(7). It is possible that a similar sequence of events operates in cells other than lymphocytes.

TINGCHUNG WANG
Department of Surgery, University of Minnesota, Minneapolis 55455
J. R. SHEPPARD
Dight Institute of Human Genetics, Department of Genetics and Cell Biology, University of Minnesota
JOHN E. FOKER
Department of Surgery, University of Minnesota

References and Notes

1. C. W. Abell and T. M. Monahan, *J. Cell. Physiol.* **59**, 549 (1973); F. J. Chlapowski, L. A. Kelly, R. W. Butcher, *Adv. Cyclic Nucleotide Res.* **6**, 245 (1976); D. L. Friedman, *Physiol. Rev.* **56**, 652 (1976).
2. I. H. Pastan, G. S. Johnson, W. B. Anderson, *Annu. Rev. Biochem.* **44**, 491 (1975).
3. R. R. Burk, *Nature (London)* **219**, 1272 (1968); W. L. Ryan and M. L. Heidrick, *Science* **162**, 1484 (1968); G. S. Johnson and I. H. Pastan, *J. Natl. Cancer Inst.* **47**, 1357 (1971); J. R. Sheppard, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1316 (1971).
4. M. L. Heidrick and W. L. Ryan, *Cancer Res.* **31**, 1313 (1971); J. Otten, G. Johnson, I. Pastan, *Biochem. Biophys. Res. Commun.* **44**, 1192 (1971); S. Bannai and J. R. Sheppard, *Nature (London)* **250**, 62 (1972).
5. J. R. Sheppard, *Nature (London) New Biol.* **236**, 14 (1972).
6. J. F. Whitfield, R. H. Rixon, J. P. MacManus, S. D. Balk, *In Vitro* **8**, 257 (1973); T. Hovi and A. Vaheri, *Nature (London) New Biol.* **245**, 175 (1973); S. Schor and E. Rozengurt, *J. Cell. Physiol.* **81**, 339 (1974); M. E. Cross and M. G. Ord, *Biochem. J.* **124**, 241 (1971).
7. J. F. Whitfield, G. P. MacManus, R. H. Rixon, A. L. Boynton, T. Yondale, S. Swierenga, *In Vitro* **12**, 1 (1976).
8. J. P. MacManus, B. M. Braceland, T. Yondale, J. R. Whitfield, *J. Cell. Physiol.* **82**, 157 (1973).
9. S. Thrower and M. Ord, *Biochem. J.* **144**, 361 (1974).
10. J. F. Whitfield, A. L. Boynton, J. P. MacManus, R. H. Rixon, P. R. Walker, U. Armato, in *Cyclic Nucleotides and the Regulation of Cell Growth*, M. Abou-Sabe, Ed. (Dowden, Hutchinson and Ross, Stroudsburg, Pa., 1976), pp. 97–130.
11. J. R. Sheppard and D. M. Prescott, *Exp. Cell Res.* **75**, 293 (1972); W. E. Seifert and P. S. Rudland, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4920 (1972); A. J. T. Millis, G. A. Forrest, D. A. Pious, *Exp. Cell Res.* **83**, 335 (1974); D. H. Russell and P. J. Stambrook, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1482 (1975); C. E. Zeilig and N. D. Goldberg, *ibid.* **74**, 1052 (1977).
12. J. P. MacManus, D. J. Franks, T. Yondale, B. M. Braceland, *Biochem. Biophys. Res. Commun.* **49**, 1210 (1972).
13. Splenic lymphocytes from (C57Bl1/6 × DBA/2) F_1 hybrid mice (8 to 12 weeks old, Jackson Laboratory) were stimulated with con A (2 μg/ml) (*25*). RNA and DNA syntheses were assessed by incorporation of [^{3}H]uridine and [^{3}H]thymidine, respectively, into trichloroacetic acid–precipitable material during a 1-hour pulse (*25*). Cells were counted on a hemocytometer. Cell viability, assessed by trypan-blue exclusion, decreased from 100 percent to 80 percent during the first 24 hours and stabilized at 70 percent for the rest of the culture time. The suspensions consisted chiefly of small lymphocytes (> 95 percent) as judged by morphology; included in the remainder were macrophages. Concanavalin A stimulates T lymphocytes to proliferate, but B lymphocytes would also be present, and the possibility that the cyclic AMP and proliferative responses originated from different cell populations cannot be excluded.
14. Intracellular cyclic AMP was determined by an adaptation of the method of Brown (*26*). Duplicate 10-ml samples of lymphocyte cultures were centrifuged at 650*g* for 10 minutes; the pellets were resuspended in 1 ml of ice-cold 5 percent trichloroacetic acid and stored at −20°C. For the assay, the cell sample was thawed and centrifuged at 1000*g* for 10 minutes. The pellet was saved for protein determination (*27*), and the supernatant was extracted five times with three volumes of water-saturated diethyl ether acidified with hydrochloric acid. The aqueous fraction was incubated at 50°C for 60 minutes to remove any residual ether. A