VARIATION IN AMYLASE HAPLOTYPES AMONG CONGENIC LINES OF THE HOUSE MOUSE

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ABSTRACT

Pancreatic amylase in the mouse displays considerable quantitative genetic variation. Agar gel electrophoresis reveals that homozygous animals have either one form of the enzyme, type A, or two forms, type AB. Only few animals have been found that contradict this statement, namely among *Mus musculus castaneus* from Thailand, which has a single-banded B type. Double-banded homozygous specimens of various origins have different relative proportions of the two isoenzymes. By measuring the A:B ratios in such animals, a number of distinct haplotypes or amylase complexes, determining ratios ranging from 61% A:39% B to 12% A:88% B, have been recognized. These complexes differ also with respect to the total amount of amylase produced. If the reference stock C3H/As is given the value 1, then other haplotypes have values ranging from 1.0 to 0.27. Nineteen amylase haplotypes have been established in congenic lines on a C3H/As background. Some of these lines contain at least four active pancreatic amylase structural genes and breeding experiments have demonstrated that the genetic elements regulating total amylase production and relative proportions of the isoenzymes are located within the amylase complex, *cis*-acting, and very closely linked to the structural genes.

GENETIC variation in both salivary and pancreatic amylases from the mouse was first described by SICK and NIELSEN (1964). Using agar gel electrophoresis, one of two salivary amylase isoenzymes, SAL-A or SAL-B, was found in homozygous animals. Breeding experiments showed that the variation was caused by two codominant alleles at a locus designated *Amy-1*. Among the animals studied the SAL-A type had only one form of pancreatic amylase, PAN-A, whereas all homozygous SAL-B mice expressed two pancreatic amylase isoenzymes, PAN-A and PAN-B. In this electrophoretic system the pancreatic bands are distinguished from the salivary amylases by their more rapid migration toward the cathode. The pancreatic variation, as with salivary, is inherited in a simple Mendelian way, but to account for the existence of two tissue-specific amylases in homozygotes, two pancreatic loci, *Amy-2* and *Amy-3*, were suggested.

In a study of the amylase variation among feral mice, NIELSEN and SICK (1975) found that these also agreed with the earlier findings that SAL-A animals were of the PAN-A type and that SAL-B coincided with both forms of pancreatic isoenzymes, namely PAN-AB. Since this is true for polymorphic populations as well, a strong linkage disequilibrium exists among the closely linked salivary and pancreatic amylase loci. However, one confirmed exception was found in animals originating from Peru. These were of the previously unknown type

SAL-B PAN-A. This finding, together with another "recombinant chromosome" described in the present paper, was taken as genetic evidence that the salivary and pancreatic amylases are determined by different structural loci.

Although that study of feral mice did not reveal any "new" electrophoretic alleles, a significant amount of quantitative variation was found in the pancreatic amylase among homozygous PAN-AB animals. Some of the specimens resembled the double-banded type described by SICK and NIELSEN (1964) with almost equal proportions of the A and B pancreatic isoenzymes. In other mice, skewed proportions were seen in which, for instance, the amount of B enzyme clearly exceeded that of the A form. Like the electrophoretic (qualitative) variation, this quantitative variation was found to be under direct genetic control.

By measuring the A:B ratios in double-banded animals of various origins, we have been able to differentiate a number of chromosome types, or amylase complexes as those haplotypes have been named (HJORTH 1979). In addition to this proportional variation between the two pancreatic amylase isoenzymes, some of the amylase complexes have been shown to differ with respect to their total pancreatic amylase production. Hence, the amylase system in the mouse contains considerably more variation than can be seen simply from the "ordinary" alleles in agar gel electrophoresis.

On the basis of a series of amylase haplotypes that have now been established in congenic lines, the aim of this paper is to describe the quantitative variation in mouse pancreatic amylase in detail and to demonstrate that it is controlled by a number of structural genes in association with regulatory elements closely linked to those genes.

MATERIALS AND METHODS

Animals: C3H/As, which has been maintained at our institute since 1967, has served as reference stock. Other inbred stocks used are: A/J, BALB/c, C57BL/6 from Bomholtgård, Laven, Denmark; CE/J, DBA/2J and RIIIS/J from the Jackson Laboratory, Bar Harbor, Maine; and YBR/Cv from DR. VERNE CHAPMAN, Buffalo, New York. In addition to these, the amylase complexes from a number of wild trapped and colony-bred animals of recent wild origin have been analyzed. In particular, the haplotype derived from *Mus musculus castaneus*, a Thailand subspecies kindly provided by DR. VERNE CHAPMAN, has been of great importance in the study.

