LINKAGE BETWEEN THE *K* BLOOD-GROUP LOCUS AND THE *Hp* LOCUS FOR HEMATIN-BINDING GLOBULINS IN PIGS¹

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TWO cases of genetic linkage have been established in pigs. The *C* and *J* blood-group loci are closely linked (ANDRESEN and BAKER 1964; RASMUSEN 1965; ANDRESEN 1966a), and IMLAH (1965) has detected a close linkage between the *Hp* locus for hematin-binding serum proteins (KRISTJANSSON 1961; HESSELHOLT 1963b) and the *K* blood-group locus. This report deals with additional information about the linkage between the *Hp* and *K* loci. The possible effect of breed, sex, and X-irradiation on the recombination frequency is examined, and the data provide an efficient estimate of the recombination frequency.

MATERIALS AND METHODS

Animals: Pigs from the three breeds, Duroc, Hampshire and Landrace, were used in this study. A herd of Duroc and Hampshire pigs was assembled by purchasing breeding animals from farms in Iowa and neighboring states. Males were purchased for each breeding season, whereas females were replaced by animals born in the experimental herd. These pigs were used in an experiment aimed at studying the genetic effects of irradiation. Details of the experimental procedures, including a diagram of the mating plan, are given by Cox (1964). The males were purchased in sets of two full brothers, and one male in each pair, chosen at random, was exposed to 300r of gonadal X-irradiation. Males were used for breeding 5 to 7 months after the exposure to insure that the sperm utilized descended from irradiated spermatogonia. Pairs of males were bred to several pairs of full sisters from the same breed. The females were not exposed to X rays but descended from exposed and unexposed males.

The Landrace pigs were selected among 120 litters of pigs subjected to blood-typing and progeny testing (ANDRESEN 1963) and comprised 27 sire progeny groups with 4 to 12 pigs in each group (172 progeny). These data were used only to study sex-linkage relative to the *K* blood-group locus, and they were included in a previous report on linkage studies (ANDRESEN 1966b). All animals considered in that report are included in the present study.

The Duroc and Hampshire data relevant to sex-linkage consisted of 19 Duroc sire families of type *Hp*/-male $\times$ -/-female, with 611 pigs, and 110 Duroc sire families of type *K*/-male $\times$ -/-female with 2566 pigs. The corresponding data from the Hampshire breed were: 24 sire families of type *Hp*/-male $\times$ -/-female, with 848 pigs, and 56 sire families of type *K*/-male $\times$ -/-female with 1623 pigs. The symbols *Hp* and *K* indicate any *Hp* or *K* allele relevant to the segregation considered and a dash, "-", symbolizes any of the alternative alleles. There were 3 to 89 pigs in each of these families.

The parental material pertinent to the *Hp-K* linkage studies included all available double backcross matings ($\equiv$ testcrosses, *Gg Tt* $\times$ *gg tt*), whereas single backcross matings and double

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intercross matings were omitted because of the limited statistical information obtained from such matings. The double backcross matings comprised 50 Duroc matings, with 353 offspring, and 59 Hampshire matings with 417 offspring.

Bloodtyping and serumtyping: The parents were 1 to 6 years old, and the progeny were 6 to 7 weeks of age when they were subjected to bloodtyping and serumtyping. Although the K antigens on the red cells can be characterized by several bloodtyping reagents, only anti- K_a and anti- K_b were used for typing the Duroc and Hampshire pigs, while the Landrace pigs were typed with K_a , K_b and K_d reagents. The serological procedures and the genetics of the K system have been described in previous papers (ANDRESEN and IRWIN 1959; ANDRESEN 1962, 1963, 1966b; SAISON 1966). The rare allele K^{ad} was observed only in a few sire families of the Landrace breed. In the Duroc and Hampshire breeds, the K_a and K_b reagents detect the red-cell antigens K_a and K_b . These antigens are products of alleles K^a and K^b . The phenotype of each pig tested so far has been $K(a+b-)$, $K(a-b+)$, $K(a+b+)$, or $K(a-b-)$. These phenotypes correspond to the genotypes K^aK^a, K^aK^- ; K^bK^b, K^bK^- ; K^aK^b , and K^-K^- . The designation K^- symbolizes the recessive allele for which no antigenic product has been discovered. Genotype K^-K^- was not observed.

