LINKAGE AMONG CATTLE BLOOD AND MILK POLYMORPHISMS¹

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IN cattle at least eleven blood group loci are currently recognized (STORMONT 1967). STORMONT (1962) reported that these eleven, the *A*, *B*, *C*, *FV*, *J*, *L*, *M*, *N*, *S*, *Z*, and *R' S'* loci, appeared to be genetically independent. Reports since that time have indicated the possible existence of one or two other independent loci (BOUW, DE GROOT, and BUYS 1965; GROSCLAUDE 1966). MORTON, STONE, and IRWIN (1956) analyzed the *A*, *B*, *C*, *FV*, *J*, *L*, *M*, *S*, and *Z* locus combinations by means of sequential probability tests to detect the presence of loose as well as close linkage. No significant evidence of linkage was found.

The discovery of additional polymorphisms detectable by gel electrophoresis has greatly expanded the number of loci known. These include hemoglobin, serum transferrin, and the milk proteins β -lactoglobulin, α -lactalbumin, α_{s1} -casein, β -casein, and k-casein (see ASCHAFFENBURG 1965; ASHTON *et al.* 1967; and KIDDY 1964 for reviews and references on these and other polymorphisms). DATTA and STONE (1963) were able to exclude linkage with recombination frequencies less than 10% between the transferrin locus and the *A*, *B*, *C*, and *FV* blood group loci and with recombination frequencies less than 5% for the *S* and *Z* loci. LARSEN (1966) found no evidence of linkage between the transferrin locus and the *A*, *B*, *C*, *FV*, *L*, *M*, *S*, *Z*, or *R' S'* loci. There was no evidence of linkage between the hemoglobin locus and the *B*, *C*, *FV*, *L*, *M*, *S*, *Z*, *R' S'*, or transferrin loci. There was, however, significant evidence of close linkage between the hemoglobin and *A* loci ($.03 \pm .017$), and a possibility of loose linkage between the hemoglobin and *L* loci.

Other workers have considered the relationships of milk protein loci to blood group, serum protein, and other milk loci. THATCHER and KIDDY (1965) examined linkage possibilities among the *B*, *FV*, *J*, *L*, *Z*, β -lactoglobulin, and transferrin loci. They found significant evidence of linkage between the β -lactoglobulin and *J* blood group loci with an estimate of 20% or more recombination. Several reports indicate nonindependent inheritance or close linkage among various casein loci (GROSCLAUDE *et al.* 1964, 1965; KING *et al.* 1965; LARSEN and THY-MANN 1966; ARAVE 1967). Whether the α_{s1} -, β -, and k-casein variants are products of a single locus or closely linked loci is not yet satisfactorily resolved.

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The purpose of this investigation was to detect and measure the linkage relationships that may exist among nine blood group, one serum protein, and four milk protein loci.

MATERIALS AND METHODS

Source of data: The total of 6,545 daughter-dam pairs which were involved in these investigations represented 259 sire progeny groups and included approximately 5,500 Holsteins, and smaller numbers of Ayrshires, Brown Swiss, Guernsey, Jerseys, and Holstein-Brown Swiss-Ayrshire two and three breed crosses. Of the total pairs, 4,894 were from herds operated by the Ohio Department of Mental Hygiene and Correction, and typed in connection with the North Central Regional Breeding Project, 1,478 were from the U. S. Department of Agriculture Dairy Cattle Research Branch at Beltsville, Md. or from herds closely cooperating with the Beltsville station including North Carolina institutional herds, the Lewisburg, Tenn. experiment station herd, and several private herds, and 173 were from Utah State University and cooperating herds.

Genetic typing: Blood antigen typing for the systems A, B, C, FV, J, L, M, S, and Z was performed by the Ohio State University Cattle Blood Typing Laboratory using standard procedures described by FERGUSON (1941). The typing of the electrophoretic variants of transferrin, β -lactoglobulin, α_{s1} -casein, β -casein, and k-casein was performed by the individual stations using starch and polyacrylamide gel techniques.

