% agar buffer solution was pipetted onto the larger slides positioned for electrophoresis across the 8.3-cm dimension. Six 5-mm slots were placed 2.5 cm from the anode when using Ionagar No. 2 and 5.5 cm from the anode with Agarose. Slots were filled with samples mixed with equal volumes of warm 2% agar.

RESULTS

1. *Electrophoretic Studies*

a) *Polyacrylamide gel system.* Figure 1 shows the common (Amy_2 A) and variant (Amy_2 B) amylase polyacrylamide zymograms of serum, pancreas, and urine. The Amy_2 A and Amy_2 B isozymes (Pa 1, 2, 3, and Pa B) appear to be identical from these sources and differ electrophoretically from the Amy_1 A isozymes (Sa 1 . . . 6) of saliva. Figure 2 demonstrates the electrophoretic pattern of another pancreatic variant, Amy_2 C, characterized by Pa C, an isozyme migrating faster than isozyme Pa B.

In our previous study of salivary amylase, the normal phenotype and three variant phenotypes were described [1]. The isozymes did not interact with each other when mixed in equal concentrations prior to electrophoresis. Similar mixing experiments utilizing urine (or serum or pancreas) and saliva demonstrate a lack of interaction of the Amy_1 common and variant components as well as the Amy_2 common and variant isozymes.

In individuals having black ancestry, Amy_1 variants similar to the Amy_2 D phenotype previously described in Caucasian Americans were noted. However, careful comparison demonstrated a slightly slower electrophoretic migration of the variant isozyme whose phenotype is designated Amy_1 E (fig. 3).

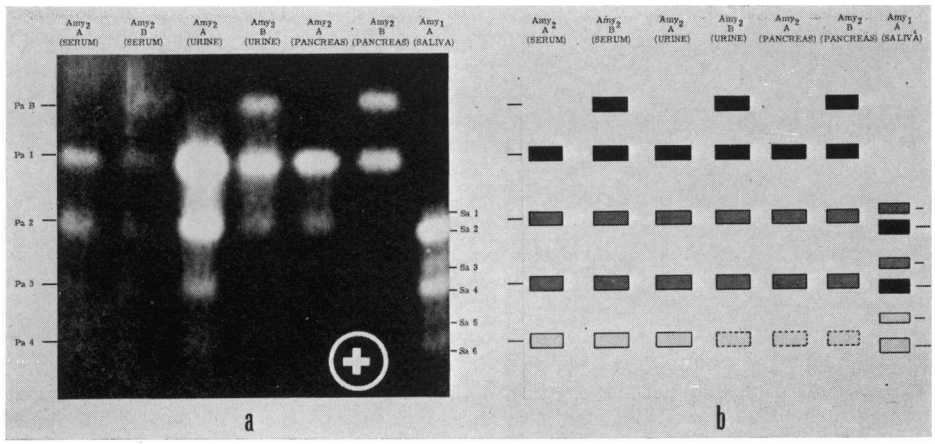

FIG. 1.—*a,* Sheet polyacrylamide amylase zymograms of serum, pancreatic homogenate, and urine from Amy_2 A and Amy_2 B phenotypes. The phenotype of Amy_1 A (salivary amylase) is shown for comparison. The relative concentrations of serum:urine:saliva:pancreas are 1:10:200:200. Pa 1, 2, 3, and 4 are the pancreatic amylase isozymes of the Amy_2 A phenotype. Pa B is the major Amy_2 B variant isozyme. Sa 1, 3, 5, and Sa 2, 4, 6 are the two series of salivary amylase isozymes of the Amy_1 A phenotype. Origin at top. *b,* Diagrammatic zymogram.

Figure 4 is a composite diagram indicating the various phenotypes seen in our polyacrylamide studies including four variant phenotypes not previously described: Amy_2 C, Amy_1 E, Amy_1 F, and Amy_1 G.