The serum proteins determined by the *Hp* locus were originally considered to be haptoglobins (KRISTJANSSON 1961; HESSELHOLT 1963a). However, these proteins are not haptoglobins, and tentatively, they have been designated as hematin-binding globulins (HESSELHOLT 1963b, GRAETZER *et al.* 1965), because this designation reflects their physiologic function. IMLAH (1965) used the term heme-binding globulins and also applied the locus designation *Hg* instead of the original designation *Hp*.

These hematin-binding proteins were detected by starch gel electrophoresis, and the procedure was a modification of that described by KRISTJANSSON (1961). Instead of porcine hemolysate, a hemolysate prepared from chicken blood was mixed with each serum sample prior to electrophoresis (HESSELHOLT 1963a). A 0.021 M Tris (hydroxymethyl)aminomethane/0.018 N hydrochloric acid buffer (pH 7.4) was used for preparing the gels, and a 0.3 M boric acid/0.1 N sodium hydroxide buffer (pH 8.7) was used in the electrode vessels. The lower surface of the gels was water-cooled during electrophoresis. On completion of electrophoresis the gels were sliced and stained with benzidine reagent as described by KRISTJANSSON (1961).

The hematin-binding globulins were represented by the products of the four previously reported codominant alleles, Hp^0 , Hp^1 , Hp^2 , and Hp^3 .

Statistical procedures: MORTON's lod score method (a sequential probability ratio test, MORTON 1955) was used for the sex-linkage analyses. Lod scores (z_1) corresponding to the recombination fractions $\theta_1 = 0.35$, 0.40, and 0.45 were computed for each sire family. Details of procedures and statements about significance tests applicable to linkage studies with genetic loci in pigs were reported previously (ANDRESEN 1966a, b). Since two or more codominant alleles are detectable in both genetic systems, information from several litters within a sire family was obtained according to the following procedure, exemplified by the K blood-group system.

Parents		Progeny			
Male	Females	Males		Females	
K^aK^b	K^aK^a	K^aK^b	K^aK^a	K^aK^b	K^aK^a
		a_1	b_1	c_1	d_1
	K^bK^b	K^aK^b	K^bK^b	K^aK^b	K^bK^b
		a_2	b_2	c_2	d_2

The letters a_1 , b_1 , c_1 , and d_1 indicate observed numbers of progeny from one or more females of the same phenotype; that is, the progeny from all K^aK^a or K^aK^- females are combined as if they were full sibs. The same is true for progeny from females of type K^bK^b and K^bK^- . Progeny from K^-K^- females may enter either of the two classes.

Hence, the values a and b , used for computing the lod score (z_1) pertinent to one sire family, are

$$a = a_1 + b_2 + c_2 + d_1$$

$$b = a_2 + b_1 + c_1 + d_2$$

In contrast to the analysis of sex-linkage, statistical methods were not required to establish linkage between the *Hp* and *K* loci, or to establish homogeneity of the linkage data. The recombination frequency was estimated as the frequency of certain recombinants among the progeny from double backcross matings. According to MORTON (1962) this method of estimation is appropriate if the linkage is close, and such an estimate is almost unbiased and has approximately a binomial distribution.

