THE C BLOOD GROUP SYSTEM IN PIGS AND THE DETECTION AND ESTIMATION OF LINKAGE BETWEEN THE C AND J SYSTEMS[1]

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PREVIOUS evidence has shown that 24 reagents for blood-typing pigs detect red cell antigens determined by 11 genetic loci (ANDRESEN 1962, 1963; BAKER and ANDRESEN 1962; NIELSEN 1961). This report deals with genetic investigations on a "new" red cell antigen, C_a, detected by an isoimmune antibody, anti-C_a. The results indicate the existence of a twelfth system of pig blood groups, the C system. In addition, the data show linkage between the loci for the C and J blood group systems and provide an efficient estimate of the recombination frequency.

METHODS AND MATERIALS

The animals used in this study were of the Duroc and Hampshire breeds and originated from farms in Iowa. There were 51 Duroc sire-families, with 176 matings (from 143 females) and 1666 offspring, and 51 Hampshire sire-families, with 230 matings (from 178 females) and 2041 offspring. The sire material consisted of littermate pairs. One male in each pair had been exposed to gonadal X-ray treatment as described by WILLHAM and COX (1962).

Red cells for blood-typing of the offspring were obtained within 24 hours after farrowing. Whenever required, repeat typing and absorptions were performed when the pigs were 6 to 12 weeks old. The methods used in typing pig blood and most of the reagents employed are described in the reports already cited. In this study, however, plastic plates replaced test tubes in both lytic and direct agglutination tests. A few reagents not previously described were included.

Antisera were obtained from two sows of the Hampshire breed. They had received intramuscular injections of a mixture of citrated whole blood from two Duroc donors. Anti-C_a, reactive in a lytic test, was isolated from one of these antisera by absorbing all previously known isoantibodies in the serum. The new blood-typing reagent was subjected to a test for purity by separate reabsorption with red cells from 18 "positive" and six "negative" individuals. Each of the absorbed serum fractions was then used in lytic tests with the 24 blood samples. A second test for purity was made by using red cells from 14 additional animals, of which 12 were "positive" and two were "negative." Such tests indicated that the C_a reagent was monospecific in tests with pig red cells. The anti-C_a isolated from the other antiserum was reactive in an indirect Coombs test. The two C_a reagents gave identical results when used in parallel in blood-typing tests involving 50 matings with 458 progeny.

The J_a reagent and the two C_a reagents were used in absorption tests involving red cells from nine crossover progeny (Table 6). The red cells from each of the five $C_a + J_a -$ recombinants removed anti-C_a but not anti-J_a. The red cells from each of the four $C_a - J_a +$ recombinants removed anti-J_a but not anti-C_a. Thus, these absorptions confirmed the results of the blood-typing tests.

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RESULTS AND DISCUSSION

Dominance of the C_a antigen: The hemolytic C_a reagent was used for blood-typing of the family material in Table 1. The mating types $C_a+ \times C_a+$ and $C_a+ \times C_a-$ had both "positive" and "negative" offspring. The mating type $C_a- \times C_a-$ had exclusively "negative" offspring. These results establish the C_a antigen as a dominant character.

The material in Table 2 includes all those $C_a + \times C_a +$ and $C_a + \times C_a -$ matings in Table 1 that had at least one "negative" offspring. The expected proportion of "negative" offspring from such selected matings will exceed the *a priori* expectation. BERNSTEIN's (1929) *A Priori Method* has been used to correct the bias. This bias is, however, negligible because of the large number of pigs per litter (average 9.1).

The observed number of C_a+ offspring (Table 2) from the 68 matings of type $C_a+ \times C_a-$ is moderately in excess of the expected ($0.05 < P < 0.10$). Two of the 30 sire-families involved represent seven matings with 79 offspring and account for 50 percent of the total deviation. The remaining 28 sire-families provided segregation results that were in good agreement with expectations ($0.30 < P < 0.50$). These results give further support for the assumption that the C_a antigen depends on a gene, C^a, capable of expressing itself in single and double dose. In addition, the results indicate that the antigen is well developed at birth and that the C_a reagent is sufficiently potent for accurate blood-typing.