Linkage analysis: The probability of the existence of linkage between pairs of the fourteen loci studied was tested by means of the sequential probability ratio test described by MORTON (1955, 1957, 1962). The informative statistic, the "lod" is derived by elision of the logarithm of the odds. The lod is the logarithm of the probability ratio formed by dividing the probability under a chosen recombination value, θ_1 , by the probability under the recombination value of one-half.

Lod scores (Z) were calculated for all available system comparisons within paternal half-sib families. All linkage determinations were performed on allele transmission patterns from sires to their offspring. The only matings utilized for any system comparison were those in which the alleles transmitted from a doubly heterozygous sire to his offspring could be determined without doubt or bias. These initial calculations considered all recombination values (θ_1) from .05 through .45 by increments of .05. For combinations in which there was evidence of linkage, lod scores were calculated for recombination fractions in the neighborhood of the peak lod score by .01 increments. For these combinations, some additional information was gained by including available maternal half-sib families. Lod scores for individual locus comparisons were summed over all sires having at least two informative daughters in those comparisons.

Lod scores greater than +3.0 were interpreted as being significant evidence of linkage (.001 probability level). Lod scores of less than -2.0 (.01 probability level) provided the basis for exclusion of linkage as close as or closer than the tested recombination fraction. Lod scores greater than -2.0 but less than +3.0 were considered to be inconclusive. The recombination fraction at which the maximum Z was calculated was regarded as the best recombination estimate.

Prior to testing for linkage relationships, single locus segregation ratios from informative matings were examined. Since the segregation ratios of the FV and J loci deviated significantly from those expected for neutral genes, an additional linkage test was sought which was not based upon the assumption of equal allelic segregation. The chi-square test described by OSTLE (1958) for contingency table analysis was chosen. The type of data examined was exactly the same as for the sequential probability test, involving only those matings in which the allele transmission from sire to offspring could be determined. For each comparison chi-square computations were performed within half-sib families and summed over families (pooled chi-square), as well as on the entire sample (total chi-square). Pooled minus total chi-square values (heterogeneity chi square) provided an indication of heterogeneity among half-sib families.

RESULTS AND DISCUSSION

Results of sequential probability tests on 88 of the 91 possible locus comparisons are presented in Table 1. For three locus combinations, A:M, C:M, and S:M,

TABLE 1

*Sequential probability linkage tests of 88 cattle blood group, serum protein,
and milk protein locus combinations*

Comparison	Number of progeny	Number of half-sib families	Recombination level at which $Z \leq -2.0$	Recombination level at which Z max. calculated	Maximum lod score
<i>A:B</i>	517	44	.40	.50	0
<i>A:C</i>	181	20	.30	.50	0
<i>A:F</i>	80	11	.20	.50	0
<i>A:J</i>	162	14	.30	.50	0
<i>A:L</i>	173	11	.30	.50	0
<i>A:S</i>	175	14	.35	.50	0
<i>A:Z</i>	135	20	.30	.50	0
<i>A:Tf</i>	106	20	.25	.50	0
<i>A:Lg</i>	60	14	.15	.50	0
<i>A:α_{81}-Cn</i>	19	6	.05	.50	0
<i>A:β-Cn</i>	88	19	.25	.50	0
<i>A:k-Cn</i>	15	5	..	.25	0.16
<i>B:C</i>	928	67	.35	.43	0.75
<i>B:F</i>	382	26	.35	.50	0
<i>B:J</i>	955	53	.40	.50	0
<i>B:L</i>	726	36	.35	.45	0.28
<i>B:M</i>	7	2	..	.50	0
<i>B:S</i>	492	29	.35	.45	0.22
<i>B:Z</i>	517	49	.35	.50	0
<i>B:Tf</i>	571	46	.30	.45	0.34
<i>B:Lg</i>	291	39	.30	.50	0
<i>B:α_{81}-Cn</i>	30	8	.05	.25	0.31
<i>B:β-Cn</i>	282	41	.30	.50	0
<i>B:k-Cn</i>	38	10	.10	.40	0.03
<i>C:F</i>	186	17	.35	.50	0
<i>C:J</i>	280	23	.30	.45	0.18
<i>C:L</i>	165	15	.30	.50	0
<i>C:S</i>	206	15	.30	.50	0
<i>C:Z</i>	164	25	.25	.50	0
<i>C:Tf</i>	231	20	.30	.45	0.05
<i>C:Lg</i>	35	9	.15	.50	0
<i>C:α_{81}-Cn</i>	9	4	..	.50	0
<i>C:β-Cn</i>	117	16	.30	.50	0
<i>C:k-Cn</i>	5	2	..	.50	0
<i>F:J</i>	96	9	.25	.50	0
<i>F:L</i>	182	13	.30	.45	0.09
<i>F:M</i>	45	2	.20	.50	0
<i>F:S</i>	139	8	.30	.50	0
<i>F:Z</i>	230	17	.30	.50	0
<i>F:Tf</i>	91	11	.20	.50	0
<i>F:Lg</i>	59	8	.15	.40	0.14
<i>F:α_{81}-Cn</i>	5	1	..	.20	0.12
<i>F:β-Cn</i>	39	10	.20	.50	0
<i>F:k-Cn</i>	7	2	..	.50	0
<i>J:L</i>	254	17	.35	.50	0
<i>J:M</i>	5	1	..	.50	0

