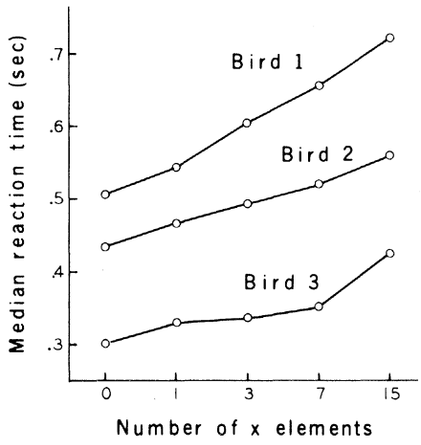

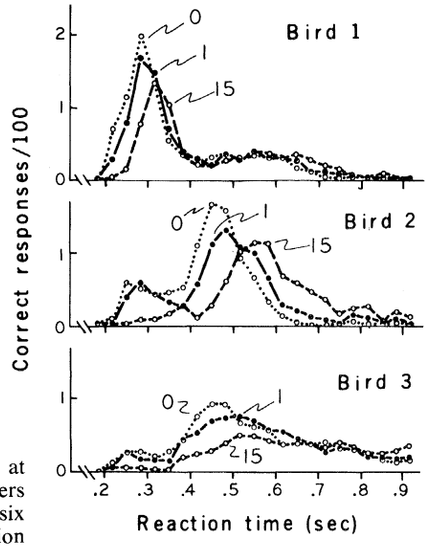

Fig. 2 (left). Median reaction times of pecks at the o when accompanied by varying numbers of x forms. Data are from 4800 trials in six sessions. Fig. 3 (right). Three of the reaction time distributions for each bird. The clearest effects of increasing the number of x stimuli appear to be a decrease in the number of responses in the first mode and a shift of the second mode to longer times.

conditions. Median reaction time was lowest when the o appeared alone, and increased with increasing number of noise elements (Fig. 2). Error rates (not shown) also were perfectly correlated with number of noise elements, rising from less than 1 percent for one x to maxima (for 15 x's) of 8.8 percent for bird 1, 5 percent for bird 2, and 2.6 percent for bird 3. The reaction time shift is seen in the distributions in Fig. 3; for clarity, only the 0, 1, and 15 x conditions appear. The distributions are all bimodal, with responses of bird 1 concentrated in the first mode and those of the other birds in the second mode. It is possible to interpret much, if not all, of the change in first-mode responses as a dropping out of the fastest responses with increasing numbers of noise stimuli, but the second mode clearly shifts in time, as best seen in the data for bird 2.

The data have several significant aspects. The pigeon search times were elevated by visual noise in much the same manner as those of humans required to search among relatively confusable or numerous items. This suggests that the birds did not process all parts of the display independently and in parallel; constant reaction times across conditions would have suggested such parallel processing. Nonetheless, the pigeons' search did proceed with relatively high speed and accuracy over a display with a very large visual angle and without notable head scanning movements (pigeon eye-movements are quite small). This finding may be related to the pigeon's complex retina, which might be specialized for acuity over a broad area.

It is tempting to associate the two modes of the reaction time distributions with a sequence of processes, such as a rapid "primary detection response" followed, if the primary process fails, by a longer-latency "secondary response" (*7*). Such an interpretation is made more complex by the finding that multimodal reaction time distributions appear to characterize pigeon peck responses in many situations, even, as Heinemann reported, those that involve no decision-making (*8*). The present data, however, are unusual in showing a clear shift in the timing of at least the second peak in the reaction time distribution; such shifts have rarely if ever been reported. Additional work with the method outlined here could provide the raw material for a model of search processes in the pigeon, as well as aiding in the identification of those aspects of search that are general across species. Such behavorial information may eventually also guide physiological studies of search and other information-processing functions.

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5. A small on-line computer, the LINC, displayed the forms on a Tektronix type 503 oscilloscope with P2 phosphor. The forms were composed of dots in a 4 by 4 matrix. The o consisted of eight dots, two on each edge of the matrix; the x also consisted of eight dots, one in each corner and four in the center of the matrix. At an apparent luminance of about 2 cd/m², the intermittency should have been well above the pigeon's critical flicker frequency (*3*). A full description of the technique is in preparation.
6. Bird 1 received most of its training in sessions with single noise conditions; that is, only one of the various numbers of x stimuli appeared on every trial for an entire session. Possibly this training variation bears some relation to the relatively fast responding of this bird.
7. This has been suggested for human search [for example, W. K. Estes, *Percept. Psychophys.* **12**, 278 (1972)].
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19 August 1976; revised 23 November 1976

GM_2 Ganglioside Lysosomal Storage Disease in Cats with β-Hexosaminidase Deficiency

