SERUM AMYLASE (THREAD PROTEIN) POLYMORPHISM IN CATTLE

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THREAD-PROTEIN polymorphism has been described in cattle (ASHTON 1958) and in pigs (ASHTON 1960). The thread-proteins (Figure 1) appear as very fine, somewhat wavy, threadlike zones on starch gel stained with protein stains. In agar electrophoresis in borate buffer at pH 8.6, the thread-proteins have the mobility of albumin, while in starch gel at pH 8.5 in phosphate buffer their mobility approximates that of the slow-alpha globulin.

The nature of the thread-proteins was not clear at the time of these publications. F. K. KRISTJANSSON (personal communication) working with pig serum noted that when stained gels were warmed for 5 to 10 minutes at 90° C the entire migration path behind the thread-proteins became depressed below the level of the remainder of the gel surface. This suggested some alteration in the

FIGURE 1.—Cattle thread-protein phenotypes as seen in phosphate buffer at pH 8.5 (ASHTON 1958). Tp 1, Tp 2, thread-proteins; Tf, transferrins (not well resolved in this buffer system); Sa, slow-alpha globulin.

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gel structure due to the migration of the thread proteins. J. W. B. KING (private communication) suggested that the thread-proteins are in fact amylases, and has confirmed this hypothesis by suitable tests (to be published). The amylase activity of pig serum is much stronger than that of cattle serum, and the degradative action of the amylases of pig serum can be seen by incubating the unstained gel in a suitable buffer following electrophoresis.

Serum amylase polymorphism in cattle is rather difficult to see on unstained gels after incubation. However KING (private communication) has found that amylases from both pig and cattle serum can be seen after prolonged immersion in 0.1 percent p-phenylenediamine dihydrochloride in acetate buffer at pH 5.7, a stain used for detecting ceruloplasmin activity. This staining reaction, carried out at alkaline rather than acid pH, has been used to type the serum amylases of several hundred cattle to establish the mode of inheritance of this polymorphism.

MATERIALS AND METHODS

Starch gel electrophoresis: Best results were obtained with phosphate buffer and electrolyte although other systems also showed the polymorphism. The electrolyte contained 21 g of anhydrous disodium hydrogen phosphate per liter. Before making to volume this was adjusted to pH 7.6 with a saturated solution of potassium dihydrogen phosphate. The buffer used for preparing the gels was a 1/50 dilution of this electrolyte in water. The gels were prepared from hydrolysed starch (Connaught Laboratories, Toronto, Canada) by the procedure described by KRISTJANSSON (1963). Usually it was found necessary to increase the starch concentration above that recommended by the manufacturers to obtain sharp zones. For example a starch batch (204-1) recommended for use at 10.2 percent w/v was used at 12.0 percent w/v.

Electrophoresis was effected in a horizontal plane with a gel 3 mm thick. Serum samples were applied on pieces of No. 17 chromatography paper, removed 15 minutes after electrophoresis commenced. The voltage between the inner edges of the filter paper wicks on the surface of the gel was 9 v per cm of gel length, and the initial current was approximately 1.5 ma per cm of gel width. Final current was about 3 ma per cm width after 90 to 105 minutes. After this time the fast amylase zone had usually migrated about 2 cm.

After removal from the apparatus, the lower surface of the uncut gel was placed uppermost in a staining tray and the stain was added. The stain was prepared just before use by dissolving p-phenylenediamine dihydrochloride in a 1/25 dilution of pH 7.6 phosphate electrolyte at a concentration of 0.1 percent w/v. The gel was transferred to an incubator at 37°C. After about one hour the ceruloplasmin zones, stained blue, were clearly visible. Following incubation overnight these zones faded (Figure 2). The following morning the gels were transferred to a wash solution containing 225 ml methanol and 50 ml glacial acetic acid per liter. The gels which were initially bluish-pink gradually lightened and the amylase zones appeared after about 15 to 30 minutes. Prolonged immersion in the wash solution caused the amylase zones to fade. It was found advisable to transfer the gels to water after about 90 to 120 minutes, the exact time being judged by the appearance of the amylase zones. Transfer to water intensified the zones.

The amylase zones appeared in reflected light as bluish-brown zones on a pink background after washing out. However phenotype identification was aided by viewing the gels in transmitted light, when the amylase zones appeared as transparent zones against the undigested translucent starch gel.