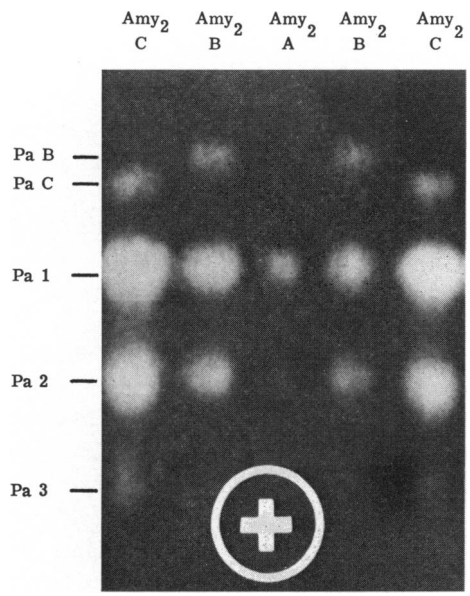

FIG. 2.—Sheet polyacrylamide zymograms of pancreatic amylase. The Amy_2 A, Amy_2 B, and Amy_2 C phenotypes as expressed in urine are illustrated. Origin at top.

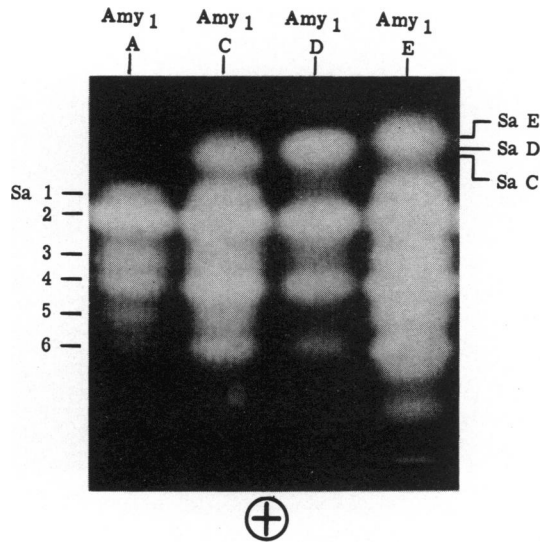

FIG. 3.—Sheet polyacrylamide amylase zymograms showing the relative migration of four Amy_1 phenotypes in saliva. Origin at top.

b) *Agar gel system.* Figure 5 shows the anodally migrating components of salivary amylase from a homozygous normal (Amy_1 A) individual and three Amy_1 variants, Amy_1 B, Amy_1 C, and Amy_1 D. In figure 6 urine and serum produce Amy_2 patterns which are identical. The variant isozymes are cathodal to the normal isozymes in agar as in polyacrylamide. However, the Amy_2 B and Amy_2 C phenotypes are not sufficiently different in migration to be readily distinguishable in agar electrophoresis. These patterns are like those seen in the Kamarýt agar gel

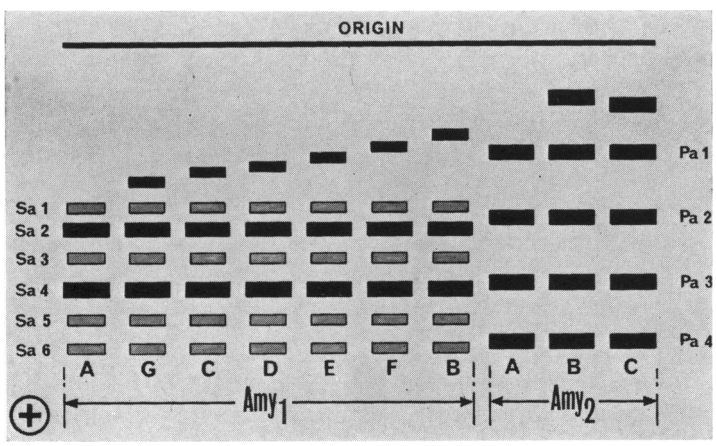

FIG. 4.—Diagrammatic representation of the relative migration rates of amylase isozymes of the Amy_1 (parotid, submaxillary, or whole saliva) and Amy_2 (pancreas, serum, or urine) phenotypes as seen in sheet polyacrylamide electrophoresis.