Abstract. *Two kittens with progressive neurologic disease had increased concentrations of GM_2 ganglioside in their cerebral cortex. Examination under the light microscope revealed cytoplasmic vacuolation of neurons and hepatocytes. Transmission and scanning electron microscopy demonstrated cytoplasmic inclusions encompassed by membranes in various central nervous system cell types and in hepatocytes. Beta-*D-N-*acetyl-hexosaminidase activity was reduced to about 1.0 percent of normal in brain, liver, and cultured skin fibroblasts of the diseased kittens; both major electrophoretic forms, A and B, of the enzyme were deficient. In fibroblasts from the parents of the diseased kittens, this enzyme activity was intermediate between that of affected and normal cats, suggesting an autosomal recessive mode of inheritance of the enzyme defect. Histopathological and ultrastructural lesions, glycolipid storage, enzyme defect, and pattern of inheritance are similar to those of human GM_2 gangliosidosis type 2.*

Ganglioside storage diseases resulting from inherited defects in lysosomal hydrolases cause devastating neurologic disorders and have been observed in man (*1*), cats, dogs, cattle, and swine (*2*). Neuronal accumulation of GM_2 ganglioside resulting from deficient activity of one or more isozymes of β-D-*N*-acetyl-hexosaminidase (E.C. 3.2.1.52) is the most common ganglioside storage dis-

ease in children. An analogous storage disorder has been reported in German shorthaired pointer dogs (*3*) and Yorkshire swine (*2*). This report describes the occurrence of GM_2 gangliosidosis in domestic cats with severe, progressive neurologic disease and deficiency of β-D-*N*-acetylhexosaminidase activity.

Two female and two male sibling kittens had progressive deterioration of motor functions including tremors, hypermetria, ataxia, and paresis. Onset of neurologic signs occurred between 4 and 10 weeks of age, and in one kitten advanced to paraplegia by 5 months of age. Other clinical signs included failure to gain weight, occasional dysphagia, and bilateral corneal opacity.

Diseased kittens were progeny of a female cat and one of her male offspring (Nos. 3 and 5, Fig. 1). The parents and seven other siblings were phenotypically normal. Two of the diseased kittens (Nos. 10 and 18, Fig. 1) were available for further study (*4*) and were killed humanely at 6 months and 11 weeks of age, respectively.

Histopathologic lesions in both kittens varied only in severity and will be described jointly. Neuronal cell bodies throughout the nervous system, including autonomic ganglia and retina, were distended, rounded, nearly devoid of Nissl substance, and had foamy cytoplasm (Fig. 2a). In frozen sections the neuronal cytoplasm stained intensely with periodic acid Schiff. Gliosis was more marked in kitten 10, but demyelination was not severe in either. Examination of neurons by transmission electron microscopy revealed multilamellar, spherical cytoplasmic inclusions with ultrastructural characteristics similar to those found in dogs with GM_2 gangliosidosis (*3*) and cats with GM_1 gangliosidosis (*5*), but slightly different from those regarded as "typical" of human GM_2 gangliosidosis (*6*) (Fig. 2b). Other cells in the central nervous system which contained inclusions appeared to be perivascular macrophages.

Vacuolated hepatocytes and distended Kupffer cells were seen in the liver of both kittens. When examined by transmission electron microscopy, the vacuoles were found to be filled with inclusions similar, but not identical, to those in neurons (Fig. 2c). The globular nature of hepatic and Kupffer cell inclusions was demonstrated by scanning electron microscopy of cryofractured specimens (Fig. 2d). Membrane-bound inclusions were also found in endothelium, smooth muscle cells of vessels, bone marrow cells, splenic macrophages, and renal interstitial cells.

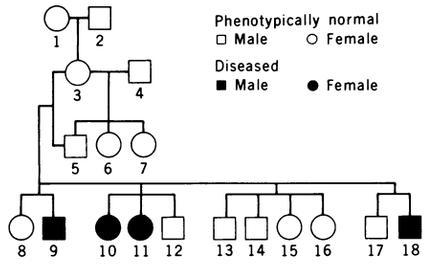

Fig. 1. Family pedigree of kittens affected with GM_2 gangliosidosis.

Gangliosides from diseased and control cat brains were extracted and quantified by the method of Suzuki (*7*). The total ganglioside content and distribution of major gangliosides from brains of diseased and normal cats is shown in Table 1. Ganglioside analysis from a cat with GM_1 gangliosidosis is shown for comparison. The total ganglioside content of the brains of the diseased kittens was two to three times that found in normal cat brain. The GM_2 ganglioside, which accounts for less than 1 percent of the total gangliosides in normal cat brain, was elevated to 38 to 44 percent of the total gangliosides in the brains of the diseased kittens. The concentration of other gangliosides was reduced. In addition, analysis of neutral glycosphingolipids (*8*) showed abnormally high concentrations of the asialo (sialic acid-free) derivative of GM_2 ganglioside in brain and liver of one diseased cat, and a 30-fold increase of the tetrahexosylceramide, *N*-acetylgalactosaminyl-digalactosyl-glucosyl ceramide (globoside), in liver. In humans, neuronal storage of GM_2 ganglioside and its asialo derivative, and visceral storage of asialo-GM_2 ganglioside and globoside is typical of GM_2 gangliosidosis type 2 (Sandhoff-Jatzkewitz disease) (*9*).