Samples: Serum samples from animals at the National Cattle Breeding Station, "Belmont," were examined. The animals sampled comprised the F_2 progeny of Brahman $\times$ Shorthorn, Brahman $\times$ Hereford, Africander $\times$ Shorthorn and Africander $\times$ Hereford cows, the cows themselves, and the sires of the F_2 progeny. A number of F_2 Shorthorn $\times$ Hereford animals were also examined.

FIGURE 2.—Diagram showing the six cattle amylase phenotypes relative to the stained ceruloplasmin zones, which fade following overnight incubation. Cp, ceruloplasmin zones; Am, amylase zones. Arrow shows direction of migration towards anode.

Samples from cows in eight milking herds from Northern New South Wales were examined. Three of these herds were wholly Jersey, three predominantly Jersey, one predominantly Guernsey, and one predominantly Australian Illawarra Shorthorn. The female progeny of some of these cows, sired by artificial insemination from Sindhi $\times$ Jersey or Sahiwal $\times$ Jersey crossbred bulls were examined, and also the bulls responsible for the progeny.

RESULTS

Phenotypes: Six phenotypes were observed in the Brahman crossbred and Africander crossbred cattle from "Belmont," but only three of these six were observed in the dairy cattle. Of the six phenotypes (Figures 2, 3) three, Am A, Am B, and Am C, consisted of single zones with differing mobilities and these subsequently proved to be homozygotes. Two of the remaining three phenotypes, Am AC and Am BC, each showed two zones corresponding to the zones produced by the homozygotes, and these proved to be heterozygotes. The sixth phenotype, Am AB, showed a single broad zone (Figure 1). In the dairy cattle only Am B, Am BC, and Am C were present.

Mating data: The appearance of the phenotypes suggested that amylase polymorphism is controlled by three codominant alleles Am^A , Am^B and Am^C , each allele producing a single zone in starch gel with the zones differing in mobility. On this hypothesis phenotypes Am A, Am B, and Am C would be the products of genotypes Am^A/Am^A , Am^B/Am^B , and Am^C/Am^C respectively, and phenotypes Am AB, Am AC, and Am BC the products of genotypes Am^A/Am^B , Am^A/Am^C ,

FIGURE 3.—Cattle serum amylase phenotypes after p-phenylenediamine staining. The zones were photographed by light transmitted through the gel and consequently appear darker than the background in the photograph instead of lighter. The amylase zones have been circumscribed in ink to improve contrast.

and Am^B/Am^C . Mating data obtained from the crossbred beef cattle (Table 1) and the dairy cattle (Table 2) confirmed this hypothesis. No exceptions to the hypothesis were encountered, and the observed and expected distribution of

TABLE 1

Amylase mating results from the "Belmont" Zebu-crossbred herd showing the observed distribution of phenotypes (numerator) and the distribution expected from the proposed three-allele hypothesis (denominator)

Phenotypes mated	Phenotypes of progeny					
	A	AB	AC	B	BC	C
A × AC	1/1	.....	1/1	.....	.....	.....
A × BC	.....	1/0.5	0/0.5	.....	.....	.....
A × C	.....	.....	2/2	.....	.....	.....
AB × AC	5/4.5	5/4.5	3/4.5	.....	5/4.5	.....
AB × BC	.....	8/3.75	0/3.75	3/3.75	4/3.75	.....
AB × C	.....	.....	6/5.5	.....	5/5.5	.....
AC × AC	6/4.75	.....	9/9.5	.....	.....	4/4.75
AC × BC	.....	6/5.75	5/5.75	.....	7/5.75	5/5.75
AC × B	.....	.....	0/0.5	.....	1/0.5	.....
AC × C	.....	.....	15/11.5	.....	.....	9/11.5
B × C	.....	.....	.....	.....	3/3	.....
BC × B	.....	.....	.....	2/2	2/2	.....
BC × BC	.....	.....	.....	0/2.5	8/5	2/2.5
BC × C	.....	.....	.....	.....	18/14/5	11/14.5
C × C	.....	.....	.....	.....	.....	6/6

TABLE 2

Amylase mating results from dairy cattle herds showing the observed distribution of phenotypes (numerator) and the numbers expected from the hypothesis proposed (denominator)

Phenotypes mated	Phenotypes of progeny		
	B	BC	C
B × B	18/18	.....	.....
B × BC	37/33.5	30/33.5	.....
B × C	.....	6/6	.....
BC × BC	13/10.5	20/21	9/10.5
BC × C	.....	3/3.5	4/3.5