Establishing the C blood group system: Assignment of two blood factors to the same blood group system or to different systems is essentially a matter of proving

TABLE 1

Summary of segregation results based on 176 matings of the Duroc breed with 1666 offspring and 230 Hampshire matings with 2041 offspring

Breed	Mating type	Number of matings	Number of offspring	
			C_a+	C_a-
Duroc	$C_a+ \times C_a+$	12	102	19
	$C_a+ \times C_a-$	76	451	318
	$C_a- \times C_a-$	88	0	776
Hampshire	$C_a- \times C_a-$	230	0	2041

TABLE 2

Observed and expected number of C_a- offspring from eight $C_a+ \times C_a+$ matings and 68 $C_a+ \times C_a-$ matings, each with at least one C_a- offspring

Mating type	Number of litters	Number of pigs	Number of C_a- offspring		Chi-square (1 degree of freedom)
			observed	expected	
$C_a+ \times C_a+$	8	92	19	23.9	1.09
$C_a+ \times C_a-$	68	681	318	342.0	3.24

allelism or nonallelism for the corresponding genes. Each 2 × 2 configuration in Table 3 includes the distribution of phenotypes among offspring from three matings of doubly heterozygous with doubly recessive parents (double backcross matings). These matings were chosen at random among those available. All four expected segregation types were observed among offspring from each mating type except from that involving the J blood group system. However, several recombinants were observed among offspring from additional double backcross matings (Table 4). This establishes that C^a is not allelic to any of the known genes.

Gene frequency estimates: The frequency of the C^a gene was calculated separately for the parental male and female material of the Duroc breed according to the method of COTTERMAN (1947). The C^a frequency based on the 51 males is 0.09 ± 0.03, and the frequency based on the 143 females is 0.21 ± 0.03. Because of the known family relationships within the material, the frequency obtained from the males probably reflects the C^a frequency within the Duroc breed in Iowa better than the value obtained from the females. The C_a antigen was not detected in the Hampshire breed (Table 1).

The evidence for linkage between the C and J systems: Examination of the segregation results in Table 3 suggested linkage between the *C* and *J* loci. Therefore, all available material pertinent to linkage involving these loci was collected (Table 4). The heterozygosity of the parents was deduced from the occurrence of at least one "negative" offspring. No correction has been made for the bias introduced by such selection since the average litter size of 9.6 in this material makes the bias negligible.

Table 4 shows the partitioning of the chi-square values for the single-factor segregations, joint segregation, and heterogeneity (MATHER 1957). With one exception (Code-No. 4), the single-factor segregations agree with the expectations. This exception concerns only two of the 45 matings and thus involves small numbers. In the same two families, the χ^2 value for CJ joint segregation approaches the 0.01 level of probability. For the remaining classes, the χ^2 values for joint segregation correspond to $P < 0.01$ or to $P < 0.001$. Thirteen of the 15 heterogeneity χ^2 values indicate homogeneity. The two exceptions concern the single backcrosses and involve few individuals. Thus, nearly all 45 matings are homogeneous for each component contributing to the total deviations from expectations. The χ^2 analysis indicates that the families agree in showing good single

TABLE 3

Summary of segregation results from double backcross matings

	Reagents																					
	A		B		E		F		G		H		I		J		K		L		M	
C-type	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
C_a+	9	5	7	4	8	12	7	7	10	7	10	6	13	7	21	1	9	5	7	5	8	4
C_a—	9	3	4	7	8	6	3	5	4	5	11	9	7	8	0	15	7	4	4	10	6	4

Each 2×2 configuration comprises the phenotypes of offspring from three matings selected at random from those available. The reagent symbols stand for any reagent relevant to the test for allelism within each family.

TABLE 4

Summary of the segregation results and the chi-square analyses relevant to detecting and estimating linkage between the blood group loci C *and* J *in pigs*

Mating type	Mating code-no.	Genotypes of matings†	Number of matings	Phenotypes of offspring			
				CJ	Cj	cJ	cj
Double backcross	1	$CJ/cj\,♂ \times cj/cj\,♀$	11	52	3	1	46
	2	$CJ/cj\,♀ \times cj/cj\,♂$	22	95	7	4	87
	3	$Cj/cJ\,♀ \times cj/cj\,♂$	1	0	2	3	0
Single backcross	4	$CJ/cj\,♂ \times Cj/cj\,♀$	2	19	5	0	3
	5	$CJ/cj \times cj/cJ$	5	29	2	11	16
Double intercross	6	$CJ/cj\,♂ \times CJ/cj\,♀$	4	29	3	0	13

Mating code-no.	Segregation			Heterogeneity			Degrees of freedom for heterogeneity
	χ^2C (1 d.f.)	χ^2J (1 d.f.)	χ^2CJ (1 d.f.)	χ^2C	χ^2J	χ^2CJ	
1	0.6	0.2	86.6***	8.3	4.6	1.1	10
2	0.6	0.1	151.5***	22.1	21.5	6.7	21
3	..	..	..	..	..	..	..
4	2.8	4.5*	6.5*	0.4	0.2	0.7	1
5	0.3	1.1	10.1**	7.4	14.0**	15.5**	4
6	0.4	2.7	46.3***	2.9	1.2	7.6	3

* $P<0.05$; ** $P<0.01$; *** $P<0.001$.
† Genotypes shown are the linkage phase most likely from offspring results.
For convenience the dominant genes C^a and J^a and their recessive counterparts C^- and J^- are designated *C*, *J*, *c*, and *j* in this table.

factor ratios, and they also agree in showing linkage between the corresponding genes.