RESULTS AND DISCUSSION

Detection and estimation of the Hp-K linkage. The previously obtained evidence for close linkage between the *K* blood-group locus and the *Hp* locus for hematin-binding globulins in pigs (IMLAH 1965) was confirmed by examining segregation results from double backcross matings of the Duroc and Hampshire breeds. Presumably, IMLAH studied Large White and Landrace pigs. In that material, three recombinants were observed among a total of 58 progeny from double backcross matings. Using FISHER's *u* score method and the formula reported by FINNEY (1940) for estimating the recombination frequency, IMLAH found a recombination of 1.7%. However, the recombination frequency based on IMLAH's data is $\hat{\theta} = 3/58$ or 5.2%, if the frequency is estimated as the ratio of certain recombinants to the total number of progeny. The latter estimate is less biased because the linkage is close (MORTON 1962). The corresponding 95% confidence interval is $1\% < \theta < 14\%$ (MAINLAND *et al.* 1956).

In the present material 28 recombinants and 745 parental types were observed among a total of 773 progeny from 109 double backcross matings. Repeated typing of additional red-cell and serum samples obtained from recombinants and their parents confirmed previous results. Moreover, the evidence for linkage was homogeneous and the single factor segregations were in accordance with expectations. Among the 109 litters, 86 comprised exclusively parental types. Among the remaining 23 litters, one recombinant was observed in each of 19 litters, two recombinants were observed in each of three litters, and three recombinants were found in one litter.

Linkage equilibrium is not expected in the present material because females were replaced by animals born in the experimental herd. Thus, the significant association actually detected among the *Hp* and *K* phenotypes in the parental material cannot be regarded as evidence against a true genetic linkage between the two loci.

Since 28 recombinants were observed among a total of 773 progeny from double backcross matings, the estimate of the recombination frequency is $\hat{\theta} = 3.62\%$ ($28/773 \times 100$), based on the combined data from both breeds. As indicated in the lower right corner of Table 1, the 95% confidence interval associated with this estimate of $\hat{\theta} = 3.62\%$ is $2.5\% < \theta < 5.3\%$. This result may be indicated as $\hat{\theta} = 3.62\% \pm 0.7\%$, but the standard error, 0.7, is slightly biased (CLOPPER and PEARSON 1934).

Testing goodness to fit: As mentioned in MATERIALS and METHODS, the procedure applied in this report for estimating the recombination frequency provides

TABLE 1

Estimates of frequencies of recombination between the Hp and K loci in pigs

Recombination in	Breed		Total
	Duroc	Hampshire	
Males	3.61 (3/83) $0.75 < \theta_m < 10.2$	4.84 (12/248) $2.5 < \theta_m < 8.2$	4.53 (15/331) $2.6 < \theta_m < 7.3$
Females	2.20 (6/273) $0.8 < \theta_f < 4.7$	4.14 (7/169) $1.7 < \theta_f < 8.2$	2.94 (13/443) $1.6 < \theta_f < 4.9$
Total	2.53 (9/356) $1.2 < \theta < 4.8$	4.56 (19/417) $2.8 < \theta < 7.0$	3.62 (28/773) $2.5 < \theta < 5.3$

The percentage of recombination, the ratio of recombinants to the total number of corresponding progeny, and the 95% confidence interval are indicated for each class.

an efficient statistic if the estimate, $\hat{\theta}$, is small. A test of goodness of fit is, therefore, appropriate in the present situation since such tests are valid only when efficient statistics are used in fitting a hypothesis to the data (FISHER 1954). The results of the latter procedure are represented by the expected values in Table 2 in which $\hat{\theta} = 0.0362$, and the total number of progeny is 773; that is, the table includes all progeny considered in Table 1.

Because two or more codominant alleles are detected in each of the two systems, a doubly heterozygous parent may represent two directly detectable linkage phases, coupling or repulsion, instead of merely one. Hence, the significant excess of matings based on parents in coupling phase (Table 2) reflects not only linkage-disequilibrium but also the author's first choice of phase when there were two possibilities.