TABLE 1—Continued

Comparison	Number of progeny	Number of half-sib families	Recombination level at which $Z \leq -2.0$	Recombination level at which Z max. calculated	Maximum lod score
<i>J:S</i>	176	10	.30	.45	0.06
<i>J:Z</i>	261	29	.30	.50	0
<i>J:Tf</i>	132	20	.30	.50	0
<i>J:Lg</i>	108	15	..	.20	5.43*
<i>J:α₈₁-Cn</i>	26	6	.15	.50	0
<i>J:β-Cn</i>	67	11	.20	.40	0.07
<i>J:k-Cn</i>	12	4	..	.50	0
<i>L:M</i>	2	1	..	.05	0.26
<i>L:S</i>	101	12	.20	.40	0.15
<i>L:Z</i>	228	20	.30	.50	0
<i>L:Tf</i>	124	13	.25	.50	0
<i>L:Lg</i>	85	13	.20	.50	0
<i>L:α₈₁-Cn</i>	6	2	..	.20	0.07
<i>L:β-Cn</i>	55	13	.20	.50	0
<i>L:k-Cn</i>	12	5	..	.30	0.13
<i>M:Z</i>	18	2	.10	.50	0
<i>M:Tf</i>	9	3	..	.50	0
<i>M:Lg</i>	6	2	..	.20	0.12
<i>M:α₈₁-Cn</i>	7	1	..	.15	0.56
<i>M:β-Cn</i>	8	3	..	.50	0
<i>M:k-Cn</i>	2	1	..	.50	0
<i>S:Z</i>	165	15	.30	.45	0.04
<i>S:Tf</i>	122	12	.30	.50	0
<i>S:Lg</i>	82	13	.20	.40	0.25
<i>S:α₈₁-Cn</i>	17	4	..	.25	0.61
<i>S:β-Cn</i>	89	11	.20	.40	0.09
<i>S:k-Cn</i>	18	5	.05	.50	0
<i>Z:Tf</i>	114	22	.20	.40	0.06
<i>Z:Lg</i>	94	19	.25	.50	0
<i>Z:α₈₁-Cn</i>	29	7	.15	.50	0
<i>Z:β-Cn</i>	43	13	.10	.35	0.13
<i>Z:k-Cn</i>	9	4	..	.50	0
<i>Tf:Lg</i>	91	18	.15	.35	0.21
<i>Tf:α₈₁-Cn</i>	23	6	.10	.50	0
<i>Tf:β-Cn</i>	107	23	.15	.35	0.59
<i>Tf:k-Cn</i>	22	7	.10	.50	0
<i>Lg:α₈₁-Cn</i>	69	12	.20	.50	0
<i>Lg:β-Cn</i>	123	30	.20	.45	0.01
<i>Lg:k-Cn</i>	79	16	.25	.50	0
<i>α₈₁-Cn:β-Cn</i>	68	18	..	.03	11.14*
<i>α₈₁-Cn:k-Cn</i>	41	5	..	.12	4.54*
<i>β-Cn:k-Cn</i>	65	18	..	.01	13.37*