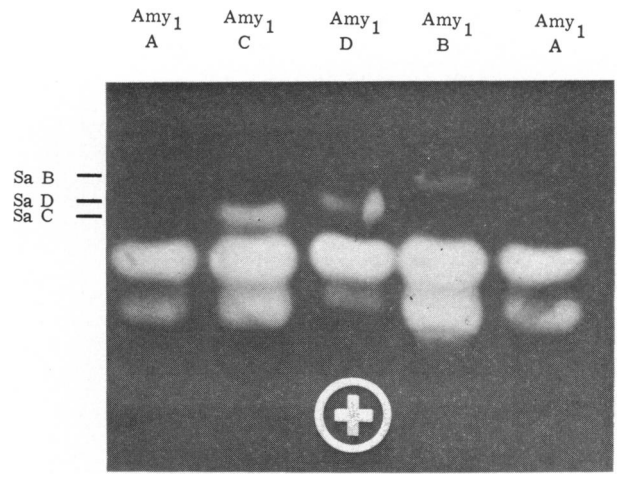

FIG. 5.—Agar (Agarose, 1%) amylase zymograms of parotid saliva showing the Amy_1 A, Amy_1 B, Amy_1 C, and Amy_1 D phenotypes. Sa B, Sa C, and Sa D are the variant salivary isozymes.

system [6]. That is, the net migration of the electrophoretically separated amylase isozymes is toward the cathode as a result of the rather marked endosmotic flow in agar of higher ion content, Ionagar No. 2.

When pancreatic homogenates (1:1 w/v in agar gel buffer) of the Amy_2 A phenotype undergo electrophoresis at full strength in an Ionagar No. 2 gel system, three isozymes are detectable which migrate identically with three Amy_2 isozymes in urine and serum. Serum and urine isozymes are electrophoretically identical (see fig. 6), and pancreatic and urine isozymes are similarly identical as seen in figure 7. The isozymes of the Amy_1 A and Amy_1 C phenotypes may be seen to migrate differently than the isozymes of Amy_2 A and Amy_2 B phenotypes. Therefore, agar electrophoresis of pancreatic homogenates, saliva, urine, and serum from the common or variant Amy_1 and Amy_2 phenotypes demonstrates that the isozymes of salivary and pancreatic amylase are electrophoretically distinguishable and that all variants analyzed thus far are cathodal isozymes as in the polyacrylamide system.

2. *Origin of Serum and Urine Amylase*

We have studied a 9-year-old boy with hyperinsulinism who required total pancreatectomy. Both he and his mother showed an Amy_2 B phenotype in their urine and serum and an Amy_1 A phenotype in their saliva. During the 2 weeks following surgery, the patient's Amy_2 B pattern in serum and urine gradually disappeared. Salivary amylase remained unchanged. However, 20-fold concentration of his urine revealed two minor amylase components corresponding to the Amy_1 A isozymes in the agar system and to the Sa 2 and Sa 4 isozymes of Amy_1 A in the polyacrylamide system. Thus, the pancreas was demonstrated as the primary

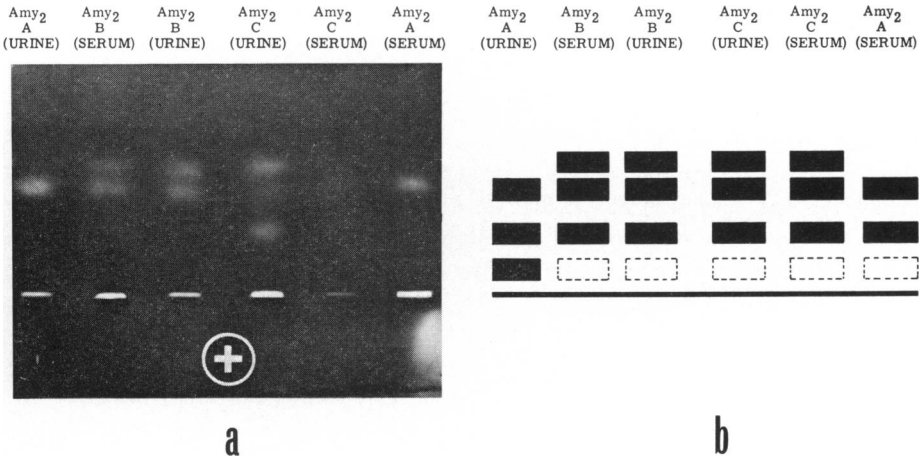

FIG. 6. *a,* Agar (Ionagar, 1%) amylase zymograms of serum and urine Amy_2 phenotypes. The variant pancreatic isozymes, Pa B and Pa C, are the cathodal components seen in the four middle zymograms. Note the marked electroendosmosis when compared with fig. 5. *b,* Diagrammatic zymogram. Origin is indicated by a solid anodal line. Open rectangles represent isozymes not clearly distinguishable in this photograph.