Establishment of congenic lines: Congenic lines of type SAL-B PAN-AB were made by crossing a selected mouse of such type to C3H/As, which is SAL-A PAN-A. F_1 animals and heterozygotes of ensuing generations were backcrossed to C3H/As for ten generations. Finally the homozygous SAL-B PAN-AB type was re-established from crosses between N_{10} heterozygotes. The resulting congenic lines are designated, for example, C3H·Amy^{CE} or C3H·Amy^{w8} where the transferred amylase complexes originate from the CE inbred stock and a wild mouse, respectively. For details of the nomenclature, see HJORTH (1979). Among wild mice the electrophoretic SAL-A PAN-A type is very common (NIELSEN and SICK 1975). If these animals are mated to C3H/As, the proper heterozygotes cannot be selected for backcrossing, so another strategy was used in producing SAL-A PAN-A lines. First the amylase complex, Amy^{w1}, from *M. m. castaneus*, SAL B PAN-B, was transferred onto the C3H/As genome as described. This congenic line, C3H·Amy^{w1}, was then used as the laboratory stock in crosses to wild animals and in the subsequent series of backcrosses from which congenic SAL-A PAN-A lines were generated. During this procedure we derived advantage from the close linkage of the salivary and pancreatic amylase loci, since the heterozygotes could be selected on the basis of their electrophoretic salivary amylase pattern.

Sample preparation: Saliva was taken from ether-anesthetized mice by flushing the mouth cavity with a drop of distilled water. After a 2-min centrifugation at 15,000 × g, the samples were used for electrophoresis. Pancreatic amylase was obtained by removing the pancreas from sacrificed animals

and freezing at −18°C in a 2× volume of water. After thawing, the glands were stirred with a rotating glass rod and centrifuged at 15,000 × g for 4 min. The supernatant was used for electrophoresis without further treatment.

Electrophoresis: The agar gel electrophoresis, performed on microscope slides in a phosphate buffer system at pH 7.3, has been described in detail previously (SICK and NIELSEN 1964; SICK 1965). Salivary amylase patterns were visualized after electrophoresis uing the starch-iodine reaction (SICK and NIELSEN 1964; NIELSEN 1969). Slides with pancreatic amylase were fixed in acetic acid, methanol and water (1:5:5), air dried and stained for protein with amido black. The relative density of amylase isoenzymes in double-banded samples were measured with a Joyce Loebl integrating scanner (NIELSEN 1977a).

Amylase determination: Amylase activity was assayed by measuring the free reducing sugar groups produced when amylase decomposes starch, following the procedure of DAHLQUIST (1962), with the modifications described by HJORTH *et al.* (1979).

RESULTS

In initial investigations of mouse amylase variation, the electrophoretic phenotypes of the animals were simply determined by inspection of the zymograms (SICK and NIELSEN 1964; NIELSEN and SICK 1975). Even by this method it was found that there were some differences in the relative proportions of the two pancreatic amylase isoenzymes from homozygous PAN-AB animals (NIELSEN and SICK 1975), but we could not give an objective description of the variation nor identify the number of different phenotypes represented.

This has now been made possible by measuring the relative amounts of amylase protein on electrophoresis slides, since it is fortuitous that amylase is the only protein in pancreas preparations which, when the agar gels are stained with amido black, migrates toward the cathode. In Figure 1, some of the quantitative variation is shown. The six slides in the figure represent the pancreatic amylase pattern from C3H/As (PAN-A), four different congenic double-banded lines (PAN-AB) and the congenic lines, C3H·Amy^{w1}, carrying the *M. m. castaneus* haplotype (PAN-B). The scan curves from the double-banded slides are given in Figure 2, and from these the relative amounts of A:B isoenzyme in the individual samples, or as quoted in this paper, the amount of