* Significant at the .001 probability level.

no information was available. Significant evidence of linkage was found for *J:Lg* ($P < 1 \times 10^{-5}$), *α₈₁-Cn: β-Cn* ($P < 1 \times 10^{-11}$), *α₈₁-Cn: k-Cn* ($P < 1 \times 10^{-4}$), and *β-Cn: k-Cn* ($P < 1 \times 10^{-13}$). No other lod scores closely approached significance. The

maximum Z value of $+0.75$ for the $B:C$ comparison corresponds to the .178 probability level for $\Theta_1 = .43$. Thus, the probability of the material under loose linkage with an estimated 43% recombination is about 5.6 times as great as under no linkage, which is far from statistical significance. Lod scores *versus* Θ_1 values are plotted in Figure 1 for the $J:Lg$, $\alpha_{s1}-Cn$: $\beta-Cn$, $\alpha_{s1}-Cn$: $k-Cn$, $\beta-Cn$: $k-Cn$, and $B:C$ locus combinations.

The results of chi-square tests of 85 locus comparisons are presented in Table 2. All of the comparisons with significant lod scores also had highly significant pooled chi-square values ($P < .001$). The evidence of non-independence of the B and C systems which is present in the pooled χ^2 value calculated within half-sib families and summed over families is not present in the total χ^2 value which was calculated across half-sib families. This and the resulting significant heterogeneity χ^2 value is as would be expected for populations in linkage phase equilibrium.

The significant chi-square values for the $Tf:\beta-Cn$ comparison considered together with the maximum lod score of $+0.59$ at $\Theta = .35$ indicate that this comparison may merit further attention when greater numbers of offspring are available. The several other significant chi-square values that are scattered through the table do not appear more frequently than would be expected by chance and probably

FIGURE 1.—Values of the accumulated lod scores (Z) at different recombination fraction levels (Θ_1) for the $\alpha_{s1}-Cn:\beta-Cn$, $\beta-Cn:k-Cn$, $\alpha_{s1}-Cn:k-Cn$, $J:Lg$, and $B:C$ system comparisons.

TABLE 2

*Chi-square linkage tests of 85 blood group, serum protein,
and milk protein locus combinations*