source of his easily detectable amylase isozyme in serum and urine prior to surgery. We should also like to note that patients with cystic fibrosis requiring large doses of pancreatic extract and presumably lacking pancreatic exocrine function have little detectable Amy_2 expression in their serum or urine. Again, minute amounts

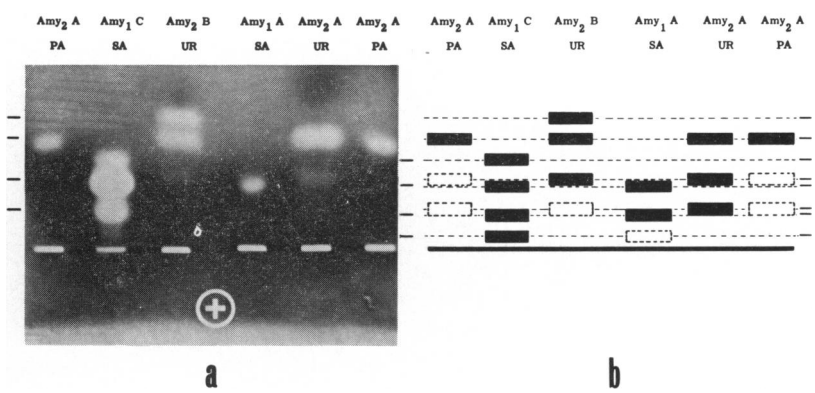

FIG. 7.—*a,* Agar (Ionagar, 1%) amylase zymograms of pancreatic homogenate (PA), diluted 1:5; urine (UR), concentrated 5×; and saliva (SA), diluted 1:100. Amy_1 and Amy_2 common and variant phenotypes show the relative migration of amylase isozymes. The four pancreatic isozymes are indicated on the left and the four salivary isozymes on the right of the photograph by horizontal lines. *b,* Diagrammatic zymogram with eight lines on the right indicating the relative rates of isozyme migration. Origin is indicated by a solid anodal line. Open rectangles represent isozymes not clearly distinguishable in this photograph. Note the marked electroendosmosis when compared with fig. 5.

of Amy_1 isozymes may be seen following 20-fold concentration of their urine. Salivary amylase is present in the saliva of cystic fibrosis patients at normal levels.

3. *Genetic Studies*

Genetic analysis of the Amy_1 phenotypes is consistent with an autosomal dominant inheritance pattern [1]. Note that each of the Amy_1 and Amy_2 variant phenotypes has been seen alone, that is, Amy_1 A/Amy_2 variant or Amy_1 variant/Amy_2 A. The clear demonstration of autosomal dominant inheritance of Amy_2 variant phenotypes (Amy_2 B and Amy_2 C) is seen in figure 8. Family 16028 provides extensive documentation of the independent inheritance of Amy_2 B as