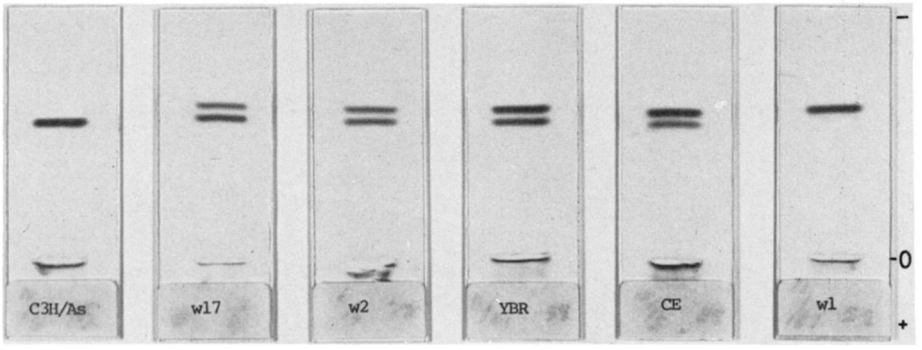

FIGURE 1.—Agar gel electrophoresis of pancreas homogenates from C3H/As (1), four congenic type PAN-AB lines, *viz.*, C3H·Amy^{w17} (2), C3H·Amy^{w2} (3), C3H·Amy^{YBR} (4), C3H·Amy^{CE} (5), and from C3H·Amy^{w1} (6). Protein was stained with amido black.

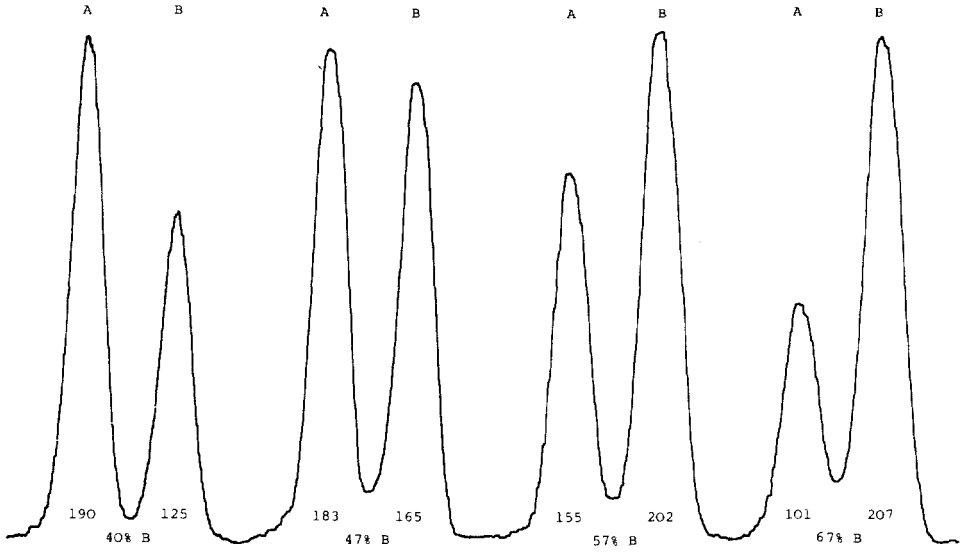

FIGURE 2.—Scanning curves of the four double-banded slides in Figure 1 representing C3H·*Amy*^{w17} (1), C3H·*Amy*^{w2} (2), C3H·*Amy*^{YBR} (3), and C3H·*Amy*^{CE} (4). Integrals of the A and B band and the calculated percentage of B amylase are given for individual samples.

PAN-B as a percentage of the total amylase can be determined. The measurements are based solely on the relative color intensity of the bands.

From studies of salivary amylase variation in the bank vole, *Clethrionomys glareola* (NIELSEN 1977a, 1977b; HJORTH *et al.* 1979), it is known that relative amounts of stained protein directly reflect relative amounts of enzyme activity. To ensure that the same relationship holds true for mouse pancreatic amylase, the A and B isoenzymes were eluted separately from double-banded agar slides after electrophoresis and amylase activity measured as described for the bank vole (NIELSEN 1977a). These results for three heterozygotes from a cross between C3H/As and a congenic PAN-B animal from C3H·*Amy*^{w1}, and three homozygous PAN-AB specimens from the congenic line C3H·*Amy*^{w2} are compared with protein measurements of samples from the same animals in Table 1. The proportions of type B amylase estimated in these two ways are very close, but as measuring activity was much more time consuming and the possibility for error arises when the electrophoretic bands are so close that it is difficult to isolate the different amylase forms, the simpler protein-scanning method has been used.