Comparison	Number of progeny	Number of half-sib families	Pooled χ^2	Total χ^2	Heterogeneity χ^2
<i>A:B</i>	490	34	32.32	0.49	31.83
<i>A:C</i>	175	18	21.14	0.49	20.65
<i>A:F</i>	71	8	9.95	0.00	9.95
<i>A:J</i>	152	9	11.13	1.03	10.10
<i>A:L</i>	168	9	11.72	0.02	11.70
<i>A:S</i>	166	10	6.75	0.87	5.88
<i>A:Z</i>	127	17	20.11	0.18	19.93
<i>A:Tf</i>	89	13	11.49	0.00	11.49
<i>A:Lg</i>	49	9	9.40	0.51	8.89
<i>A:α_{s1}-Cn</i>	12	3	5.06	0.00	5.06
<i>A:β-Cn</i>	77	14	15.32	0.63	14.69
<i>A:k-Cn</i>	13	4	7.56	0.12	7.44
<i>B:C</i>	914	61	97.99**	0.02	97.97**
<i>B:F</i>	375	24	26.76	0.30	26.46
<i>B:J</i>	932	45	54.49	1.29	53.20
<i>B:L</i>	714	32	38.86	0.04	38.82
<i>B:M</i>	7	2	1.33	0.88	0.45
<i>B:S</i>	470	22	35.07*	1.61	33.46*
<i>B:Z</i>	493	41	53.38	7.11**	46.27
<i>B:Tf</i>	556	41	65.86**	1.97	63.89**
<i>B:Lg</i>	265	32	35.22	2.03	33.19
<i>B:α_{s1}-Cn</i>	26	6	10.71	0.00	10.71
<i>B:β-Cn</i>	243	25	34.95	2.98	31.97
<i>B:k-Cn</i>	29	8	13.44	0.85	12.59
<i>C:F</i>	159	11	19.25	0.00	19.25*
<i>C:J</i>	272	20	21.89	2.52	19.37
<i>C:L</i>	156	11	13.10	0.20	12.90
<i>C:S</i>	199	13	12.03	0.21	11.82
<i>C:Z</i>	148	19	26.21	0.05	26.16
<i>C:Tf</i>	226	17	23.64	3.74	19.90
<i>C:Lg</i>	23	4	4.75	1.05	3.70
<i>C:β-Cn</i>	105	11	9.54	0.36	9.18
<i>C:k-Cn</i>	3	1	0.75	0.75	...
<i>F:J</i>	90	7	3.68	0.99	2.69
<i>F:L</i>	177	11	19.10	0.44	18.66*
<i>F:M</i>	45	2	0.81	1.07	-0.26
<i>F:S</i>	118	6	7.20	0.05	7.15
<i>F:Z</i>	225	15	13.69	0.18	13.51
<i>F:Tf</i>	81	8	11.29	0.03	11.26
<i>F:Lg</i>	59	8	13.79	3.31	10.48
<i>F:α_{s1}-Cn</i>	5	1	2.22	2.22	...
<i>F:β-Cn</i>	30	6	3.73	0.56	3.17
<i>F:k-Cn</i>	7	2	0.14	0.63	-0.49
<i>J:L</i>	238	11	10.25	0.66	9.59
<i>J:M</i>	5	1	0.83	0.83	...
<i>J:S</i>	166	8	6.39	0.07	6.32
<i>J:Z</i>	249	24	31.80	0.32	31.48

TABLE 2—Continued

Comparison	Number of progeny	Number of half-sib families	Pooled χ^2	Total χ^2	Heterogeneity χ^2
<i>J:Tf</i>	119	15	16.74	2.42	14.32
<i>J:Lg</i>	103	13	47.78***	23.36***	24.42*
<i>J:α_{s1}-Cn</i>	21	4	3.64	1.68	1.96
<i>J:β-Cn</i>	62	9	8.82	0.52	8.30
<i>J:k-Cn</i>	6	2	3.33	0.00	3.33
<i>L:S</i>	84	8	13.25	0.05	13.20
<i>L:Z</i>	214	14	16.39	0.46	15.93
<i>L:Tf</i>	121	12	16.20	0.44	15.76
<i>L:Lg</i>	75	8	10.12	0.00	10.12
<i>L:α_{s1}-Cn</i>	4	1	1.33	1.33	...
<i>L:β-Cn</i>	44	8	6.66	0.36	6.30
<i>L:k-Cn</i>	6	2	6.00*	0.67	5.33*
<i>M:Z</i>	18	2	0.64	0.14	0.50
<i>M:Tf</i>	4	1	0.44	0.44	...
<i>M:Lg</i>	3	1	0.75	0.75	...
<i>M:α_{s1}-Cn</i>	7	1	3.73	3.73	...
<i>M:β-Cn</i>	4	1	1.33	1.33	...
<i>S:Z</i>	155	12	19.55	0.58	18.97
<i>S:Tf</i>	110	7	7.11	0.88	6.23
<i>S:Lg</i>	62	6	9.10	0.53	8.57
<i>S:α_{s1}-Cn</i>	13	2	7.42*	6.96**	0.46
<i>S:β-Cn</i>	82	8	13.29	5.06*	8.23
<i>S:k-Cn</i>	14	3	4.42	2.57	1.85
<i>Z:Tf</i>	98	16	23.28	0.95	22.33
<i>Z:Lg</i>	78	13	13.99	0.47	13.52
<i>Z:α_{s1}-Cn</i>	21	4	3.34	0.04	3.30
<i>Z:β-Cn</i>	30	8	13.70	3.33	10.37
<i>Z:k-Cn</i>	4	2	2.00	0.44	1.56
<i>Tf:Lg</i>	80	14	22.35	0.07	22.28
<i>Tf:α_{s1}-Cn</i>	23	6	5.23	0.35	4.88
<i>Tf:β-Cn</i>	70	9	21.60*	8.40**	13.20
<i>Tf:k-Cn</i>	10	2	1.71	0.08	1.63
<i>Lg:α_{s1}-Cn</i>	59	8	11.67	1.73	9.94
<i>Lg:β-Cn</i>	82	17	25.25	0.39	24.86
<i>Lg:k-Cn</i>	59	9	11.29	0.00	11.29
<i>α_{s1}-Cn:β-Cn</i>	57	12	53.12***	49.09***	4.03
<i>α_{s1}-Cn:k-Cn</i>	42	5	26.36***	16.48***	9.88*
<i>β-Cn:k-Cn</i>	60	16	56.00***	6.88**	49.12***