series of unlabeled cyclic AMP standards ranging from 0 to 10 pmole were extracted in parallel with the cell samples. The assay mixture consisted of 0.1 ml of bovine adrenal protein kinase, 0.1 ml of unlabeled cyclic AMP standard or sample, 0.2 ml of buffer (0.1*M* tris-HCl, *p*H 7.4, 8 m*M* theophylline, and 6 m*M* mercaptoethanol), and 2 pmole of cyclic [^{3}H] AMP (36.6 Ci/mmole) in 0.05 ml of distilled water. After the mixture was incubated at 4°C for 3 hours, 0.3 ml of assay buffer with 10 percent (weight to volume) charcoal and 2 percent (weight to volume) bovine serum albumin was added, and the mixture was centrifuged at 4°C in a Sorvall GLC-1 centrifuge for 10 minutes at 3000*g*. A sample (0.4 ml) of the supernatant was mixed with 5 ml of Bray's solution and the radioactivity was determined. Samples and standards were assayed in triplicate. For each assay, appropriate blanks containing 0.1 ml of water were included, and a standard curve was constructed by plotting the amount of cyclic AMP standards against radioactivity in the supernatant. Intracellular cyclic AMP was expressed as the number of picomoles per 10^6 viable cells or per milligram of protein. The authenticity of the cyclic AMP in the sample was confirmed by its susceptibility to degradation by phosphodiesterase.
15. J. R. Vane, *Nature (London) New Biol.* **231**, 232 (1971); J. B. Smith and A. L. Willis, *ibid.*, p. 235; S. H. Ferreira, S. Moncada, J. R. Vane, *ibid.*, p. 237.
16. J. W. Hinman, *Annu. Rev. Biochem.* **41**, 161 (1972); B. Samuelson, E. Granstrom, K. Green, M. Hamberg, S. Hammarstrom, *ibid.* **44**, 669 (1975).
17. J. W. Smith, A. L. Steiner, C. W. Parker, *J. Clin. Invest.* **50**, 442 (1971); C. W. Parker, M. L. Baumann, M. G. Huber, *ibid.* **52**, 1336 (1973); D. J. Franks, J. P. MacManus, J. F. Whitfield, *Biochem. Biophys. Res. Commun.* **44**, 1177 (1971).
18. V. A. Ferraris and F. R. DeRubertis, *J. Clin. Invest.* **54**, 378 (1974).
19. H. Sheppard and G. Wiggan, *Biochem. Pharmacol.* **20**, 2181 (1971).
20. The cyclic AMP analog, 8-bromo cyclic AMP, was added to parallel cultures of con A–stimulated lymphocytes at the times indicated. At 48 hours after con A addition, DNA synthesis was assessed by [^{3}H]thymidine incorporation. The rate of incorporation in unstimulated control lymphocytes was 3620 count/min per hour per 10^6cells. Net incorporation was calculated by subtracting the control value from total incorporation.
21. Cell cycle phase distribution was measured by flow microfluorometry. Samples (2 ml) of the culture were centrifuged and the lymphocytes were resuspended in 2 ml of a solution of sodium citrate (1.2 mg/ml) containing 50 μg of propidium iodide per milliliter (*28*). After staining for 10 minutes, cell fluorescence, proportional to the amount of cellular DNA, was measured by a Cytofluorograf (model 4801 Bio/physics Systems).
22. J. W. Smith, A. L. Steiner, W. M. Newberry, Jr., C. W. Parker, *J. Clin. Invest.* **50**, 432 (1971).
23. C. W. Parker, T. J. Sullivan, H. J. Wedner, *Adv. Cyclic Nucleotide Res.* **4**, 2 (1974).
24. A. E. Powell and M. A. Leon, *Exp. Cell Res.* **62**, 316 (1970); A. Novogradsky and E. Katchalski, *ibid.* **97**, 1 (1976); L. Furcht and S. Gentry, *Cell. Immunol.* **22**, 225 (1976). Cysteine (2 m*M*) inhibits lymphocyte proliferation completely if added up to 8 hours after neuraminidase–galactose oxidase (NAGO) treatment. Thereafter cells gradually become irreversibly stimulated and are totally insensitive to cysteine by 15 hours after NAGO stimulation.
25. T. Wang, C. Marquardt, J. Foker, *Nature (London)* **261**, 702 (1976).
26. B. L. Brown, *Biochem. J.* **121**, 561 (1971).
27. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
28. J. Fried, A. G. Perez, B. D. Clarkson, *J. Cell Biol.* **71**, 172 (1976).
29. We thank C. Fitzpatrick for technical assistance, Drs. R. F. Howard and T. H. Hudson for valuable discussions, and Drs. N. D. Goldberg and J. G. White for reviewing the manuscript. Supported by grants from the National Leukemia Association, St. Paul Ramsey Medical Education and Research Fund, and the Minnesota Leukemia Task Force.

