Blood Groups of the I System in Pigs: Association with Variants of Serum Amylase

Abstract. *The Am 1 variant of porcine serum amylase was detected only in pigs having the red-cell antigen I_b, whereas several I_b positive pigs were found without Am 1. Family and population studies show that the chromosomal loci,* I *and* Am, *are identical or closely linked. The phenotype of one exceptional progeny from a double backcross mating possibly was a result of paternal irradiation or genetic recombination during oögenesis.*

Examination of blood types and serum types of pigs of the Danish Landrace breed has indicated an association between blood groups of the *I* system and variants of serum amylase (*1*). Independent investigations carried out in this laboratory with pigs of the Hampshire breed also indicate an association. The results suggest that the *I* blood-group locus and the *Am* locus for serum amylase are closely linked. However, the results also prompt a discussion of possible pleiotropic effect of alleles at one locus.

The red-cell antigens I_a and I_b are products of allelic autosomal genes, I^a and I^b, and the phenotype of each pig tested so far has been either I(a+b−), I(a−b+), or I(a+b+). These phenotypes correspond to the genotypes I^aI^a, I^bI^b, and I^aI^b, based on present evidence. Details of the genetics of the I blood groups and their detection have been described (*2*). The three variants of serum amylase, Am 1, Am 2, and Am 3, appear to be controlled by codominant alleles, Am^1, Am^2, and Am^3, in all breeds of pigs tested so far. Various phenotype designations are indicated in Table 1. Workers who have studied these amylase types agree that they represent the thread proteins reported earlier by Ashton and by Schröffel and Hojný (*3*).

To preserve maximum amylase activity, the serum samples were obtained shortly after the blood clotting was completed. The serum was stored at −25°C before being examined by starch-gel electrophoresis. Buffers and initial procedure have been described (*4*).

Each gel was sliced immediately after electrophoresis, and the lower gel slab (2½ mm thick) was immersed in 0.1 percent *p*-phenylenediamine dihydrochloride in acetate buffer at *p*H 5.7 and incubated at 37.5°C for 16 to 18 hours. Although this staining method primarily serves to detect ceruloplasmin after 1 to 1½ hours incubation, it also aids in detecting the results of amylase activity (*5*, *6*).

All available segregation results pertinent to analyzing genetic association between the *Am* and *I* loci are shown in Table 1. No Am-1-positive (or Am-3-positive), I_b negative progeny was observed among all progeny from the five mating types, but one Am-1-negative, I_b-positive female was detected among offspring from a double backcross mating (*7*), in which the mother was doubly heterozygous. This exceptional progeny is indicated by a dagger (†) in the upper row of Table 1. Correct genotyping of the parents was supported by tests of a second litter from this sow and seven additional litters sired by the boar. Typing of blood obtained from the exceptional progeny at various intervals during its first year of life confirmed the previous results that the phenotype was I(a+b+), Am 2-2. The red-cell typing included titrations and absorptions, and there was no evidence of mismating, adoption, or superfecundation, on the basis of complete blood types and serum types of all possible parents. Moreover, this exceptional female was mated with an I(a+b+), Am 2-2 boar. Ten pigs were born and were typed when they were 6 weeks of age. Two pigs were I(a+b−), four I(a−b+), four I(a+b+), and each of the ten pigs had the same amylase phenotype as the parents, that is, Am 2-2.

If the results in Table 1 are considered alone, they suggest that the *Am* and *I* loci are closely linked. Since the Am^1 (or Am^3) and I^b genes of all doubly heterozygous parents were in coupling phase (*cis*-position), it is possible to estimate the recombination frequency by use of the maximum-likelihood method on the combined data in Table 1 (*8*). This estimate of the recombination frequency is 0.8 percent, if the exceptional progeny is regarded as a *bona fide* recombinant. The 95 percent confidence interval for this estimate is 0.02 percent $< \theta <$ 2.4 percent. This estimate corresponds to the estimate of 2.4 percent (3/125) obtained by typing pigs of the Danish Landrace breed (*1*). However, Hesselholt *et al.* (*1*) considered the possibility that the three ostensible recombinants had been misclassified.