The majority of inbred mouse strains have the amylase type SAL-A PAN-A, whereas only a few, including CE and YBR, are SAL-B PAN-AB (CHAPMAN and PAIGEN 1979). CE has a clearly skewed pancreatic amylase pattern dominated by the B form [identical to the type in Figure 1 (5)]. When the proportions were measured in several CE individuals representing various age groups and both sexes, all values clustered around 68% B (Figure 3). The amylase haplotype from CE was introduced into the C3H/As genome as described in MATERIALS AND METHODS, and the A:B ratio was measured for heterozygotes from all ten backcross generations. Ten F_1 offspring from the initial cross between C3H/As

TABLE 1

Comparison of relative pancreatic amylase enzymatic activity and relative amounts of stained protein in samples from PAN-AB animals

	% activity in B band		% stained protein in B band	
C3H × castaneus heterozygotes	35		41	
	41	40.0	42	41.3
	44		41	
C3H·Amy^{w2} homozygotes	49		47	
	48	47.0	45	46.0
	44		46	

and CE showed 29–33% B amylase, with an average of 31.2% (SE 0.34). No systematic deviation from the F_1 value could be traced among the heterozygotes in the subsequent generations (Figure 3) and in the last group measured, heterozygotes from an $N_{10} \times N_{10}$ cross, the average B value was 30.7% (SE 0.43). Figure 3 also gives data for mice from the established congenic line, C3H·Amy^{CE}. The distribution within this group is very similar to that found for the pure-bred animals just as the average B values are almost identical.

The second inbred SAL-B PAN-AB stock used to construct a congenic line, *viz.*, YBR, gave a value of 57.6% (S.E. 0.46) for the B amylase form, the same as the average value measured in the derived congenic line, C3H·Amy^{YBR}. From wild or colony-bred PAN-AB mice a total of 12 congenic lines have been made. The percentages of B amylase in these homozygous lines, ranging from 39% to 88%, are shown in Table 2. In all individual lines the heterozygote values were constant during the backcrossing process and the ratio among the two isoenzymes in homozygous animals also remained the same. These results demonstrate that the genetic determinants causing the specific relative proportions in animals homozygous for a double-banded pancreas amylase pattern are *cis*-acting and are restricted to the transferred chromosome segment that carries the amylase structural genes.

So far, only from *M. m. castaneus* have we detected a chromosome coding solely for the pancreatic amylase type B. In crossing a PAN-A animal with such a PAN-B, one would expect to find equal amounts of the two isoenzymes in heterozygotes. However, this was not found to be true for offspring from C3H/As × *M. m. castaneus*, because all animals gave a value near 40% B amylase, and this value remained unchanged throughout ten backcross generations ($\bar{X}$ 39.4% B, S.E. 0.25).

This finding led us to question whether other crosses would show a similar trend; that is, an unequal contribution from the chromosomes carrying the amylase genes. A series of matings was therefore made between the congenic C3H·Amy^{w1} line and each of the inbred stocks A/J, BALB/c, C57BL/6, DBA/2J and RIIIS/J, all being of type SAL-A PAN-A. Without exception, the offspring groups had close to 40% pancreatic B amylase, indicating that these strains all have a chromosome type with the same relative "strength" as C3H/As.