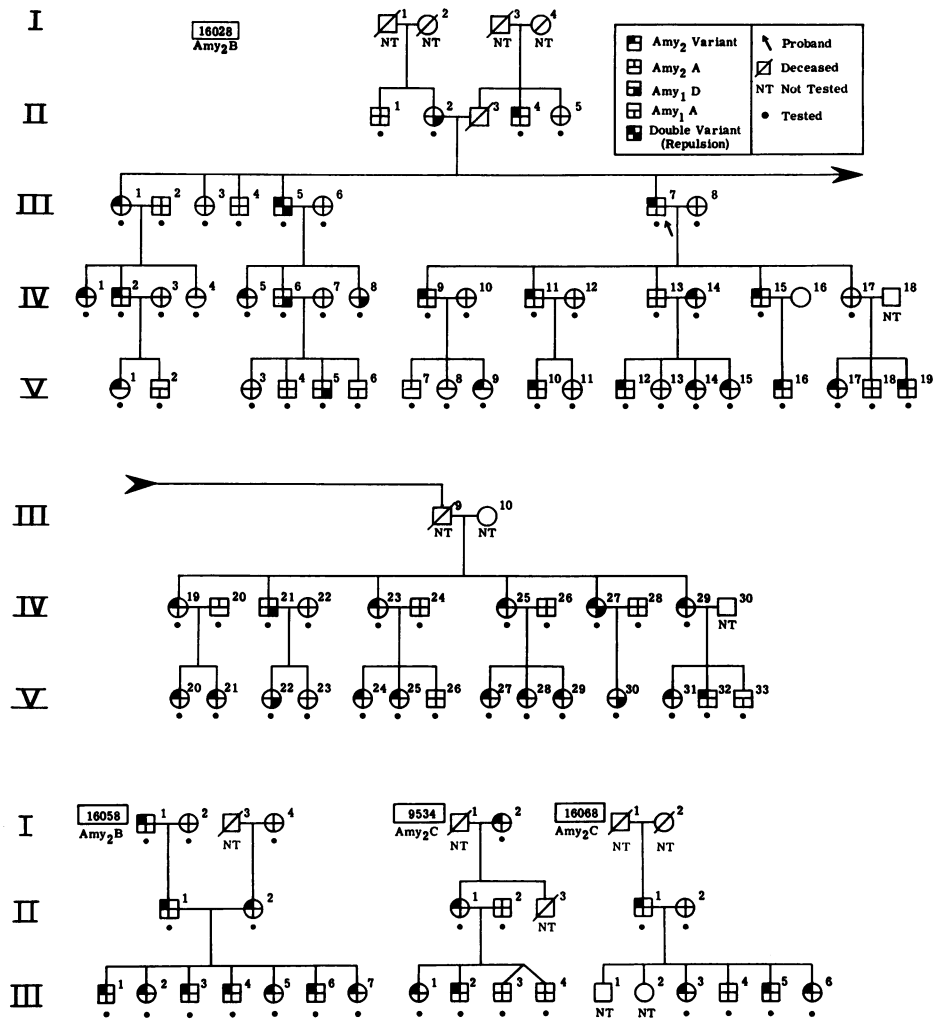

FIG. 8.—Pedigrees of families with pancreatic amylase (Amy_2) variants

well as Amy_1 D. Male-to-male transmission of the Amy_2 variant is seen in subjects III-7 to IV-9, 11, and 15. In families 16068 and 9534 the autosomal dominant inheritance of the Amy_2 C variant is demonstrated.

In family 16058 both parents are Amy_2 B heterozygotes. Six Amy_2 B $\times$ Amy_2 B matings have been ascertained thus far. In a few of these families Amy_1 A offspring allow us to state that both parents were, in fact, heterozygous, while in others the possibility remains that one or both of the parents could have been homozygous for the variant allele. At present, among 21 offspring at risk for being homozygous, all amylase zymograms have been indistinguishable from those of known heterozygotes. The offspring of these matings were tested for the 3:1 ratio expected for a trait expressing dominance when both parents are heterozygous; no significant deviation was found ($\chi^2_{(1)} = 1.08$, $.30 < P < .40$).

To date, we have had only one opportunity to examine a mating of two Amy_1 heterozygotes. One offspring showed a normal pattern and five showed patterns of known heterozygotes. We have not yet had the opportunity to study a mating type of the mixed heterozygous variety such as Amy_2 B $\times$ Amy_2 C.

Segregation data at the Amy_2 locus (table 1) shows no significant deviation from

TABLE 1

SEGREGATION ANALYSIS OF THE Amy_2 PHENOTYPE

MATING TYPE				OFFSPRING			
				Male		Female	
Male	Female	No. MATINGS	TOTAL No. OFFSPRING	Variant	Normal	Variant	Normal
Amy_2 A $\times$ Amy_2 B		28	85	19	20	28	18
Amy_2 B $\times$ Amy_2 A		23	79	18	19	20	22
Amy_2 A $\times$ Amy_2 C		1	3	1	1	1	0
Amy_2 C $\times$ Amy_2 A		1	4	1	1	2	0
Total		53	171	39	41	51	40

NOTE.—$\chi^2_{(1)} = 0.474$, $.40 < P < .50$ (total variants:total normal = 90:81).

the 1:1 ratio expected for an autosomal dominant trait ($\chi^2_{(1)} = 0.474$, $.40 < P < .50$).

