diseased cats, a preliminary characterization of β-hexosaminidase in normal cat tissues has shown a remarkable degree of similarity between the human and the feline enzyme systems (*15*). Thus, the pattern of neural and visceral glycosphingolipid storage and the type of enzyme deficiency strongly suggest that the feline disease is analogous to human GM_2 gangliosidosis type 2.

This report describes the second gangliosidosis and the eighth lysosomal storage disease known to occur in domestic cats (*2*). Feline GM_2 gangliosidosis, like some of the previously described feline lysosomal disorders, affords an opportunity for the study of the pathogenesis and treatment procedures applicable to these devastating diseases in humans.

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11. M. C. Rattazzi, P. J. Carmody, R. G. Davidson, in *Isozymes II. Physiological Function*, C. L. Markert, Ed. (Academic Press, New York, (1975), p. 439. A 10 percent (weight : volume) extract of normal liver, and 30 percent extract of liver from the affected animal were prepared by sonic disruption of the tissues in 0.05*M* citrate-phosphate buffer, *p*H 5.5. The supernatants obtained after high-speed centrifugation (40,000*g* for 30 minutes at 4°C) were used for electrophoresis.
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14. Test for frequency distribution of 1 : 3 ($P < .05$).
15. M. C. Rattazzi, in preparation. Feline β-hexosaminidase is normally present in two major forms in nervous tissue and visceral organs. Both forms are associated with lysosome-rich subcellular fractions and exhibit latency. The molecular weights of the feline isozymes, and their *p*H optima, substrate specificities, and kinetic characteristics with artificial substrates are very similar to those of human β-hexosaminidase. Isoelectric points and thermal denaturation properties of feline β-hexosaminidase, however, are different from those of the human isozymes.
16. Supported in part by USPHS grants HD 09842, RR-208-014, and NS 10967; by a grant (project 417) from the HEW Maternal and Child Health Service; and by grant RR-05493 from the NIH Biomedical Research Support Program, M.C.R. is the recipient of an NIH Research Career Development Award (K04-GM-70638). We are grateful to R. J. Desnick of the Department of Pediatrics, University of Minnesota, for neutral glycosphingolipid determinations. We thank T. Garrison and C. J. Downing for their cooperation.
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3 August 1976; revised 26 October 1976

Lymphocyte-Defined Loci in Cattle

Abstract. *Using the results of all paired one-way mixed lymphocyte culture tests on families of half-sibs, we have established that the lymhocyte-defined system in cattle contains a minimum of two loci. The methodology presented is applicable to studies of the lymphocyte-defined systems of other species.*

A major histocompatibility complex (MHC) has been reported in at least 11 mammalian species (*1*), but it has not been described in cattle despite extensive immunogenetic research on this species (*2*). Assuming that the various breeds of cattle are analogous in some respects to the various races of humans, we believe that a description of the MHC of cattle will help in understanding the human histocompatibility system. Furthermore, because of their size and availability, cattle can provide an almost unlimited source of material for chemical characterization of the histocompatibility antigens.

While studies of the serologically defined (SD) systems with lymphocytotoxic typing serums have been relatively straightforward, studies of the lymphocyte-defined (LD) systems have been complicated because they are defined by cell culture techniques (mixed lymphocyte culture, MLC) which may yield capricious results and which do not readily identify individual specificities. Indeed, the enumeration of the number of alleles and loci of LD systems is extremely difficult (*3*), although some recent progress has been made with newer techniques (*4*). More than a single locus has been detected only in humans and mice (*5*), which are the only two species that have been studied extensively. In both species the existence of two loci has been demonstrated primarily by the detection of rare genetic recombinations or by the use of homozygous typing cells.

We have developed an analytical method (*6*) which detects multiple LD loci with relative simplicity. We have applied this method to 7 families of cattle containing an average of 11 adult paternal half-sibs. The results indicate the existence of at least two LD loci. In this report, we present a detailed analysis of one of these families (*7*).

Table 1 shows the results of all paired

Table 1. Mean 30-second counts of [^{3}H]thymidine uptake in triplicate paired one-way MLC tests on seven paternal half-sibs. The counts in each column (A through G) are compared to the isogeneic controls along the diagonal (underscored).

X-irradiated stimulating cells	Responding cells						
	A	B	C	D	E	F	G
A	13,833	53,642	43,002	57,728	49,437	64,838	19,268
B	51,020	9,623	4,386*	9,780*	12,087*	13,334*	36,461
C	29,035	19,820	2,908	18,788	15,766*	16,665*	19,699
D	61,813	20,931	39,132	6,666	37,243	82,168	50,079
E	54,520	33,074	50,136	50,195	10,465	91,146	79,313
F	60,331	66,151	15,281	14,461	43,379	16,637	47,118
G	47,570	27,687	41,755	37,749	51,321	70,214	4,824

*Response not significantly different from controls ($P > .05$).