In most Danish mouse populations the predominant amylase type is SAL-A

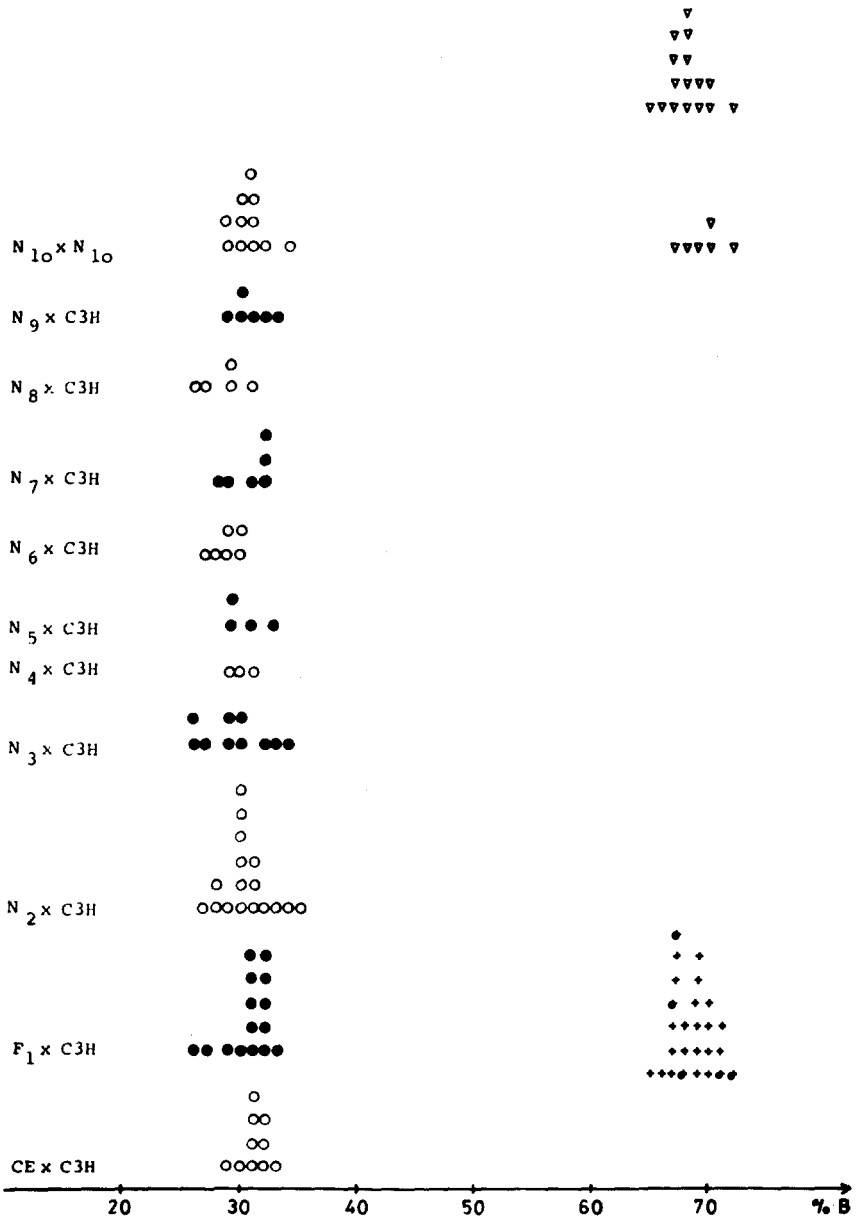

FIGURE 3.—Results from the establishment of the congenic line C3H·*Amy*^{CE}. Percentages of B amylase was measured in heterozygotes from 10 backcross generations. Values for each individual are indicated by ○ or ●. The two symbols are used alternately to distinguish successive generations. $\bar{X}_{F_1}$ = 31.2% B (S.E. 0.34). Mean value for heterozygotes from $N_{10} \times F_{10}$ equals 30.7% B (S.E. 0.43). $\bar{X}_{total}$ = 30.3% B (S.E. 0.20). +, Inbred CE ($\bar{X}$ = 68.6% B, S.E. 0.34); ▽, congenic C3H·*Amy*^{CE} ($\bar{X}$ = 68.4% B, S.E. 0.38).

PAN-A (NIELSEN and SICK 1975) and, as often occurs, a different picture appeared when such feral animals were used in the crosses. The results from a mating of a wild PAN-A male and a C3H·*Amy*$^{w\,1}$ female are shown in Figure 4.

TABLE 2

Percentage of pancreatic B amylase in homozygotes and heterozygotes of 12 congenic type AB lines and of one type B line

Origin	Congenic lines (amylase complex)	% B in homozygotes			% B in heterozygotes			Relative "strength"
		$\bar{X}$	SE	N	$\bar{X}$	SE	N	
Zealand	w17	39.1	0.47	30	15.2	0.25	51	0.64
Zealand	w21	44.1	0.31	18	16.9	0.32	38	0.68
Pet shop	w11	44.1	0.48	32	11.1	0.23	67	0.34
Japan	w34	46.3	0.40	24	11.3	0.26	91	0.32
Pet shop	w2	46.1	0.32	21	16.9	1.13	16	0.58
Holland	w3	56.8	0.24	26	25.1	0.35	16	0.79
Inbred	YBR	57.6	0.46	40	12.2	0.33	64	0.27
N. Jutland	w22	64.7	0.44	46	26.2	0.30	71	0.68
S. Jutland	w19	67.3	0.44	39	29.6	0.32	43	0.79
Inbred	CE	68.5	0.25	48	30.2	0.20	91	0.79
S. Jutland	w5	81.2	0.35	30	35.4	0.34	59	0.77
S. Jutland	w9	88.3	0.47	46	39.2	0.37	25	0.80
Thailand	w1	100			39.4	0.25	86	0.67

The heterozygotes all have one parent from the inbred stock C3H/As. For each amylase complex the enzyme production, relative to C3H/As, is estimated.