* Significant at the .05 probability level.

** Significant at the .01 probability level.

*** Significant at the .001 probability level.

represent that type of deviation from expectations. Numbers in some of the cells were small. Since the distribution of the test statistic, χ^2 , closely approximates the chi-square distribution only when the subclass numbers are large, the test is not ideally suited for many of the comparisons.

Homogeneity of Θ values among families was tested as described by MORTON (1962): $\mathcal{L} = 4.605 \left(\sum_{i=1}^n \hat{z}_i - \hat{Z} \right)$ where n = the number of families, $\hat{z}_i$ = the maxi-

mum lod score for the i th family, and $\hat{Z}$ is the maximum Z accumulated within Θ values over all families. $\mathcal{L}$ has the chi-square distribution with $n-1$ degrees of freedom. The $B:C$ comparison tested in this manner appeared to consist of homogeneous data ($.70 < p < .80$). Judgment about linkage, however, will have to be suspended until additional data permit a decision to be rendered.

Tests for homogeneity of Θ were also conducted for the $J:Lg$, $\alpha_{s1}-Cn$: $\beta-Cn$, and $\alpha_{s1}-Cn$: $k-Cn$ comparisons with the following results:

$\mathcal{L} J:Lg = 4.605$ ($9.34-5.43$) = 18.0 for 14 df; $.20 < P < .30$

$\mathcal{L} \alpha_{s1}-Cn: \beta-Cn = 4.605$ ($12.61-11.14$) = 6.8 for 17 df; $.975 < P < .99$

$\mathcal{L} \alpha_{s1}-Cn: k-Cn = 4.605$ ($61.01-4.54$) = 6.8 for 4 df; $.10 < P < .20$.

Thus, the data appear to be homogeneous. The $\beta-Cn$: $k-Cn$ comparison was not tested since no recombinant types were found.

For all of the linked systems, recombination fractions, standard errors, and 95% confidence intervals were calculated. Recombination fractions were determined by the sequential probability estimates and by calculating the fraction, number of crossover gametic types.

total number of gametes. The 95% confidence limits were determined by plotting the antilogs of the lod scores *versus* the Θ values, determining the area under the curve, and cutting 2.5% of the area from each tail. These values are presented in Table 3.

