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.

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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.

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These data clearly establish an SD locus, but the question remains whether or not the SD antigens of cattle are controlled by a single gene or several closely linked genes as is true of most other mammalian species whose MHS are well defined. In our studies of families we found that about 10 percent of the "haplotypes" carried more than one specificity. We do not know if these represent the products of closely linked genes in a haplotype or simply cross-reactions of our oligospecific typing serums. However, segregation data on antigen 4 suggest that it is controlled by a linked locus. Also, we have 15 additional antiserums which detect specificities that do not appear to be associated with any of the 11 groups reported here.

Whereas previous studies (*5*) have described some lymphocyte antigens of cattle, the present report, along with the one on LD loci (*2*), makes it possible to add cattle to the list of mammalian species in which an MHS has been described (*1*). Our data on transplantation strongly suggest that the SD antigens described here play an important role in histocompatibility. Reciprocal skin grafts between three cows with identical SD antigens survived for 19 days, while all those between each of these cows and another with different SD antigens were rejected within this same period. In addition, the LD loci of these cows did not appear to influence the fate of these grafts at least within the 19 days of observation.

Unfortunately, we do not have enough data to clearly demonstrate linkage between the *BoLA-A* and the LD loci. However, in at least two sire families they behaved as if linked (*1, 6*).

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Loss of Hippocampal Theta Rhythm Results in Spatial Memory Deficit in the Rat

Abstract. *Rats learned, using distal room cues, to run to a goal on an elevated, circular track starting from any position on the track. The goal was one of eight equidistant, recessed cups set around the track, the goal cup being distinguished from the others solely by its position in the room. After learning, electrolytic lesions were made in the medial septal nucleus eliminating hippocampal theta rhythm in some animals but not in others. Rats without theta rhythm were no longer able to perform the spatial task, whereas rats with undisturbed theta rhythm retrained normal performance. Although rats without theta rhythm could not find their way directly to the goal, they recognized its location when they came upon it by chance. This type of spatial deficit appears similar to that shown by hippocampally lesioned patient H.M. Subsequent tests demonstrated that rats deprived of theta rhythm before training could nevertheless learn the task.*

Hippocampal damage in humans results in a pervasive amnesia for ongoing events as well as a deficit in spatial orientation (*1, 2*). Two types of findings in rats suggest that the hippocampus may be closely associated with the processing of spatial information in this species. First, correlations have been reported between the firing of hippocampal neurons and the position or orientation of animals in space (*3*). Second, lesions of the hippocampus or associated structures have been found to result in deficits in spatial learning (*4*) and memory (*5*). If, as implied by these last studies, the ability of rats to acquire and use spatial information is dependent on normal hippocampal function, then it is possible that disruption of hippocampal theta rhythm might result in deficits in the performance of spatial tasks, since (i) theta rhythm invariably accompanies exploratory behavior in the rat (*6*), and (ii) theta rhythm is associated with intracellular potential changes which should be capable of modifying the excitability of hippocampal neurons (*7*) and might be presumed to play a role in normal hippocampal function. To investigate this possibility, behavioral tests were undertaken in which rats were trained in a spatial task and the effect of medial septal lesions just sufficient in extent to eliminate theta rhythm was determined.

Rats were trained on a circular maze (*4*) consisting of a circular track 2.4 m in diameter with a track width of 30.5 cm. The track was elevated 60.6 cm above floor level and had attached side walls 8.9 cm high. From any position on the track, the animal was afforded a full view of the testing room, which contained diverse landmarks (Fig. 1A). Spaced equidistantly around the track and set into its surface were eight cups, each 5.1 cm in diameter, to which water could be delivered from a remote source or from which water could be withdrawn by tubes connected to the bottoms of the cups. Each cup was equipped with a photocell detector which signaled remotely when an animal poked its nose into the cup to a depth of more than 2.5 cm for 2 seconds or longer. Each animal was maintained with free access to food and water and, on the day prior to training, was allowed to explore the maze for four sessions, each 10 minutes long. Thereafter, the animal had free access to food but was partially water-deprived. Water was available only for brief periods on the maze and for a 15-minute period in the animal's home cage each day when tests were complete. For each rat, eight trials spaced 20 minutes apart were carried out each day during the hours of 9 a.m. to 1 p.m. In each trial, the rat was released at a random position on the track, facing alternately in successive trials either toward the center of the maze or outward. The animal was given water when it activated the photoelectric detector at a designated goal cup by poking its head into it. The animal was then allowed to drink for 10 seconds and the water was withdrawn, completing the run. Alternatively, the trial was terminated if the goal cup was not activated within 5 minutes of release. The goal cup was distinguished from the others only by its con-

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