TABLE 3

Distribution of amylase phenotypes in eight dairy herds, the derived gene frequencies, and the expected phenotype distribution calculated from these frequencies

Herd	Breed		Total	Phenotypes			Gene frequencies	
				B	BC	C	Am ^B	Am ^C
B	Jersey	Observed	60	27	27	6	0.675	0.325
		Expected		27.3	26.3	6.4		
G	Jersey	Observed	120	40	63	17	0.596	0.404
		Expected		42.6	57.8	19.6		
Sg	Jersey	Observed	75	37	31	7	0.700	0.300
		Expected		36.7	32.0	6.3		
W	Mainly Jersey	Observed	76	26	39	11	0.599	0.401
		Expected		27.3	36.5	12.2		
D	Mainly Jersey	Observed	173	42	98	33	0.526	0.474
		Expected		47.9	86.2	38.9		
O	Mainly Jersey	Observed	142	60	57	25	0.623	0.377
		Expected		55.1	66.7	20.2		
	Total of Jerseys	Observed	646	232	315	99	0.603	0.397
		Expected		234.9	309.3	101.8		
Sc	Guernsey	Observed	46	34	9	3	0.837	0.163
		Expected		32.2	12.6	1.2		
T	Illawarra Shorthorn	Observed	72	26	39	11	0.597	0.403
		Expected		27.3	36.5	12.2		

progeny did not differ significantly in any mating group.

Breed distribution of amylase types: Table 3 shows the distribution of cow genotypes for the eight dairy herds, and the derived gene frequencies for each herd. The observed and expected distribution do not differ significantly.

The gene frequencies for the crossbred beef cattle are strongly influenced by the experimental mating program in use at "Belmont," and for this reason are not considered in detail in this report. However it seems likely that Am^A is a feature of Brahman and Africander cattle and may be absent from the common

British breeds of cattle. It was not present in the Hereford $\times$ Shorthorn crosses and their descendants in the "Belmont" herd, nor was it found in the dairy cattle examined.

DISCUSSION

J. W. B. KING (personal communication) has suggested the locus symbol *Am* in place of *T* for thread-protein amylase activity in pigs. This more descriptive symbol has been adopted in the present work with cattle.

Four thread-protein phenotypes were recognized in British breeds of cattle by ASHTON (1958), T1 with a single slow-moving zone, T2 with a single fast-moving zone, T1/T2 with both zones, and T0 with no discernible zones (Figure 1). Comparison of thread-protein patterns after nigrosine staining, and amylase patterns after p-phenylenediamine staining, showed that thread-protein phenotype T2 is equivalent to amylase phenotype Am B, T1 to Am C, and T1/T2 to Am BC. Thread-protein phenotypes classed as T0 (no discernible zones) were found to show zones by the more sensitive phenylenediamine procedure. In most cases T0 phenotypes were found to be Am BC phenotypes, but in some cases they proved to be Am B or Am C.

It is apparent that the genetic hypothesis proposed for thread-protein polymorphism in cattle, involving a "silent" allele *T^o* to account for the phenotypes not having detectable thread-protein zones, is incorrect. Phenotypes lacking amylase zones were not seen in the present study, although sometimes very faint zones detectable with certainty only in transmitted light were obtained.

Apart from cattle and pigs, thread-proteins, presumably amylases, have been seen in sheep, goats, dogs and horses, but they have not been found to be polymorphic in these species. BERK, KAWAGUCHI, ZEINEH, UJIHIRA and SEARCY (1963) have produced chromatographic evidence that at least three molecular forms of amylase occur in normal rabbit serum.

Serum amylase has not been detected in human serum by the method described, although very strong zones are obtained with human saliva. Results obtained by WILDING (1963) show that amylase exists unbound in human serum and the possibility of serum or salivary amylase polymorphism in humans should be considered.

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SUMMARY

The results of 178 matings in a beef cattle herd and 140 matings in eight dairy herds show that amylase polymorphism in cattle is controlled by three autosomal codominant alleles, *Am^A*, *Am^B*, and *Am^C*. Amylase polymorphism is equated with previously described thread-protein polymorphism. *Am^A* was present in the progeny from Brahman $\times$ British and Africander $\times$ British crossbreds, but not in the dairy cattle examined. *Am^B* and *Am^C* were present in all breeds examined.

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