A positive association was found between the C and J blood types in the parental material. However, this result cannot be regarded as evidence against a true genetic linkage between the two loci, because linkage equilibrium is not expected in the present material.

The results in Table 5 involving sex-segregation are extracted from the class of double backcross matings in Table 4, Code-No. 1. The four litters were sired by the same C_a, J_a positive boar. The C_a phenotypes as well as the J_a phenotypes are evenly distributed among the male and female offspring. Hence, neither the *C* nor the *J* locus is completely X-linked or Y-linked. These findings are compatible with the evidence for linkage between *C* and *J*.

The estimation of linkage: Twenty-two of the double backcross matings indicated in Table 4 produced exclusively parental combinations, and one mating (Code-No. 3) produced exclusively nonparental combinations. Crossover recombinants were observed in each of the remaining 11 sibships from double backcross matings (Table 6). Each of six litters had one recombinant, each of four litters had two recombinants, and one litter had three recombinants. Taking the number of pigs per litter into account, the segregation results clearly demonstrate close linkage and also show that all the double backcross matings, except one (Code-No. 3 in Table 4), were in coupling phase. This was supported by results

TABLE 5

Data relevant to a test for complete sex-linkage of the C *and* J *blood group loci. The material consists of 35 offspring from one doubly heterozygous male mated with four doubly recessive females*

Parental genotypes		Offspring phenotypes and numbers			
Male	Females	Males		Females	
CJ/cj	*cj/cj*	$8C_a+$	$10C_a-$	$9C_a+$	$8C_a-$
		$8J_a+$	$10J_a-$	$8J_a+$	$9J_a-$

of pedigree studies. For example, one boar, that presumably had been homozygous at both loci, was either the grandsire or the great-grandsire of ten of the doubly heterozygous parents.

The selection of two of the double backcross matings was based exclusively on the occurrence of a $C_a - J_a +$ recombinant offspring from each mating (Table 6). After excluding these two propositi, 15 crossover recombinants were observed among 300 offspring from double backcross matings. This corresponds to a recombination frequency of $\hat{\theta} = 0.05$. The proportions of recombinants originating from crossover events in males and females within this mating type are 4/102 and 11/198, respectively. These proportions suggest that crossing over occurs with equal frequency in both sexes. Likewise, no effect of gonadal X-ray treatment on the recombination frequency was observed. The proportions of crossovers among offspring from doubly heterozygous X-ray treated boars and control boars are 2/54 and 2/48, respectively.

The distribution of offspring among the four segregation types from single backcross matings ($CcJj \times Ccjj$ or $CcJj \times ccJj$) and double intercross matings

TABLE 6

Summary of segregation results form the 11 double backcross matings having crossover progeny

Litter no.	Phenotypes of offspring			
	CJ	Cj	cJ	cj
2662	8	1	0	4
7862*	5	1	0	6
11162	2	2	1	6
16262	2	0	2	6
16662	5	1	0	7
1863	4	1§	0	4
8263	3	2§	0	4
14563†	5	0	0	1
22163	2	0	1§	5
24763	4	1§	1§	4
26963‡	3	1§	0	2

*,† Two litters from one sow.
†,‡ One cJ propositus discarded from each litter. Red cells from both propositi were used in absorptions.
§ Red cells from these recombinants were used in the absorptions described in MATERIALS AND METHODS.

($CcJj \times CcJj$) indicates that all doubly heterozygous parents must have been in coupling phase. This could easily have been possible since the doubly heterozygous parents were either closely related or offspring from double backcross matings. Thus, the linkage phase is known for all the available data. This means that the recombination value can be estimated by using the maximum likelihood method on the combined data from the three mating types (MATHER 1935). Since crossing over seems to occur with equal frequency in both sexes and no effect of gonadal X-ray treatment on the recombination frequency has been observed, the data and formulae in Tables 4 and 7 lead to the following joint logarithm likelihood expression for estimating $\hat{\theta}$:

$$\begin{aligned} L = {} & 285 \log (1 - \hat{\theta}) + 15 \log \hat{\theta} + 48 \log (2 - \hat{\theta}) \\ & + 16 \log (1 + \hat{\theta}) + 2 \log \hat{\theta} + 19 \log (1 - \hat{\theta}) \\ & + 29 \log [2 - (1 - \hat{\theta})^2] + 3 \log [1 - (1 - \hat{\theta})^2] \\ & + 13 \log (1 - \hat{\theta}). \end{aligned}$$