TABLE 2

Distribution of phenotypes among the 773 progeny from 109 double backcross matings considered in Table 1

Linkage-phase of parent		Phenotypes of progeny				Chi-square
		Hp K	Hp-	-K	--	
Coupling	Observed	254	7	9	243	
	Expected†	247.2	9.3	9.3	247.2	0.8 (2 df)
	Formulae for expectations‡	$\frac{n_1}{2} (1-\hat{\theta})$	$\frac{n_1}{2} \times \hat{\theta}$	$\frac{n_1}{2} \times \hat{\theta}$	$\frac{n_1}{2} (1-\hat{\theta})$	
Repulsion	Observed	3	132	116	9	
	Expected	4.7	125.3	125.3	4.7	1.8 (1 df)
	Formulae for expectations§	$\frac{n_2}{2} \times \hat{\theta}$	$\frac{n_2}{2} (1-\hat{\theta})$	$\frac{n_2}{2} (1-\hat{\theta})$	$\frac{n_2}{2} \times \hat{\theta}$	

† The expected numbers are calculated for $\hat{\theta} = 0.0362$.

§ $n_1 = 513$; $n_2 = 260$.

The classes in which the expected frequencies are less than five are considered jointly in Table 2 for calculating chi-square. The two χ^2 values of 0.8, 2 df and 1.8, 1 df correspond to the probabilities $0.50 < P < 0.70$ and $0.10 < P < 0.20$. Thus, this test shows that the observed departure from expectations, assuming good single factor segregations and no linkage, is accounted for by the assumption of close linkage.

Influence of breed and sex: Table 1 shows the recombination frequencies observed within each breed. The difference between the recombination estimates of $\hat{\theta} = 4.56\%$ in Hampshires and $\hat{\theta} = 2.53\%$ in Durocs is not significant, as indicated by the overlapping 95% confidence intervals associated with these estimates.

RENWICK and SCHULZE (1965) have reviewed the literature on the influence of sex on the recombination frequency between linked loci in various species. HALDANE (1922) noted a general tendency for linkage to be closer in the heterogametic sex in the animal kingdom. This observation has been confirmed in various species, but the effect is relatively slight in mammals. In man, the two linkages nail-patella: ABO and Lutheran: Secretor show the same trend (RENWICK and SCHULZE 1965; COOK 1965).

In the first study of linkage between the *C* and *J* blood-group loci in pigs, the proportions of recombinants originating from crossover events in males and females were 4/102 and 11/198 (ANDRESEN and BAKER 1964). In the material reported by RASMUSEN (1965), the corresponding proportions were $\hat{\theta}_m = 3/36$ and $\hat{\theta}_f = 1/21$. Additional published data (ANDRESEN 1966a) should not be considered here because only doubly heterozygous females were subjected to investigation. The combined data from the first two studies on the *C-J* linkage show that $\hat{\theta}_m = 7/138 (= 0.051)$ and $\hat{\theta}_f = 12/219 (= 0.055)$. These frequencies fit expectations according to a hypothesis that the female to male ratio $\theta_f:\theta_m$ is 1:1.

The results in Table 1 show that the $\hat{\theta}_m$ is greater than $\hat{\theta}_f$ for the *Hp-K* linkage within each breed. However, the differences are not statistically significant. The latter is also true for the combined estimates of $\hat{\theta}_m = 4.53\%$ and $\hat{\theta}_f = 2.94\%$, based on both breeds. Thus, pigs, in contrast to man, do not support the conclusion that the homogametic sex shows more recombination for a given pair of loci than does the heterogametic sex.

Effects of radiation. Numerous experiments with *Drosophila*, as surveyed by HANNAH-ALAVA (1966), have shown that X rays can induce crossing over during spermatogenesis. The recombinants may be recovered as singles, small clusters or large clusters among the progeny from X-ray-exposed *Drosophila* males. Similar investigations have not been reported for laboratory mammals, although suitable stocks of several species are available (JAY 1963). In the first study of linkage between the *C* and *J* blood-group loci in pigs, no difference was observed between the recombination frequencies based on progeny from irradiated and nonirradiated sires.