4. *Population Studies*

The Amy_1 variants are relatively uncommon in Caucasians. Our previous studies gave a combined frequency of variant phenotypes of .007 [1]. Present studies (table 2) now based on 961 unrelated Caucasian Americans provide a combined frequency of .0073. In 208 Afro-Americans the combined frequency of Amy_1 variants was .0769. Among 27 individuals of Oriental ancestry (Chinese, Japanese,

TABLE 2

POPULATION DISTRIBUTION OF Amy_1 PHENOTYPES

ETHNIC GROUP	TOTAL NO. TESTED	Amy_1 PHENOTYPES						
		A	B	C	D	E	F	G
Caucasian Americans ..	961	954 (99.3%)	1 (0.10%)	5 (0.52%)	1 (0.10%)	0	0	0
Afro-Americans	208	192 (92.3%)	0	1 (0.48%)	2 (0.96%)	11 (5.29%)	0	2 (0.96%)
Oriental	27	26 (96.3%)	0	0	0	0	1 (3.7%)	0
Black Nigerians	10	8 (80.0%)	0	0	0	2 (20.0%)	0	0

and Korean), one autosomal dominant variant (Amy_1 F) was detected in a Chinese.

Only two Amy_2 variant phenotypes have been found to date; Amy_2 B is present in both Caucasian Americans and Afro-Americans. Thus far Amy_2 C has been seen only in Afro-Americans and in the few Nigerians studied. The single Caucasian previously reported as Amy_2 C [4] proved to be Amy_2 B on retesting. The frequencies of variant Amy_2 phenotypes are summarized in table 3. The frequency of Amy_2 B variant phenotypes is .105 in Caucasian Americans and .029 in Afro-Americans. The frequency of Amy_2 C in Afro-Americans is .047. No Amy_2 variant has been seen in individuals of Oriental ancestry.

DISCUSSION

The only comparable electrophoretic studies of human amylase have been reported by de la Lande and Boettcher [9, 10]. They, too, used a polyacrylamide electrophoretic system, but with different buffers. Resolution of the odd (Sa 1, 3, 5 . . .) and even (Sa 2, 4, 6 . . .) salivary amylase bands using their buffer is less clear when compared with those obtained using the present buffer system [1].

TABLE 3

POPULATION DISTRIBUTION OF Amy_2 PHENOTYPES

ETHNIC GROUP	TOTAL NO. TESTED	Amy_2 PHENOTYPES		
		A	B	C
Caucasian Americans	673	602 (89.5%)	71 (10.5%)	0
Afro-Americans	383	354 (92.43%)	11 (2.87%)	18 (4.70%)
Oriental	27	27	0	0
Black Nigerians	9	6	0	3 (33.3%)

Nonetheless, their serum amylase zymograms consisting of at least three bands decreasing in intensity toward the anode are similar to ours. They also reported that pancreatic and salivary gland extracts gave markedly different electrophoretic patterns [9]. We agree with their conclusion that individual isozymes from the pancreas and serum differ electrophoretically from salivary amylase isozymes. When they compared normal amylase isozymes from duodenal aspirate and serum with those of a patient with acute pancreatitis, the serum Amy_2 isozymes were markedly increased in the individual with pancreatitis. This strongly suggested to them that the Amy_2 isozymes originate in the pancreas.

Other workers have shown that the liver is a major source of serum and urinary amylase in the rat [11]. We conclude, however, that such is not the case in humans since individuals with pancreatic insufficiency or total pancreatectomy have little demonstrable serum or urinary amylase. We have also demonstrated that the small amounts of amylase activity seen in serum and urine of the patients are identical by electrophoresis to the major salivary amylase isozymes, Sa 2 and Sa 4, suggesting that small amounts of salivary amylase do find their way into the serum and are excreted by the kidney.

Previous studies of amylase in serum, urine, pancreas, and saliva in agar showed from one to four isozymes. When serum showed four bands, the pair nearer the cathode was construed as being of pancreatic origin and the pair nearer the anode of salivary gland origin [6, 7]. We have confirmed that in Amy_2 variant phenotypes the two cathodal bands are indeed the normal and variant pancreatic isozymes. However, the isozymes nearer the anode, variably seen in serum and urine, usually are not, as originally thought, of salivary origin. These isozymes in serum (urine) which seem to migrate like the major salivary amylase isozymes, in fact differ appreciably in their migration and are electrophoretically identical with pancreatic amylase isozymes. Agar electrophoresis of salivary amylase (Amy_1 A) shows that the common isozymes migrate slightly faster than the more anodal isozymes seen in serum and urine (figs. 6, 7). Further, salivary amylase variants have their expression cathodal to the normal components of salivary amylase in both agar (fig. 5) and polyacrylamide gels (fig. 3), not anodal as previously described [2, 6, 7] (fig. 5). We therefore feel that serum and urine should be utilized only for the identification of pancreatic amylase (Amy_2) variation since all of their major isozymes appear to be of pancreatic origin.