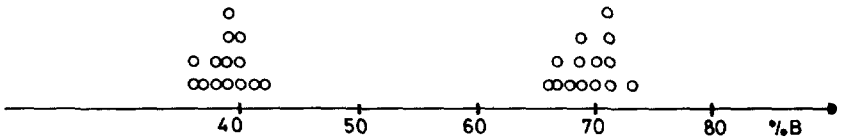

FIGURE 4.—Offspring from a cross between C3H·*Amy*^{w1} ♀ (PAN-B) × wild ♂ (PAN-A). Percentage of B amylase in individual animals is indicated by ○. Mean values for the two groups are 38.9% B (S.E. 0.47) and 69.2% B (S.E. 0.45). From this cross two congenic lines, C3H·*Amy*^{w15} and C3H·*Amy*^{w16}, have been made starting with an animal from each group.

As expected, all F_1 animals from three litters were PAN-AB, but they clearly segregated into two groups indicating that the wild parent was a heterozygote. One group had an average of about 40% B and the other about 70% B amylase. Using C3H·*Amy*^{w1} as the laboratory parental animals, two congenic lines, C3H·*Amy*^{w15} and C3H·*Amy*^{w16}, have been constructed with a heterozygote from each of the two groups as starting points. Six different SAL-A PAN-A amylase complexes are now, *via* C3H·*Amy*^{w1}, on a C3H/As background. The heterozygote values for the lines are given in Table 3.

Since the same tester chromosome was used in the production of all these lines and the genetic background can be considered to be uniform for the lines at generation 10, the observed differences must result from different PAN-A amylase complexes. If the enzyme production determined by the C3H/As chromosome is arbitrarily set to 1, the relative "strengths" of the other haplotypes can be calculated from the proportions of the two amylase forms in heterozygotes. In C3H/As × C3H·*Amy*^{w1} animals the ratio is approximately 60% A:40% B, which means that the *Amy*^{w1} complex has a production of ⅔ or 0.67 relative to that of the C3H/As chromosome. In *Amy*^{w1}/*Amy*^{w16} heterozygotes with 30% A and 70% B (Figure 4), the amylase synthesis from the wild-

TABLE 3

Percentages of pancreatic B amylase in heterozygotes between C3H·Amy^{w1} and six congenic PAN-A lines

Origin	Congenic line	% B heterozygotes	Relative "strength"
Peru	w4	39.6	1.0
N. Jutland	w15	39.4	1.0
C. Jutland	w33	49.0	0.70
C. Jutland	w13	53.9	0.57
Greece	w34	58.8	0.47
N. Jutland	w16	70.6	0.28

For each amylase complex the enzyme production, relative to C3H/As, is estimated.

derived PAN-A chromosome is 3/7 or 0.43 of that of *Amy*^{w1}. Relative to C3H/As, this PAN-A complex therefore has a "strength" of only 0.29 (the product of 0.43 by 0.67), or less than 1/3. The values for the congenic PAN-A lines are calculated in Table 3. Additional lines are being made but up to now C3H/As represents the amylase complex associated with the highest enzyme production.

The argument that deviations from a 1:1 ratio in PAN-A × PAN-B heterozygotes are a reflection of differential amylase production from the two chromosomes can also be used indirectly for offspring from PAN-A × PAN-AB animals. CE, for instance, is characterized by having 68% B amylase, whereas the C3H/As × CE heterozygotes have 30% B (Figure 3). The CE complex thus contributes 30/68 or 0.44 of the total amylase whereas 0.56 is contributed by the C3H/As chromosome, giving a "strength" of 44/56 or 0.79 for CE, relative to C3H/As.

The two congenic SAL-B PAN-AB lines created from the inbred YBR, C3H·*Amy*^{YBR}, and a feral mouse from Holland, C3H·*Amy*^{w3}, appeared similar when judged from their homozygous values of 57.6% (S.E. 0.46) and 56.8% B (S.E. 0.24), respectively (Table 2). However, when the parameter, amylase production, was included, these two haplotypes turned out to be quite different. In C3H/As × C3H/*Amy*^{w3} heterozygotes 25.1% (S.E. 0.35) of the pancreatic amylase was of type B, putting the "strength" of the *Amy*^{w3} chromosome of 0.79. The offspring from C3H/As × C3H·*Amy*^{YBR} showed a very skewed amylase pattern with only 12.2% B (S.E. 0.33), so the YBR chromosome has a relative value as low as 0.27. The "strengths" of the amylase complexes in the congenic SAL-B PAN-AB lines are included in Table 2.