Many of the animals included in the parental Hampshire material were related. Linkage equilibrium is therefore not expected, and, hence, the observation on 27 parents in coupling phase (Table 1) is compatible with the linkage hypothesis. The situation is similar to that reported in the first publication of linkage between the C and J blood-group loci, where merely one repulsion-phase individual was observed among parents from 45 matings applicable to linkage studies, despite a recombination frequency of 5 to 6 percent (*9*). The strong association between Am 1 (or Am 3) and I_b has been observed among all pigs tested so far in this laboratory (Table 2).

Table 1. Summary of data on joint segregation of blood groups of the I system and variants of serum amylases (Am) among 269 progeny from 36 matings of Hampshire pigs.

Mating types	Matings (No.)	Progeny phenotypes and numbers*			
		1	2	3	4
Double backcrosses					
$\frac{Am^1I^b}{Am^2I^a} \times \frac{Am^2I^a}{Am^2I^a}$	14	49 Am 1-2, I_b+	0 Am 1-2, I_b−	1† Am 2-2, I_b+	52 Am 2-2, I_b−
$\frac{Am^3I^b}{Am^2I^a} \times \frac{Am^2I^a}{Am^2I^a}$	1	4 Am 2-3, I_b+	0 Am 2-3, I_b−	0 Am 2-2, I_b+	2 Am 2-2, I_b−
Single backcrosses					
$\frac{Am^1I^b}{Am^2I^a} \times \frac{Am^2I^a}{Am^2I^b}$	18	65 Am 1-2, I_b+	0 Am 1-2, I_b−	44 Am 2-2, I_b+	25 Am 2-2, I_b−
$\frac{Am^3I^b}{Am^2I^a} \times \frac{Am^2I^a}{Am^2I^b}$	1	2 Am 2-3, I_b+	0 Am 2-3, I_b−	3 Am 2-2, I_b+	3 Am 2-2, I_b−
$\frac{Am^1I^b}{Am^2I^a} \times \frac{Am^1I^b}{Am^2I^b}$	2	6 Am 1-2, I_a+	4 Am 1-2, I_a−	0 Am 1-1, I_a+	9 Am 1-1, I_a−

* Only reactions with the I reagent (antibody to I_a or antibody to I_b) relevant to the segregation in question are shown because some progeny were not tested with the I_a reagent. Phenotypes Am 1-1, Am 1-2, Am 2-2, and Am 2-3 correspond to the genotypes Am^1Am^1, Am^1Am^2, Am^2Am^2, and Am^2Am^3. † Recombinant, according to a linkage hypothesis.

Table 2. Summary of data showing positive association between the Am 1 variant of serum amylase and the red cell antigen I_b in pigs. Data in Table 1 are included. Variant Am 3 was observed only in three Hampshire sows and 23 of their 41 progeny from six litters. All Am 3 pigs were I_b-positive.

Animals*	Distribution of phenotypes			
	A+ I_b+	A+ I_b−	A− I_b+	A− I_b−
H { Parents	33	0	83	68
H { Progeny	262	0	744	580
H Males	25	0	49	28
L,Y,L-Y Females	17	0	39	11

* H, Hampshire; L, Landrace; Y, Yorkshire; and L-Y, indicates L-Y crosses. † A+ and A− indicate presence or absence of Am 1 (or Am 3).

However, in the Danish Landrace breed, Am 3 was positively associated with I_a (*1*).

Apart from the exceptional progeny already considered, all data in Tables 1 and 2 are compatible with a hypothesis of pleiotropy—that is, a series of alleles at one locus each of which has two effects. For example, one allele might be responsible for an I_b-positive red-cell phenotype in conjunction with type Am 1 of serum amylase. Another allele might be responsible for an I_b-positive red-cell phenotype in conjunction with amylase type Am 3. Based on the present material, additional alleles, each with pleiotropic effect, may be visualized.