24 January 1978; revised 7 March 1978

Microcirculation of the Spleen: An Open or Closed Circulation?

Abstract. *By injecting plastic microspheres of a specific size (3 to 4 micrometers) into the circulation and following their movement and distribution in the spleen, it was revealed how blood travels from the arterial capillaries to the venous sinuses. This method demonstrated that both open and closed circulation exist in the spleen and that about 90 percent of the blood takes the open route of circulation in the normal unanesthetized rabbit.*

Although the microcirculation of the spleen is central to its function as a blood filter, the intermediary vascular connection between the arterial capillary and the venous sinus has remained a subject of debate for the last 100 years. Three major theories about intermediary splenic circulation exist: (i) The open circulation theory states that the arterial capillaries open directly into the cordal meshwork of the red pulp and the blood is then forced into the venous sinuses (*1*); (ii) the closed circulation theory states that the arterial capillaries connect directly to the venous sinuses (*2*); (iii) the third theory holds that both open and closed circulation exist in the spleen (*3*). These differences in interpreting the nature of splenic circulation suggest that the methods for previous studies may have been inadequate, since both morphological observation of fixed spleen and direct observation of the living spleen produce conflicting results and are unable to settle the question. In addition, the ambient temperature of the exteriorized spleen, anesthesia, and manipulation of the spleen during experimentation affect the organ's microcirculation (*4*). We now describe a new method for studying the splenic circulation. This method demonstrates that both open and closed circulation exist in the spleen, and that about 90 percent of the blood takes the open route in normal unanesthetized rabbits. This method does not require direct observation of the vascular connection between the arterial capillary and the venous sinus and avoids invasive manipulation of the spleen and anesthesia.

Blood cells in the spleen pass through the reticular meshwork of the cords and pores (the interendothelial spaces) of the sinus wall. These pores measure be-

0036-8075/78/0714-0157$00.50/0

tween 0.5 and 2.5 μm (*5*). It was postulated that any rigid microspheres that were slightly larger than the pore and were introduced into the splenic circulation would not be able to pass through the sinus wall from the sinus into the cord or vice versa. Such microspheres would appear in the cords only if they traveled by open circulation, and would appear in the sinuses only if they traveled by closed circulation. If both open and closed circulation existed in the spleen, microspheres would appear in the cords as well as in the sinuses. Therefore, it was feasible to determine how blood traveled from the arterial capillaries to the venous sinuses by introducing rigid microspheres into the circulation and studying their distribution in the spleen at appropriate times. Since the microspheres were distributed to the capillary beds according to the blood flow (*6*), the relative blood flow to the open and closed circulation could be determined by enumerating the microspheres within and without the sinuses at appropriate times after their introduction into the circulation.

This method requires (i) injection of microspheres of suitable size into the bloodstream, (ii) termination of the blood flow upon the arrival of the microspheres in the spleen, and (iii) preparation of histological sections of the splenic tissue for studying microsphere distribution in the spleen. The ideal size of the microsphere was 3 to 4 μm (*7*). Such microspheres are small enough to travel in the capillaries and the cordal meshwork of the spleen, yet are large enough to be prevented from passing through the sinus wall. Unanesthetized New Zealand White rabbits weighing between 3.5 and 4 kg were injected in the marginal ear vein with 3×10^8 carbonized plastic microspheres (3 to 4 μm) in 0.5 ml of saline. At either 8 to 9 seconds or 15 to 18 seconds after the microsphere injection, blood flow and heartbeat were stopped by injecting an overdose of pentobarbital into the marginal vein of the other ear (*8*). The spleens were removed and processed for histological studies (*9*). The microspheres were readily identified in histological sections and were found in the cords as well as in the sinuses (Fig. 1).