In our total material, the YBR haplotype is associated with the lowest amylase synthesis (0.27), closely followed by the wild-derived PAN-A complex, *Amy*^{w16}, with a relative value of 0.28. The expected percentage of B amylase in heterozygotes carrying these two chromosomes should therefore be a little less than half the 58% B recorded in YBR homozygotes. When the cross was made, the actual measured average value, based on 16 individuals from two litters, was 27.9% B (S.E. 0.25). A similar result was obtained from a cross between the two SAL-B PAN-AB lines C3H·*Amy*^{w3} having 57% B and C3H·*Amy*^{w5} with 81% B. The two complexes from these lines have almost the same relative amylase production (Table 2), and in accordance with this the amount of B amylase in the heterozygotes was 70.3% (S.E. 0.46), close to the mean of the parental values.

cis-acting genetic elements, closely linked to the amylase structural genes, have been mentioned previously as one necessary condition for the constant proportions among the A and B pancreas isoenzymes observed in heterozygotes from the backcross generations during the production of congenic SAL-B PAN-AB lines. The invariable proportions show also, however, that the relative "strengths" of the different chromosomes remain unchanged during this process. Therefore, a set of genetic determinants responsible for the overall pancreatic amylase production must be associated with this chromosome segment. Since no correlation seems to exist among relative isoenzyme proportions specified by the amylase complexes and their "strengths", the two parameters may have different genetic bases and an adequate description of a particular mouse pancreas amylase type should give information about both parameters.

During the production of congenic lines we have accumulated data from over 2500 animals in which a possible recombination within the amylase segment could be detected. In addition, the offspring of more than 500 of these animals were tested, but in no case was a recombinant type seen in the electrophoretic pattern, isoenzyme proportions, amylase production or among those parameter and salivary amylase. So, in classic genetic terms, the size of the amylase complex, regulating both the qualitative and the quantitative variation in the mouse amylases, is very limited.

DISCUSSION

Partly because of nongenetic factors such as age and feeding conditions and partly because of the genotype of the animals, the overall amylase content of the pancreas may vary considerably from one specimen to another. To circumvent the interspecimen variation our measurements have been based solely on the relative ratio of the two isoenzymes in double-banded animals. This has proved to be a very powerful tool in the analysis and has enabled us to characterize a number of amylase complexes that differ with respect to the relative proportions of A and B pancreatic amylase and/or with respect to the amount of enzyme they determine.

In the initial presentation of genetic variation in mouse amylase (SICK and NIELSEN 1964), we assumed that the pancreatic amylase was determined by two loci. Such a model may explain the presence of two isoenzymes in homozygous animals, but it can account for the quantitative variation described here only if both loci have a series of multiple alleles with the following properties: 1) they all determine amylase of either the agar gel electrophoretic form A or B; 2) they determine different amounts of amylase. For instance, the congenic line C3H·Amy^{w3} has 57% B amylase whereas C3H·Amy^{w5} has 81% (Table 2), but the amylase complexes from both lines have the same overall amylase production relative to C3H/As. On the other hand, if C3H·Amy^{w3} is compared with C3H·Amy^{YBR} they are seen to have the same A:B ratio but quite different "strengths" (Table 2).

Bank vole salivary amylase displays quantitative variation with elements distinctly resembling the variation in mouse pancreatic amylase, such as skewed proportions of isoenzymes in homozygous strains and chromosomes with

different amylase production (NIELSEN 1977a, 1977b; HJORTH *et al.* 1979). The genetic basis for this system is a variation in the number of (active) salivary amylase structural genes and a single chromosome can have from one to three such genes (NIELSEN 1977b). The described variation in the mouse could be explained by a similar model, where the individual structural genes may code for either the electrophoretic A form or the B form of pancreatic amylase. In such a model the undefined expression "genetic determinants", used in the RESULTS section, refers to clusters of structural genes. The number of genes in the cluster determines the total amylase production, whereas the ratio of A to B genes is responsible for the isoenzyme proportions. Making the assumption that each gene specifies identical enzyme production, then the number has to be fairly high for the model to be valid. Again YBR or C3H·Amy^{YBR}, with 42% A and 58% B amylase, may serve as an example. To give this (almost) 2:3 ratio, the chromosome should have a minimum of 2 A and 3 B genes. Yet the YBR complex has an enzyme production of 0.27 compared to the C3H/As chromosome, which means that the C3H/As haplotype should have 18–19 active pancreatic amylase structural genes.