If pleiotropy rather than linkage is the correct interpretation of the data, then the observed exception to this hypothesis poses an interesting problem. A paternal mutation of I^a to I^b might be postulated because the boar had been exposed to gonadal x-irradiation, whereas the genetic material of the sow had not been exposed to x-rays (*10*). A change of Am^1 to "not Am^1" would require a spontaneous mutation during oögenesis, or such a change could be a result of gene interaction. If so, apparently only the enzyme variant was affected but not the blood factor determined by the same allele having pleiotropic effect.

Since the observations might have important theoretical implication, evidence on whether the ostensible amylase polymorphism is real or spurious is needed. According to Uriel (*11*) enzymes may be carried by, or form complexes with, other proteins or protein fragments. This possibility has been considered previously in connection with studies of amylase variations in pigs (*5*); because the three variants have not been observed in saliva and pancreas fluid (*6*), the latter is assumed to be the main source of serum amylase. Thus, amylase might be attached to, and follow, the migration of variants of a polymorphic system of other proteins in serum, and merely serve as an indicator for these proteins in the starch gel. Hence, the data show that the *I* blood-group locus and the *Am* locus for serum amylase, or possibly for amylase-binding constituents of serum, are closely linked or identical. The distinction between the two hypotheses depends on the correct interpretation of the phenotype of one exceptional pig out of the 1939 pigs considered.

The observations in pigs are comparable to the genetic association detected between the Ss (*12*) serum protein variants and the H-2 histocompatibility traits in the mouse (*13*). The *H-2* locus controls a set of red blood cell antigens and is also the strongest of several loci affecting tissue transplantation incompatibility. The Ss protein is a euglobulin, but tests for enzymatic activities have been negative. Although specific Ss and H-2 types always are associated, it is not clear whether the association reflects close linkage or multiple effects of the *H-2* locus.

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Isolation of Nuclei from a Marine Dinoflagellate

Abstract. *By means of a medium containing dextran, nuclei were isolated in high yield from cells of* Gymnodinium nelsoni, *a marine dinoflagellate. Most of the DNA, but less than one-tenth of the RNA, of the original cells was recovered in the purified nuclei. The nuclei appeared substantially intact as observed by light or electron microscopy. The isolated nuclei were capable of incorporating tritiated uridine triphosphate into material insoluble in cold acid. The general procedure was found to be applicable also to two species of diatoms.*

In order to test the generality of nucleic acid-mediated control systems hypothesized on the basis of studies on bacteria and bacterial viruses, it would be highly desirable to be able to obtain intact nuclei and other organelles from microorganisms that can be grown axenically, under controlled conditions, and in a defined medium. With few exceptions it has proved difficult to obtain intact nuclei from microorganisms.

We looked among the algae for species with large, naked cells and with a prominent nucleus. The large dinoflagellate (Fig. 1a) *Gymnodinium nelsoni* Martin (strain GSBL) (*1*) appeared the most promising, and we report here the characterization of nuclei obtained from this organism.

Subcultures of *Gymnodinium nelsoni*, maintained in algal culture at the Woods Hole Oceanographic Institution (WHOI), were grown with aeration and 11,000 lumens/m² of fluorescent

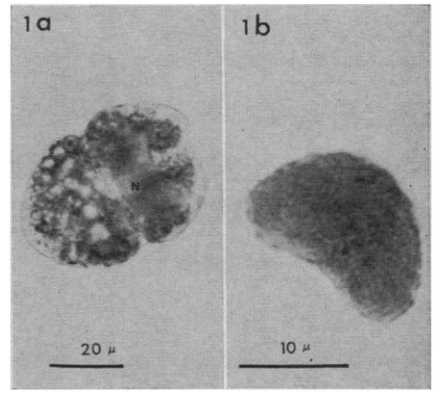

Fig. 1. (a) *Gymnodinium nelsoni* stained with methyl green in bright-field illumination. The nucleus is the lobed, opaque structure in the lower half of the cell. (b) Isolated nucleus, stained with methyl green in bright-field illumination.