Table 1. Distribution of plastic microspheres (3 to 4 μm) in the red pulp of rabbit spleen between the time of injection of microspheres into the ear vein and termination of heartbeat. For each spleen, 2000 to 3000 microspheres were counted from random tissue sections, and the percentage of the microspheres in the cords and the sinuses was calculated. Five rabbits were in each time group.

Time (seconds)	Percentage of microspheres* in		Microspheres counted (No.)
	Sinuses	Cords	
8–9	8.6 ± 1.3	91.4 ± 1.3	12,074
15–18	2.5 ± 1.2	97.5 ± 1.2	12,460

*Mean ± standard deviation.

The relative distribution of the microspheres in the cords and the sinuses at succeeding intervals after microsphere injection was determined (Table 1). About 91 percent of the microspheres were localized in the cords, and 9 percent were in the sinuses of rabbits whose heartbeats were stopped at 8 to 9 seconds (*10*); however, only 2.5 percent of the microspheres were found in the sinuses in the rabbits whose heartbeats were stopped at 15 to 18 seconds. These results demonstrate that (i) both open and closed circulation exist in the spleen, (ii) about 90 percent of the blood passing through the spleen of a normal unanesthetized rabbit takes the open route, and (iii) microspheres entering the spleen through open circulation are trapped in the cords while those that use closed circulation leave the spleen. A small number of microspheres were still observed in the sinuses 15 to 18 seconds after the microsphere injection. This may be due to the fact that a portion of the blood passing through the spleen may remain in its sinuses for various periods of time, as was observed by Knisely (*2*).

Our microsphere method for studying the intrasplenic microcirculation can be used to determine whether there are any changes in the intrasplenic microcirculation in pathological spleens, and to correlate the nature of the circulation with certain pathological states.

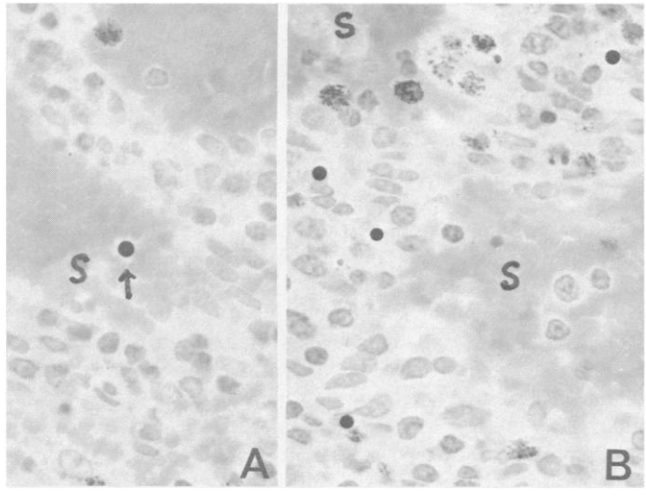

Fig. 1. Distribution of plastic microspheres (3 to 4 μm) in the red pulp of rabbit spleen. Microspheres were injected into the ear vein 15 seconds before termination of the heartbeat. (A) One microsphere (arrow) is shown in the lumen of the sinus. (B) Four microspheres are scattered in the cord. Abbreviation: *S*, sinus (× 800).

LI-TSUN CHEN

Department of Cell Biology and Anatomy, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

