GENETIC CONTROL OF TWO PRE-ALBUMINS IN PIGS[1]

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THREE loci controlling the synthesis of serum proteins in the pig have been identified to date. KRISTJANSSON (1960a, b) has identified two alleles controlling the synthesis of transferrins in the pig and has demonstrated (1961) three alleles involved in the synthesis of pig haptoglobins. ASHTON (1960) has described three "thread protein" phenotypes and on the basis of preliminary mating data has suggested that two alleles are involved in determining these phenotypes. In the same paper, ASHTON reports three β-globulin phenotypes which appear to be the same as the transferrin types described by KRISTJANSSON. KING (1961, personal communication) has identified a third transferrin allele and a fourth haptoglobin allele. Serum protein polymorphisms in several species have been reviewed by OGDEN (1961). Plasma protein polymorphisms in man have been reviewed by HARRIS (1961).

In the course of starch gel electrophoresis technique experiments, we have observed differences between individuals with respect to protein fractions (pre-albumins) which migrate in advance of the serum albumin fraction.

This report describes a starch gel electrophoresis procedure which yields discrete separation of pre-albumins, transferrins and haptoglobins in pig serum, and presents evidence concerning the genetic mechanism underlying the pre-albumin polymorphism which has been observed.

MATERIALS AND METHODS

Sera were obtained from the parents and from random samples of progeny produced from matings of seven Yorkshire sires to 34 Yorkshire and 13 Landrace dams, and from matings of five Landrace sires to 29 Landrace and eight Yorkshire dams.

Sera were subjected to horizontal starch gel electrophoresis in gels made with 58.25 g hydrolysed starch (Connaught Medical Laboratories, Toronto) in 500 ml of a .014 M Tris(hydroxymethyl)aminomethane - .004 M citric acid buffer solution (pH 7.5). It was necessary to alter the amount of starch slightly when shifting to a new starch lot number. In order to provide uniform gel preparation

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conditions, 390 ml of buffer was heated to 100°C and added rapidly to the starch suspended in 110 ml of buffer at 21°C. The resulting viscous mass was shaken vigorously for about 15 seconds, degassed for one minute by applying a vacuum to the flash, and then poured into the gel forms. In our experience, gels prepared in this manner give more uniform electrophoretic results than gels prepared by heating the entire starch-buffer suspension over an open flame. In addition, the process takes much less time and effort.

Gel forms with interior dimensions of 22.4 cm × 12 cm × 0.6 cm were used and the above starch mixture was sufficient to fill two forms of this size. A lightly oiled sheet of plexiglass was placed over the hot mixture and held firmly in place by weights distributed around the perimeter of the form. The gels were allowed to set and cool for 90 minutes at room temperature before loading serum samples.

After cooling, the gels were cut across their width parallel to and 5.8 cm from one end to form the insertion line. The smaller portion of the gel was carefully pushed back and pieces of Whatman No. 3 chromatography paper 1 cm × 0.6 cm soaked in the serum to be analysed were placed approximately 1.0 mm apart against the exposed cut surface of the larger portion of the gel. The opened cut was then carefully closed against the line of inserts.

A piece of Saran wrap (Dow Chemical Company) was used to cover the gel, eliminating all air spaces. The Saran wrap was folded back to expose 2.2 cm of the gel at the insert end and 2.0 cm at the other end to provide contact surfaces for the filter paper wads used to connect the gel to the electrode chambers. Platinum wire electrodes were used.

Gels were connected to the electrode chambers as described by SMITHIES (1955). The electrode chambers contained a 0.3 M boric acid - 0.1 M NaOH solution (pH 8.7). An initial voltage of 165 v was applied for 15 minutes, after which the current was shut off, the paper inserts removed, and the insert cut carefully closed. After recovering the gel with the Saran wrap, 165 v was applied for a further 15 minutes, after which the voltage was increased to 350 v for the remainder of the electrophoresis. At 350 volts, current drawn was 50 ± 2 ma per gel.

The brown borate boundary, which is observed to migrate in this and similar discontinuous starch gel systems, was allowed to migrate exactly 11.2 cm from the insert line. This is accomplished in about two hours from the time the system is switched to high voltage. In our experience, a deviation of 0.1 cm in either direction from this distance will result in very poor resolution of the pre-albumins. The gels were not cooled during electrophoresis and became quite warm as the borate boundary passed through the gel. In our experience, optimum resolution is achieved at a room temperature of 21°C. The gels were carefully removed from the form, sliced and stained in the manner described by SMITHIES (1955).

Each serum sample was classified for pre-albumin phenotype and this information related to pedigree information on all animals tested.