Based on the presumption that salivary and pancreatic amylases are the products of two closely linked, genetically independent loci [12], an attempt has been made to provide an acceptable nomenclature for their isozymes, alleles, and phenotypes. It was not possible to number the isozymes consecutively with the form having highest mobility toward the anode being numbered 1 as suggested by the subcommittee on isoenzymes of the International Union of Biochemistry. Both Amy phenotypes are characterized by an indefinite series of isozymes with least activity in those of greatest mobility and are thus variably present or absent. For this reason the normal isozymes were sequentially numbered from the origin (e.g., Sa 1, Sa 2, . . . , and Pa 1, Pa 2, . . .).

We have followed the literature where possible and have indicated alleles on a continuing alphabetic basis: Amy_1 refers to the locus for salivary amylase and Amy_2 refers to the locus for pancreatic amylase. The normal alleles for each of these loci were previously designated as Amy_1^A and Amy_2^A. Consistent with previous usage, Amy_2^B has been reserved for the common variant allele seen in Caucasian populations. The additional Amy_2 allele seen in individuals of African descent has been designated as Amy_2^C. The Amy_1 locus has given rise to electrophoretically different phenotypes; we now designate as alleles Amy_1^B, Amy_1^C, Amy_1^D (originally called Bn, Tn, and A1), Amy_1^E, Amy_1^F, and Amy_1^G. Variant phenotypes are named with respect to the variant isozyme, for example, Amy_1 D and Amy_2 B (fig. 4).

The acrylamide electrophoretic system easily distinguishes Amy_1 and Amy_2 phenotypes providing the basis for demonstrating autosomal dominant inheritance. Neither variant homozygosity nor doubly mutant heterozygosity has been seen to date as a clearly distinguishable phenotype. Since among offspring at risk for being homozygous for the Amy_2^B allele all were indistinguishable from known Amy_2 B heterozygotes, dominance is present with respect to the variant phenotypes. It is important to note that each of the variant phenotypes has been seen alone in the presence of a homozygous normal phenotype at the other amylase locus, and it is further important to note the independent inheritance of the expressions of these two loci as seen in family 16028 (fig. 8).

One might assume that originally there was a single locus for amylase expression. If the locus were duplicated, then the expression of each locus could have undergone modification such that each was limited to a specific organ, for example, the pancreas and salivary glands. Either before or, more likely, after these events the genes could have mutated, giving rise to independent and different Amy_1 and Amy_2 expressions. Our rationale for this hypothesis is based on the necessity of explaining the frequent occurrence of the Amy_1^E and Amy_2^C alleles in coupling in Afro-Americans and Nigerians and the relatively infrequent occurrence of heterozygotes for these alleles at only one locus in these populations. The Amy_1^E:Amy_2^C chromosome could have originated in their ancestors by sequential mutations at these loci. It would have been extremely unlikely to have resulted from recombination in a double heterozygote in repulsion. Since the Amy_1 E and Amy_2 C phenotypes are not seen in Caucasians, it is attractive to speculate that the specific mutations in question are not of recent origin and that the heterozygotes of the type Amy_1 E:Amy_2 A or Amy_1 A:Amy_2 C are a result of crossing over in a double heterozygote in coupling rather than a recurrent identical mutation.

The frequencies of the phenotypes are summarized in tables 2 and 3. It is interesting that the Amy_2 B variants previously reported occur at similar frequencies, 10.3% in southern Moravians [8] and 10% in Caucasian Australians [10]. The new variants, Amy_1 E, Amy_1 G, and Amy_2 C, are present exclusively in those of black African descent, while Amy_1 F has been seen in one Chinese family. The involvement of possible selective factors such as diet [13] in maintaining these polymorphisms remains an enigma.