References and Notes

1. A. Hartmann, in *Handbuch der mikroskopischen Anatomie des Menschen*, V. Mollendorfs, Ed. (Springer, Berlin, 1930), vol. 6, p. 397; D. Mackenzie, Jr., A. O. Whipple, M. P. Wintersteiner, *Am. J. Anat.* **68**, 397 (1941); W. W. Fleming and A. K. Parpart, *Angiology* **10**, 28 (1959).
2. T. Billroth, *Virchows Arch. Pathol. Anat. Physiol.* **23**, 457 (1862); K. Helly, *Arch. Mikrosk. Anat. Entwicklungsmech.* **59**, 93 (1902); M. Knisely, *Anat. Rec.* **65**, 23 (1936); G. Simon and R. Pictet, *Acta Anat.* **57**, 163 (1964); T. Murakami, T. Fugita, M. Miyoshi, *Experientia* **29**, 1374 (1973). A modified theory of the closed circulation was proposed by R. Pictet, L. Orci, W. Forssmann, and L. Girardier [*Z. Zellforsch. Mikrosk. Anat.* **96**, 372 (1969)].
3. F. Weidenreich, *Arch. Mikrosk. Anat. Entwicklungsmech.* **58**, 247 (1901); J. W. McNee, *Trans. Med. Soc. London* **54**, 185 (1931); T. Snook, *Anat. Rec.* **130**, 711 (1958); L. Weiss, *Am. J. Anat.* **113**, 51 (1963); P. Vaupel *et al.*, *Res. Exp. Med.* **164**, 223 (1974).
4. H. M. Peck and N. L. Hoerr, *Anat. Rec.* **109**, 479 (1951).
5. P. F. Leblond, *Nouv. Rev. Fr. Hematol.* **13**, 771 (1973); L. T. Chen and L. Weiss, *Blood* **41**, 529 (1973).
6. A. M. Rudolph and M. A. Heymann, *Circ. Res.* **21**, 163 (1967); R. J. Buchin, R. F. Edlich, T. P. Hennessy, M. S. Tung, M. V. Prevost, O. H. Wangensteen, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **26**, 496 (1967).
7. Plastic microspheres of various sizes have been tested for their ability to pass through the red pulp of the spleen. It was found that microspheres of 50 μm were capable of reaching the central arteries, microspheres of 15 μm reached the arterioles, and microspheres less than 7 μm reached the sinuses as well as the cords. Although 5-μm microspheres were capable of reaching the capillary terminations and entering the cords, they were not able to move freely within the meshwork of the cords. Microspheres of 3 to 4 μm were found to be small enough to be able to move within the cordal meshwork and large enough to be detained in the cords by the sinus wall.
8. The microspheres were injected into the marginal ear vein through a 22-gauge hypodermic needle. After the injection of microspheres the blood flow and the heartbeat were stopped at different intervals. It was found that the microspheres began to enter the spleen by 6 seconds, and that, by 8 to 9 seconds, microspheres were randomly distributed throughout the red pulp of the spleen. Blood flow and heartbeat were stopped by an overdose of pentobarbital (100 mg per kilogram of body weight), which was injected into one of the ear veins through a small polyethylene tube. The heartbeat stopped 4 to 5 sec-

onds after initiation of the pentobarbital injection. The termination of the heartbeat was detected by a stethoscope placed beneath the rabbit in the restraining box.

9. After immersion for 2 to 3 days in cold Karnovsky's fixative, the spleens were cut into slices (1.5 mm thick). The tissue was then dehydrated in alcohol and embedded in a mixture of butoxyethanol glycol methacrylate (Polysciences). Sections, 3 μm thick, were stained with hematoxylin and eosin.
10. A few of the microspheres in the sinuses may have left the spleen by 8 to 9 seconds after microsphere injection. Therefore, the percentage of microspheres that entered the sinuses may be slightly underestimated.
11. I thank D. Anderson for technical assistance. This work was supported in part by NSF grant PCM 76-20367, and by a Leukemia Society of America scholarship to L.T.C.

23 February 1978

Serologically Defined (SD) Locus in Cattle

Abstract. *Using cytotoxic serums obtained from multiparous cows or by alloimmunization, we have detected 11 lymphocyte antigens controlled by codominant alleles at a serologically defined locus called* BoLA-A *(bovine lymphocyte antigens). This locus, along with the lymphocyte defined loci previously reported, establishes the existence of a major histocompatibility system of cattle.*

Major histocompatibility systems (MHS) have been defined in at least 11 mammalian species (*1*). In every species that has been studied extensively, these systems have consisted of loci producing at least two kinds of lymphocyte antigens: those which are detected by cytotoxic antiserums [serologically defined (SD)], and those detected by mixed lymphocyte culture tests [lymphocyte defined (LD)]. Recently, our laboratory described two LD loci in cattle (*2*) and we now present data describing an SD locus called *BoLA-A* (bovine lymphocyte antigens). These loci together define the MHS in cattle.

A modification of the two-step cytotoxicity test (*3*) was used for typing fresh peripheral blood lymphocytes isolated by gradient centrifugation with Ficoll-Hypaque (density, 1.075). Two microliters of undiluted antiserum and approximately 1500 lymphocytes were transferred by pipette into Terasaki plates containing one drop of mineral oil per well. After 30 minutes at 22° ± 2°C, 5 μl of undiluted rabbit serum were added as a source of complement. Trypan blue was added after an additional 30 minutes of incubation. Finally, formalin was added and cytotoxicity was recorded as positive if 50 percent or more of the lymphocytes were killed. All tests were set up in duplicate; the concordance between the results in these duplicate tests was greater than 90 percent.