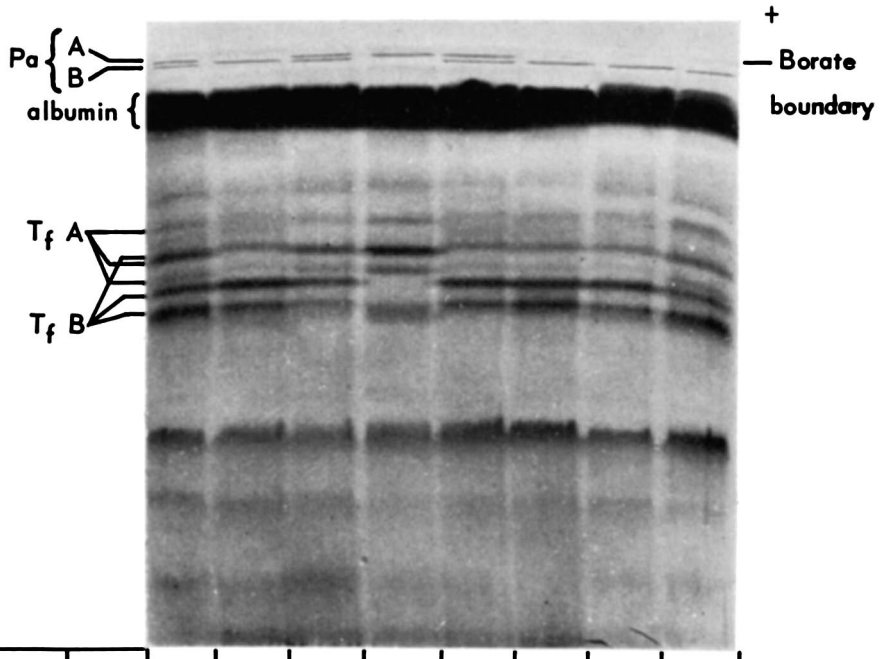
RESULTS AND DISCUSSION

Figure 1 is a photograph of a gel illustrating the resolution which is achieved

for pre-albumins and transferrins. It will be noted that some protein occupies the same position as the borate boundary. In addition there are three other protein fractions which migrate immediately behind the borate boundary and in front of the albumin. The most rapidly migrating of these three fractions is tentatively designated as pre-albumin A (Pa A) and the intermediate fraction as pre-albumin B (Pa B). Minor variations in slowest of these fractions do not appear to be related to variations in Pa A or Pa B.

Three phenotypes have been observed with respect to Pa A and Pa B. The first possesses a small amount of both Pa A and Pa B and is designated as phenotype Pa AB. The second has a large amount of Pa A but no Pa B and is designated Pa AA while the third has a large amount of Pa B but no Pa A and is designated Pa BB. Analyses of sera collected from the same animals a year apart indicate that an individual's phenotype does not change between six and 18 months of age.

The distribution of progeny phenotypes resulting from mating sires and dams of the three different phenotypes are presented in Table 1. It was hypothesized that the syntheses of Pa A and Pa B are under the control of alleles Pa^A and Pa^B respectively, and that Pa^A Pa^A individuals synthesize only Pa A (phenotype Pa



Phenotypes									
Pa	AB	BB	AB	AA	AB	BB	BB	BB
Tf	AB	BB	AB	AA	BB	BB	BB	AB

FIGURE 1.—Starch gel stained with Amido Black 10B showing resolution of pre-albumins (Pa) and transferrins (Tf) after electrophoresis. Phenotypes for Pa and Tf are indicated. Pre-albumins were retouched on the original photograph.

TABLE 1

Distribution of progeny phenotypes (observed/expected) from various mating classes and the results of chi-square tests of their fit to the hypothesis that phenotype Pa AA = Pa^A Pa^A, Pa BB = Pa^B Pa^B and Pa AB = Pa^A Pa^B

Matings		Progeny phenotypes			
Male	Female	Pa AA	Pa AB	Pa BB	P of χ^2
AA	× AA	15/15	.....	.....	..
	AB	16/19.5	23/19.5	.....	> .30
	BB	.....	20/20	.....	..
AB	× AA	27/33	39/33	.....	.15
	AB	38/35	71/70	31/35	> .70
	BB	.....	13/13	13/13	..
BB	× AA	.....	26/26	.....	..
	AB	.....	10/8.5	7/8.5	..
	BB	.....	.....	9/9	..
Totals		96/102.5	202/190	60/65.5	> .45

AA), and that Pa^A Pa^B individuals synthesize both Pa A and Pa B (phenotype Pa AB), and that Pa^B Pa^B individuals synthesize only Pa B (phenotype Pa BB). Only those progeny phenotypes expected from this hypothesis were observed.

The goodness of fit of the observed progeny phenotypes to those expected on the basis of this hypothesis was determined by the χ^2 tests reported in Table 1. These tests indicate that our observations are consistent with the hypothesis presented.

To provide uniformity of nomenclature for transferrins across species, it is proposed that the pig transferrin designations Tf–7 and Tf–1 (KRISTJANSSON 1960b) be changed to Tf A and Tf B respectively. In the above starch gel electrophoresis system, each of these transferrins migrate as three discrete zones as illustrated in Figure 1. Homozygotes exhibit three transferrin zones and heterozygotes six. Binding of Fe^{59} by each of these zones has been demonstrated using the technique of GIBLETT, HICKMAN and SMITHIES (1959). Haptoglobin polymorphisms can be accurately identified by adding a small amount of hemoglobin to the sera before electrophoresis and staining one half of the gel with benzidine at the conclusion of the electrophoresis, as described by KRISTJANSSON (1961). Thus, the genotypes at three different loci controlling the synthesis of pig serum proteins can be determined from a single electrophoretic analysis.

SUMMARY

Starch gel electrophoresis conditions which yield good resolution of the transferrins, haptoglobins and pre-albumins of pig serum have been described.

Three different phenotypes with respect to pre-albumin A (Pa A) and pre-albumin B (Pa B) have been observed. The first possesses a small amount of both Pa A and Pa B and is designated phenotype Pa AB. The second has a large amount of Pa A but no Pa B and is designated phenotype Pa AA, while the third has a large amount of Pa B but no Pa A and is termed phenotype Pa BB.

Data are presented which support the hypothesis that the syntheses of Pa A and Pa B are under control of alleles Pa^A and Pa^B respectively, and that an individual possessing only one pre-albumin is homozygous for the allele controlling the synthesis of that pre-albumin (phenotype Pa AA has the genotype $Pa^A\ Pa^A$ and phenotype Pa BB has the genotype $Pa^B\ Pa^B$), while the phenotype possessing both pre-albumins (Pa AB) has the genotype $Pa^A\ Pa^B$.

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