It is both interesting and perplexing to note that all of the linked systems have significant total chi-square values. Only the $\beta-Cn$: $k-Cn$ comparison has a heterogeneity chi-square value which is more highly significant than the total chi-square value. These significant total chi-square values are interpreted as indications that the systems are not in linkage phase equilibrium in the population, although in multiple allelic system comparisons the value may not be meaningful. The indicated disequilibrium is most surprising for the $J:Lg$ linkage system, where a recombination estimate of 20% was determined. By the formula, $\text{Disequilibrium} = (1-2\Theta)^n$, where n = the number of generations of random mating following

TABLE 3

*Estimates of recombination fractions for the $J:Lg$, $\alpha_{s1}-Cn$: $\beta-Cn$,
 $\alpha_{s1}-Cn$: $k-Cn$ and $\beta-Cn$: $k-Cn$ linkage systems*

Linkage comparison	Recombination fraction		Standard error*	95 percent confidence interval
	Sequential probability estimate	Crossover gametes Total gametes		
$J:Lg$	.20	.194	.038	$.135 < \theta < .295$
$\alpha_{s1}-Cn: \beta-Cn$	.03	.029	.020	$.010 < \theta < .095$
$\alpha_{s1}-Cn: k-Cn$	.12	.122	.051	$.060 < \theta < .255$
$\beta-Cn: k-Cn$	.00†	.000	...	

* S.E. = $\sqrt{\frac{\theta(1-\theta)}{N}}$, where N = Total number of progeny.

† The smallest recombination fraction considered in sequential probability estimates was .01. However, since no recombinants were found, it is obvious that the best estimate of θ is 0.

complete disequilibrium, it may be determined that when $\Theta = .2$ only ten generations will be required to reduce the disequilibrium to less than .01. If $\Theta = .01$ approximately 200 generations will be required to approach equilibrium this closely. It appears likely that if the observed non-independent system segregations are truly a result of chromosomal linkage, either there has been a marked deviation from random breeding in the population, the loci (particularly *J* and *Lg*) are much more closely linked than suspected, or both conditions are true.

Since it is possible that a few sires of similar phase with disproportionately large families might have biased the total chi-square, it is perhaps profitable to examine the linkage phase condition of the sire samples. Based upon the observed distribution of alleles into the four gametic classes, it is possible to determine, at a specified probability level, the linkage phase of any randomly chosen, doubly heterozygous sire. The *a posteriori* probability that such a sire is in the coupling

phase is $P = \frac{\Theta^{b+c} (1-\Theta)^{a+d}}{\Theta^{b+c} (1-\Theta)^{a+d} + \Theta^{a+d} (1-\Theta)^{b+c}}$, where a,b,c, and d represent the progeny phenotype classes described by MORTON (1955). For the *J:Lg* comparison, there is a 96% probability that of ten random sires with data like those of the ten most predictable sires in this comparison, nine will be in the *J'Lg^B* phase and one in the *J'Lg^A* phase. The probability of finding at least nine of ten unrelated sires in the same phase is given by: $P = 22 (1/2)^{10} = .021$. Since two of these sires were half-sibs the probability would be raised to approximately .027. Of the entire group of 15 sires, 13 appear to be in the *J'Lg^B* phase, one in the *J'Lg^A* phase, and one in unknown phase. Additional evidence against sampling error is presented by BRUM *et al.* (1968). They reported significant evidence of non-random occurrence of *J* and *Lg* genotypes in the Ohio portion of this sample.

The possibility of a chemical or metabolic relationship between *J* and *Lg* gene products should certainly be considered. The finding of significant deviation of *J* system alleles from expected segregation ratios (Table 4) favors this viewpoint. However, in the 108 progeny involved in the *J:Lg* linkage comparison, single locus segregation ratios did not significantly differ from expectations (50*J'*:58*J'*; 54*Lg^A*: 54*Lg^B*), β -lactoglobulin segregation in the entire sample of animals was also near expectations. It is evident that any unequal *J* system segregation did not bias the lod score results *per se*, although the unequal segregation may be related to the *J:Lg* loci dependency phenomenon.