Amylase variants occur at a frequency high enough to make them particularly

useful for population genetic studies, as for example in estimating the proportion of racial admixture (M). Since the Amy_2 B phenotype occurs in Afro-Americans but has not been seen in black Africans, the Amy_2 locus is useful in estimating this admixture in present Afro-Americans. An estimate of M can be obtained from $M = (q_n - q_a)/(q_c - q_a)$, where q_n is the frequency of the Amy_2^B allele in Afro-Americans, q_c is the frequency of Amy_2^B in Caucasian Americans, and q_a is the frequency of Amy_2^B in black African ancestors [14]. If the Amy_2^B allele arose in the ancestors of Caucasian Americans and did not occur in the ancestral population of Afro-Americans ($q_a = 0$), then the frequency of Amy_2^B in present-day American blacks suggests a Caucasian admixture of 0.27. This estimate is in agreement with those reviewed by Reed [14] in various populations of nonsouthern American blacks.

SUMMARY

Discontinuous sheet polyacrylamide gel and agar gel electrophoresis have been utilized to demonstrate the origin of amylase phenotypes and to identify seven salivary (Amy_1) and three pancreatic (Amy_2) amylase phenotypes that are expressions of two closely linked loci on chromosome 1. The combined frequencies of the Amy_1 variants in Caucasian Americans and Afro-Americans is .0073 and .0769, respectively. The frequency of Amy_2 B in Caucasian Americans and Afro-Americans is .105 and .029, respectively. The Amy_2 C phenotype is present only in Americans of African descent at a frequency of .047 and is found also in black Africans. Family data demonstrate an autosomal dominant mode of inheritance for each of the variants. The reasons for avoiding agar gel electrophoresis as a single system to type Amy_1 and Amy_2 phenotypes are discussed.

ACKNOWLEDGMENTS

We wish to thank L. Noack, L. Jackson, and B. Thompson for their technical assistance and help in collection of specimens. Dr. P. M. Conneally provided helpful and constructive criticism.

REFERENCES

1. WARD JC, MERRITT AD, BIXLER D: Human salivary amylase: genetics of electrophoretic variants. *Am J Hum Genet* 23:403–409, 1971
2. KAMARÝT J, LAXOVÁ R: Amylase heterogeneity variants in man. *Humangenetik* 3:41–45, 1966
3. BOETTCHER B, DE LA LANDE FA: Electrophoresis of human saliva and identification of inherited variants of amylase isozymes. *Aust J Exp Biol Med Sci* 47:97–103, 1969
4. MERRITT AD, BIXLER D: Genetic polymorphism in human urinary amylase. *Excerpta Medica Int Congr Ser* 233:120, 1971
5. MALACINSKI GM, RUTTER WJ: Multiple molecular forms of α-amylase from the rabbit. *Biochemistry* 8:4382–4390, 1969
6. KAMARÝT J, LAXOVÁ R: Amylase heterogeneity: some genetic and clinical aspects. *Humangenetik* 1:579–586, 1965
7. LAXOVÁ R, KAMARÝT J: Genetic aspects of human α-amylase heterogeneity. *FEBS Symposium* 18:335–339, 1970
8. KAMARÝT J, ADÁMEK R, VRBA M: Possible linkage between uncoiler chromosome (Un-1) and amylase polymorphism (Amy_2) loci. *Humangenetik* 11:213–220, 1971

9. DE LA LANDE FA, BOETTCHER B: Electrophoretic examination of human serum amylase isozymes. *Enzymologia* 37:335–342, 1969
10. BOETTCHER B, DE LA LANDE FA: Unusual salivary and serum amylase isoenzymes in a man. *Enzymologia* 40:234–242, 1971
11. SANDERS TG, RUTTER WJ: Molecular properties of rat pancreatic and parotid α-amylase. *Biochemistry* 11:130–136, 1972
12. MERRITT AD, RIVAS ML, WARD JC: Human amylase loci: evidence for close linkage. *Nature* (Lond) 239:243–244, 1972
13. DE JONG G, HOORN AJW, THÖRIG GEW, SCHARLOO W: Frequencies of amylase variants in *Drosophila melanogaster*. *Nature* (Lond) 238:453–454, 1972
14. REED TE: Caucasian genes in American Negroes. *Science* 165:762–768, 1969