Since $\log (p)^2$ is equal to $2 \log p$ and $\log (1 - p^2)$ is equal to $\log (1 + p) + \log (1 - p)$, the expression can be simplified as follows:

$$\begin{aligned} L = {} & 330 \log (1 - \hat{\theta}) + 20 \log \hat{\theta} + 51 \log (2 - \hat{\theta}) \\ & + 16 \log (1 + \hat{\theta}) + 29 \log (3 - 2\theta + \hat{\theta}^2). \end{aligned}$$

Differentiating and equating to zero we obtain:

$$-\frac{330}{1 - \hat{\theta}} + \frac{20}{\hat{\theta}} - \frac{51}{2 - \hat{\theta}} + \frac{16}{1 + \hat{\theta}} - \frac{29(2 - 2\hat{\theta})}{3 - 2\hat{\theta} + \hat{\theta}^2} = 0.$$

This equation may be solved by arithmetic approximation and interpolation. A close approximation for the solution is $\hat{\theta} = 0.05286$. Intermediate steps of the procedure indicate that a change of 0.001 in the value of $\hat{\theta}$ alters the sum of the left side of the equation by 7.644. Then, according to MATHER (1935), the information, $I_{\hat{\theta}}$, is equal to 7644. This gives the standard error $(I_{\hat{\theta}})^{-1/2} = 0.0114$. Therefore, the estimate of the recombination value based on the combined data is $\hat{\theta} = 0.053 \pm 0.011$.

Testing goodness of fit: MATHER (1935) has shown that the method of maximum likelihood on the combined data leads to an efficient statistic. Hence, we can apply the chi-square test of goodness of fit (FISHER 1954). For this purpose, the segregation data in Table 4 were rearranged in Table 7. For convenience, however, the five offspring from a repulsion heterozygote were not included. The degrees of freedom are two less than the number of classes because the expected numbers have been calculated from those observed by means of an adjustable parameter ($\hat{\theta}$) (FISHER 1954).

The classes in which the expected frequencies are less than five are considered jointly in Table 7 for calculating the three chi-square values. These χ^2 values correspond to probabilities between 0.10 and 0.50. Thus, this test confirmed the conclusion from the χ^2 analysis in Table 4. Nearly all of the original departure

TABLE 7

Distribution of phenotypes among progeny from three mating types

Mating		Phenotypes of offspring CJ	Cj	cJ	cj	Chi-square
Double backcross	Observed	147	10	5	133	2.3
	Expected	139.7	7.8	7.8	139.7	(2 d.f.)
	Formulae for expectations	$\frac{n_{11}}{2}(1-\hat{\theta})$	$\frac{n_{11}}{2}\times\hat{\theta}$	$\frac{n_{11}}{2}\times\hat{\theta}$	$\frac{n_{11}}{2}(1-\hat{\theta})$	
		CJ	Cj + cJ		cj	
Single backcross	Observed	48	18		19	2.4
	Expected	41.4	23.5		20.1	(1 d.f.)
	Formulae for expectations	$\frac{n_{31}}{4}(2-\hat{\theta})$	$\frac{n_{31}}{4}(1+\hat{\theta})+\frac{n_{31}}{4}\times\hat{\theta}$		$\frac{n_{31}}{4}(1-\hat{\theta})$	
Double intercross	Observed	29	3		13	1.4
	Expected	32.6	2.3		10.1	(1 d.f.)
	Formulae for expectations	$\frac{n_{33}}{4}[2+(1-\hat{\theta})^2]$	$2\times\frac{n_{33}}{4}[1-(1-\hat{\theta})^2]$		$\frac{n_{33}}{4}(1-\hat{\theta})^2$	

The expected numbers are calculated for $\hat{\theta}=0.053$. The table includes all progeny indicated in Table 1 except the five from a repulsion-heterozygote (Code No. 3).
$n_{11}=295$; $n_{31}=85$; $n_{33}=45$.

from expectations, assuming good single factor segregations and no linkage, is accounted for by the assumption of close linkage.

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SUMMARY

A "new" red cell antigen, C_a, has been detected in pigs of the Duroc breed, and a twelfth blood group system, the C system, has been established. Linkage between the *C* and *J* blood group loci has been detected by using all available data pertinent to linkage studies. The data are homogeneous with respect to the single-factor segregations and their joint segregation. Complete X or Y linkage has been excluded for both loci. Crossing over seems to occur with equal frequency in the two sexes. The recombination value of 5.3 percent ± 1.1 percent was estimated by using the maximum likelihood method on the combined data.

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