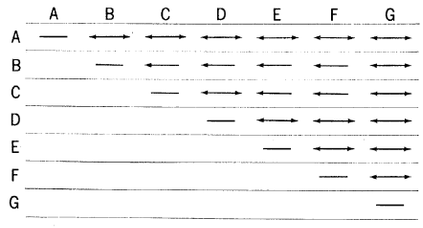

Fig. 1. Response matrix summarizing the data in Table 1, with arrows indicating the direction of stimulation. For example, F stimulates B, but B does not stimulate F, as represented by a single-headed arrow. The absence of arrows in the diagonal represents background responses.

combinations of one-way MLC tests (*8*) performed on a family of seven paternal half-sibs. Stimulation was determined by use of a *t*-statistic (*9*) with a significance level of .05. Figure 1 summarizes these results in matrix form, where the arrows signify stimulation. For example, individuals A and B are mutually stimulatory as indicated by a two-headed arrow (A ↔ B). In contrast, C stimulates B, but not vice versa, as indicated by a one-headed arrow (B ← C). The main diagonal (as in Table 1) represents the isogeneic controls to which the allogeneic reactions are compared.

A stimulation in an MLC is generally interpreted to mean that the stimulating cell has at least one antigenic specificity not present in the responding cell. It follows that a unidirectional stimulation means that all antigens in one animal are present in the other, but not vice versa. For example, B ← C (Fig. 1) indicates that the antigens of B are a proper subset of the antigens in C. Similarly, C ← F indicates that the antigens of C are a proper subset of the antigens of F. Therefore, the antigens of B must be contained in the set of antigens of F. It follows that B ← F.

This transitive property of unidirectional response allows us to transform our data into a diagram (Fig. 2) which depicts the seven members of this family in a hierarchy. Reading across the rows in Fig. 1, A is mutually stimulatory with all of the other animals and thus is isolated in our diagram (Fig. 2). The same is true for individual G. In the next row, B is stimulated unidirectionally by C, D, E, and F (Fig. 1); therefore, these four individuals are placed above and connected to B (Fig. 2). The third row tells us that E and F unidirectionally stimulate C and so they are placed above and connected to C. Since the remaining relations are mutual stimulations, no further construction is necessary. The animals are now ordered such that an individual located above and connected to another individual must contain all of the antigens of the latter and at least an additional one. Any two individuals not connected through a unidirectional pathway are mutually stimulatory and must each possess an antigen not possessed by the other.

What is a reasonable genetic interpretation of these data if we assume from our genetic model (*6*) that one locus (allele) controls a single antigenic specificity? Let us focus on individuals E, F, C, and B in Fig. 2. Because B stimulates A and G, B must possess an allele whose antigen is detectable (that is, B is not homozygous for a null allele). Since C must contain all alleles of B and, in addition, an allele not present in B, C must be heterozygous at a locus for which B is homozygous. In comparing E or F with C, the same argument requires C to be homozygous at some other locus. To explain these relationships in terms of a single LD locus would require an impossibility: that the locus be simultaneously homozygous and heterozygous in individual C. In other words, the genetic relationship of these four individuals cannot be explained on the basis of a single LD locus. Hypothetical genotypes compatible with our data are presented in Fig. 2. Note that our interpretation requires a third allele at one of the loci. In fact, if D were included in our genetic analysis, the two loci would need a total of no less than six alleles, though these could be distributed among the two loci in a number of different ways.

Two independent sources of data lend support for our interpretation. Assuming strength of response to increase with the number of antigenic differences, we would expect E and F to stimulate B to a greater degree than does C. These quantitative differences in response were generally observed (see Table 1). Further, using serological data for SD antigens obtained in our laboratory (*10*), we were able to assign one of the two paternal haplotypes to five of the seven half-sibs (tests on the other two half-sibs were uninformative). Cows D, B, and F all shared one paternal haplotype; A and G shared the other. While there is no hard evidence that these LD loci are part of a MHC in cattle, it is a reasonable working hypothesis by analogy to other species (*1*). Thus, assuming close linkage of the LD and SD loci, we would expect the segregation of the sire's haplotypes into two mutually stimulatory groups, if the

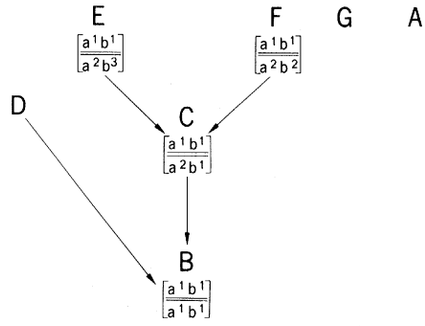

Fig. 2. The seven animals are shown diagrammatically in a hierarchy where an animal located above and connected to a lower animal stimulated the latter unidirectionally. Animals not connected by a unidirectional pathway mutually stimulated one another. Hypothetical genotypes are given within brackets.

sire were heterozygous for the LD loci, as was observed here (Fig. 2).

These data confirm the expected property of transitivity of nonstimulation. Furthermore, the existence of three animals in a chain of unidirectional stimulation has established that the LD system in cattle contains at least two loci.

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16 November 1976; revised 11 January 1977