The results of measuring the possible effects of X rays on the *Hp-K* recombination frequency are shown in Table 3. This table includes the 713 progeny considered in Tables 1 and 2. The classification of progeny is based on the assump-

TABLE 3

The ratios of recombinants to the total number of progeny from each of four mating types

Doubly heterozygous parents	Mating types*			
	$X\sigma \times x\varphi$	$X\sigma \times c\varphi$	$C\sigma \times x\varphi$	$C\sigma \times c\varphi$
Males	3/82	4/58	5/132	3/59
Females	4/98	3/116	3/86	3/142

* $X\sigma$ = X-ray-exposed male; $C\sigma$ = male not exposed to X rays. $x\varphi$ = female whose sire was exposed to X rays; $c\varphi$ = female whose sire was not exposed to X rays.

tion that an effect of X-ray exposure will be detected if the exposed animal is doubly heterozygous. However, this classification is also justified by the fact that the frequency of crossing over might be regulated by mutable genes, not necessarily located adjacent to the linked genes considered (JESSOP and CATCHESIDE 1965).

No significant difference was observed between combinations of any two of the eight ratios shown in Table 3. The extreme ratios 4/58 and 3/142 correspond to the recombination frequencies 6.90% and 2.11%, and the corresponding 95% confidence intervals are $1.9\% < \theta < 16.0\%$ and $0.4\% < \theta < 6.0\%$.

Since X-irradiation had no detectable effect on the recombination frequency, the previously derived estimate of $\hat{\theta} = 3.6\% \pm 0.7\%$ can be regarded as an efficient estimate of the spontaneous recombination frequency for the *Hp-K* linkage based on the present data.

Searching for partial sex-linkage: The occurrence of heterozygous males precludes complete sex-linkage of the *Hp* and *K* loci. Therefore, the results in Table 4 refer to the possibility of detecting partial sex-linkage of the two loci, even though the existence of homologous loci on mammalian sex chromosomes is merely hypothetical (STERN 1960). The accumulated *lod* scores, *Z*, are indicated in this table for the three selected recombination values $\theta_1 = 0.35, 0.40$, and 0.45 . Since $Z = 0$ for $\theta_1 = 0.50$, the two *lod* score curves for the *Hp*-Sex and *K*-Sex comparisons can easily be constructed; cf. Figure 1 in Andresen (1966b). The recombination values $\theta_1 = 0.42$ and 0.43 corresponding to $Z = -2$ are obtained by graphical interpolation. This procedure indicates that the recombination value relative to the *Hp*-Sex linkage relationship is greater than 0.42 at the chosen significance level of $P = 0.01$, which corresponds to $Z = -2$. At this significance level, the recombination frequency relative to the *K*-Sex comparison is greater

TABLE 4

Summary of information relative to possible partial sex-linkage of the Hp and K loci

Linkage relationship	<i>Z</i> for θ_1			Value of θ_1 for $Z = \text{approx. } -2$
	.35	.40	.45	
<i>Hp</i> -Sex	-14.6	-4.8	-0.7	.42
<i>K</i> -Sex	-31.5	-8.1	-0.2	.43

than 0.43. Thus, if the *Hp* and *K* loci are on the homologous section of the sex chromosomes, they must be at the distal end. A similar conclusion was made relative to the location of the closely linked *C* and *J* blood group loci (ANDRESEN 1966a). Moreover, it was postulated that such location probably would have been detected by the accumulated data, since the Y chromosome in pigs is very short (McCONNELL, FECHHEIMER and GILMORE 1963). The same argument applies to the *Hp-K* data. Consequently, a second case of autosomal linkage has now been established in pigs.

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SUMMARY

Close linkage between the *K* blood-group locus and the *Hp* locus for hematin-binding globulins in pigs was confirmed. Crossing over seems to occur with equal frequency in the Duroc and Hampshire breeds and also in males and females. No effect of gonadal X-irradiation on the recombination frequency was observed. Complete sex-linkage was excluded, and there was no evidence for partial sex-linkage of the two loci. The estimate of the recombination frequency is $3.6\% \pm 0.7\%$, based on the combined data.

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