The *J* system differs from other blood group systems in that the *J* antigen is a

TABLE 4
Segregation of J system alleles from J^J/Jⁱ × Jⁱ/Jⁱ matings

Progeny genotype	Observed number	Expected number	Chi-square
<i>J'/J'</i>	478	558.5	11.60
<i>J'/Jⁱ</i>	639	558.5	11.60

23.20*

* $p < .0005$.

serum soluble substance which is adsorbed to the surface of the cells (STORMONT 1949). It also displays considerable quantitative variation (STONE 1962), a fact which imparts no additional accuracy to the qualitative tests. The possibility that the strength of the J hemolytic reaction might be influenced by the β -lactoglobulin type was examined by comparing the strengths of J positive reactions of 20 J positive randomly chosen animals from each of the three Lg groups (A, AB, and B). An analysis of variance revealed no significant difference.

An additional alternative to linkage that is discussed by MORTON (1955) is nonrandom segregation of nonhomologous chromosomes. Presumably, this phenomenon might be expected to produce a disequilibrium condition.

The apparent linkage phase disequilibrium of the various casein combinations, indicated by the significant total chi-square values, was corroborated by examination of probable sire linkage phases. Because of the low frequency of recombination, linkage phase could be determined with virtual certainty.

Of 14 sires and 4 dams whose progeny were involved in the α_{s1} -Cn: β -Cn comparison, 17 were of linkage phase α_{s1} -Cn^B β -Cn^{A2}/ α_{s1} -Cn^C β -Cn^{A3}. The remaining sire was of genotype α_{s1} -Cn^A β -Cn^{A1}/ α_{s1} -Cn^B β -Cn^{A2}. Thus, in this Holstein sample only three linkage combinations were found. In other breeds, not involved in this comparison because of lack of sires doubly heterozygous at these systems, other combinations were found. The β -Cn: *k*-Cn and the α_{s1} -Cn: *k*-Cn system combination appeared to be much nearer equilibrium. Some deviation from equilibrium is less surprising for the casein loci because the loci appear from the results of this and other investigations (GROSCLAUDE *et al.* 1964, 1965; LARSEN and THYMAN 1966) to be very closely linked. The apparent disequilibrium, nevertheless, indicates closer linkage than has been estimated for the α_{s1} -Cn: β -Cn and the α_{s1} -Cn: *k*-Cn combinations. Only two apparent crossover types were found among the 68 progeny involved in the α_{s1} -Cn: β -Cn comparison, six among the 41 progeny of the α_{s1} -Cn: *k*-Cn comparison, and none among the 65 progeny in the β -Cn: *k*-Cn comparison. Retyping on these progeny and their dams was performed when possible, and other systems were again compared with parental genotypes to check for the possibility of parentage error. Nevertheless, all of the possible errors in typing, sample handling, and record keeping cannot be eliminated, and since they tend to bias the recombination estimate toward $\frac{1}{2}$ it is easy to believe that linkage could be closer. At any rate it seems evident that the DNA segments coding the amino acid sequences of the polypeptide chains of α_{s1} -, β -, and *k*-casein are located very close to each other on the same chromosome either within the same locus or in very closely linked loci. The order on the chromosome of these coding sequences, as indicated by this investigation, is α_{s1} -Cn, β -Cn, *k*-Cn.

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SUMMARY

Sequential probability tests were employed to test for genetic linkage among the cattle *A*, *B*, *C*, *FV*, *J*, *L*, *M*, *S*, and *Z* blood group loci, the serum transferrin

locus, and the milk protein loci β -lactoglobulin, α_{s1} -casein, β -casein, and k -casein. Significant evidence of linkage was found between the J and Lg loci, the α_{s1} - Cn and β - Cn loci, the α_{s1} - Cn and k - Cn loci, and the β - Cn and k - Cn loci. Recombination fraction estimates for these respective combinations are .20, .03, .12, and 0.00. Linkage could be excluded for most other systems at the 20% recombination level. Apparent disequilibrium of linkage phase for the J and Lg system combination aroused suspicion of closer linkage or other factors producing the non-independent segregation.

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