Over 1600 serums were tested against a standard panel of cattle lymphocytes and against several sire families (dams, their calves and, when available, sires). A serum was selected as a typing reagent only if it gave a clear-cut and narrow spectrum of reactivity. Of the 67 serums selected, over half were from Holsteins; 42 were from multiparous cows, 21 were produced by alloimmunization with lymphocytes, and 4 were obtained from colostrum. Most of the serums were oligospecific. Each serum was assigned to one of 11 serologically distinct groups on the basis of its reactivity pattern with the lymphocytes from over 900 cattle belonging to 60 sire families.

Each of the 11 antigens defined by these groups of serums was studied for segregation (allelism) in sire families of half sibs. As shown in Table 1, each antigen behaved as if it were controlled by an autosomal codominant gene. Of the 55 possible heterozygous combinations, we were able to study 27, all of which showed 1:1 segregation. While we have not directly tested every pair of antigens for allelism, all 11 appear to be controlled by alleles at a single locus, because we have seen segregation of all 11 putative alleles from at least one of the others. We have called this locus the *BoLA-A* locus.

Table 2 shows the gene frequency distribution among 931 cattle in five dairy breeds. The breeds show marked differences; in particular, some breeds appear to have at least one allele in a significantly higher frequency than any other breed; for example, Holstein, *6b*; Jersey, *7*; Guernsey, *6n*; and Ayrshire, *1*. The frequencies given in Table 2 may not be completely representative of the different breeds because of the limited number of sires used in each breed and the relatively small number of herds from which the samples were drawn (*4*). It is clear that the *BoLA-A* locus is highly polymorphic and exhibits a high degree of heterozygosity. In the two most studied breeds, Holstein and Brown Swiss, the frequency of null alleles (antigens not detectable with our antiserums) is .097 and .087, respectively.

Table 1. Segregation ratios of alleles at the *A* locus of the *BoLA* system in cattle sire families. We used a total of 60 heterozygous sires; each provided data for two alleles.

Allele	Number of sires	Ratio of (+) to (−) offspring*
1	4	17:15
2	18	62:59
3	12	48:40
4	0	N.T.†
5n‡	7	30:32
5b‡	11	53:50
6n	10	29:40
6b	8	29:26
7	5	22:15
8	5	33:34
10	4	11:16
null	12	39:36

*None of the ratios was significantly different from the expected 1:1 ($P > .1$). †Not tested. Although there were no sires carrying allele *4*, its segregation was studied in dam-calf pairs. ‡The *n* refers to a narrow and *b* to a broad specificity.

Table 2. Gene frequencies determined by direct gene counting (*4*) in five cattle breeds for the alleles at the *BoLA-A* locus.

Allele	Breeds				
	Holstein	Brown Swiss	Jersey	Guernsey	Ayrshire
1	.097 ± .011*	.019 ± .005	.013 ± .007	.005 ± .005	.197 ± .048
2	.201 ± .015	.222 ± .017	.042 ± .013	0	.088 ± .034
3†					
4	.022 ± .005	.008 ± .004	.142 ± .023	0	.059 ± .029
5n‡	.185 ± .014	.038 ± .008	.052 ± .015	.109 ± .023	.029 ± .020
5b‡	.120 ± .012	.134 ± .014	.017 ± .008	.032 ± .013	0
6n‡	.008 ± .003	.053 ± .009	.162 ± .024	.257 ± .032	.093 ± .035
6b‡	.110 ± .012	.006 ± .003	.021 ± .009	.005 ± .005	0
7	.055 ± .008	.003 ± .002	.131 ± .022	.037 ± .014	0
8†					
10	.004 ± .002	.110 ± .012	0	.016 ± .009	.015 ± .015
null	.097 ± .011	.087 ± .011	.207 ± .026	.220 ± .030	.245 ± .052
Sires/total	25/368	17/316	7/118	7/95	4/34

*Standard error = $[p(1 - p)/2N]^{1/2}$ where p is the gene frequency and N is the number of individuals. †These gene frequencies were not estimated because early in these studies only multispecific typing serums were available. This accounts for the fact that the sum of the gene frequencies within each breed does not add up to one. ‡See Table 1.

0036-8075/78/0714-0159$00.50/0