By employing other techniques, e.g., polyacrylamide gel electrophoresis and peptide mapping, a number of the congenic lines have been used to test whether multiple structural amylase genes could be detected. The individual lines showed from one to four pancreatic amylase forms in polyacrylamide, demonstrating the presence of at least four structural genes (HJORTH *et al.* 1980; HJORTH 1982). Additional variation among the lines was seen in peptide maps from purified pancreatic amylase, which indicated an even higher number of genes (HJORTH *et al.* 1980). In polyacrylamide gel electrophoresis both C3H·Amy^{CE} and C3H·Amy^{w3} have the same four isoenzymes but in different constant relative proportions (BLOOR *et al.* 1981). Again these skewed enzyme patterns suggest the existence of some kind of regulatory elements associated with the single structural genes.

In the light of these findings, the first proposed model, in which the entire pancreatic amylase variation could be determined by only two loci, must be abandoned.

It was possible that the amylase proportions measured by our protein scannings could be defective on account of differential degradation of the isoenzymes. However, this possibility was ruled out by *in vivo* pulse-labeling experiments, in which it was observed that the relative rate of amylase isoenzyme synthesis closely reflected the relative proportions measured by protein scanning (BLOOR *et al.* 1981). Thus, the differences must occur at the transcription or translation level and are most likely caused by the production of different amounts of mRNA. A three-fold variation in pancreatic amylase mRNA content was seen in the two congenic SAL-A PAN-A lines, C3H·Amy^{w15} and C3H·Amy^{w16}, whose amylase production relative to C3H/As is 0.28 and 1.0, respectively, a ratio close to 1:3 (OWERBACH *et al.* 1981). In the same study, the number of amylase genes was estimated by hybridizing total mouse DNA to a ^{32}P-labeled rat pancreatic amylase cDNA probe. The rat has at least four amylase genes (MACDONALD *et al.* 1980) and the hybridizations showed that those congenic mice investigated had 2–2.5 times more, or approximately ten genes.

Apparently the two lines did not differ in number, but active and nonactive genes (or pseudogenes) cannot be distinguished by this method, so the observed differences in mRNA (and enzyme) content may result from differences either in the number of active genes with the same mRNA production, or to different rates of synthesis and/or degradation of amylase mRNA from the same number of active genes.

If the mouse has in the order of ten amylase genes, of which one is the salivary amylase structural gene (HJORTH 1982) and some pseudogenes (SCHIBLER *et al.* 1982), the second model proposed, with about 20 active pancreatic amylase structural genes, is obviously not valid either.

HAGENBÜCHLE *et al.* (1980) found only one kind of pancreatic amylase mRNA in the inbred mouse strain A/J and argued that only a single pancreatic amylase gene is active. Later the same authors (SCHIBLER *et al.* 1982) reported that A/J contains two identical pancreas amylase genes. Both in agar and in polyacrylamide electrophoresis this stock offers proof of just one isoenzyme, identical to that produced by C3H/As. The A/J chromosome is, however, responsible for a very high relative amylase production, similar to that of the C3H/As chromosome (see RESULTS). In addition to the pancreas, amylase mRNA can be isolated from mouse salivary glands and from liver, although concentrations in these three tissues are very different: 10^5 molecules/cell in pancreas, 10^4 molecules/cell in salivary gland, and only 10^2 molecules/cell in liver (SCHIBLER *et al.* 1980). YOUNG *et al.* (1981) and HAGENBÜCHLE *et al.* (1981) observed that salivary and liver mRNAs vary only with respect to their 5′-non-translated sequences and concluded that they were transcribed from the same gene. As one explanation for the different mRNA concentrations in salivary glands and liver, they suggested that different initiation sites for the transcription were utilized by the two tissues. The apparent discrepancy between the report by HAGENBÜCHLE BOVEY and YOUNG (1980) of the existence of only one (or two) active pancreas amylase gene(s) for the A/J chromosome and our finding of a high amylase production determined by that chromosome may in part be settled if the gene (or genes) have different initiation sites from the pancreatic amylase genes of most congenic lines.

Clearly, in order to satisfy all aspects of the genetic variation in mouse pancreatic amylase, one or two loci appear to be too few and twenty to be too many. The amylase complex must include at least four pancreatic amylase structural genes with adjacent *cis*-acting sequences regulating the expression of the individual genes. A similar model has been proposed to account for the qualitative variation of mouse salivary amylase (HJORTH 1979). Many of these sequences are likely to be present in our collection of congenic lines, and we are continuing the study of the various amylase complexes, using recombinant DNA techniques, in the hope of defining and understanding the function of the regulatory elements.

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