Activities of β-D-acetyl hexosaminidase (*p*H 4.0 and 4.5) and β-galactosidase (*p*H 3.8) were measured spectrofluorometrically by the method of Ho and O'Brien (*10*), the substrates used consisting of β-D-*N*-acetyl glucosaminyl and β-D-galactosyl derivatives of 4-methyl-umbelliferone (see Table 2). The

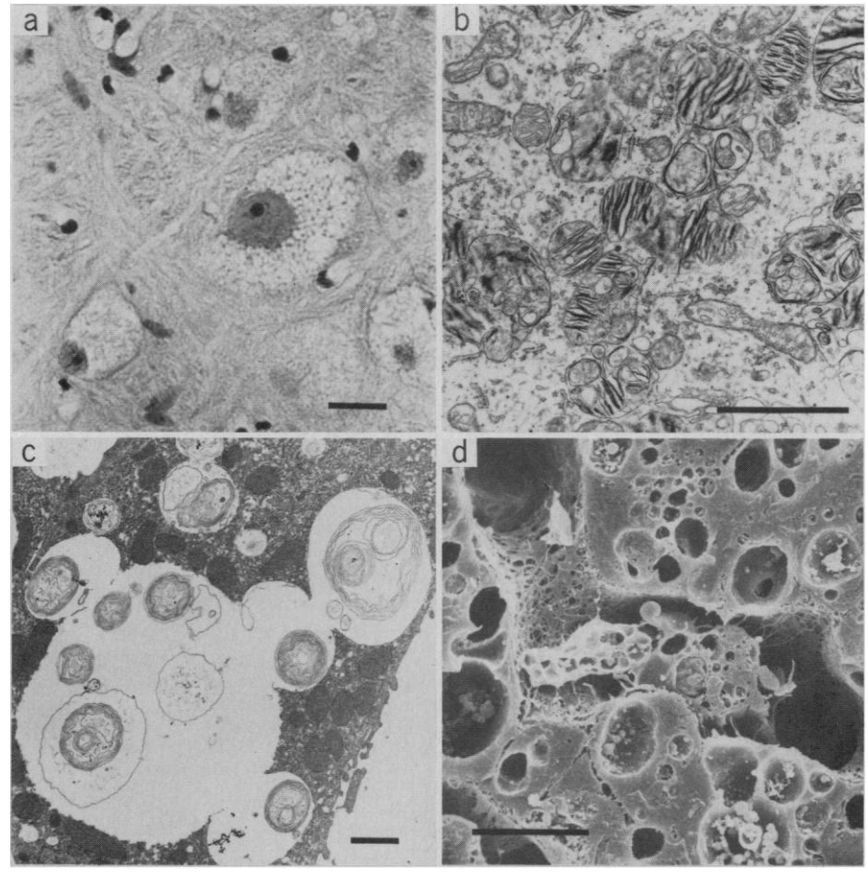

Fig. 2. (a) Neurons with foamy cytoplasm and eccentric nuclei from diseased kittens. Scale bar 20 μm. (b) Laminated inclusions encompassed by membranes within neuronal cytoplasm of diseased kitten. Scale bar, 1 μm. (c) Hepatocyte from diseased kitten with vacuoles containing membranous and vesicular inclusions similiar to those described in glycosphingolipidoses (*3*, *5*). Scale bar, 1 μm. (d) Scanning electron micrograph of cryofractured surface from liver of diseased kitten. Kupffer cell (center) contains many vacuoles. Large vacuoles in hepatocytes contain globular inclusions. Scale bar, 10 μm.

total β-hexosaminidase activity in brain, liver, and fibroblasts from diseased kittens was 0.5 to 2.0 percent of normal. The activity of β-galactosidase in diseased brain was equal to or slightly higher than the activity of this lysosomal hydrolase in normal cat brain. β-Galactosidase activity in liver from cats with GM_2 gangliosidosis was markedly higher than normal. This is consistent with the observation that activity of lysosomal hydrolases other than the mutant enzyme is increased in most lysosomal storage diseases, including feline GM_1 gangliosidosis (*2*).

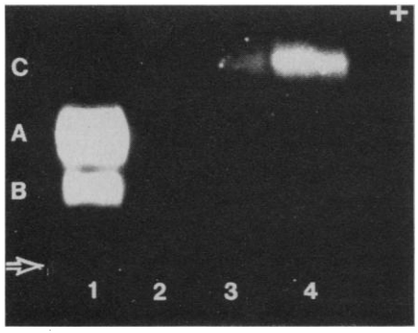

Fig. 3. Fluorescent bands of β-D-*N*-acetylhexosaminidase activity after electrophoresis of cat liver extracts on cellulose acetate gel at *p*H 6.0, and incubation with the fluorogenic substrate, 5 methylumbelliferyl-β-D-*N* acetylglucosaminide (*11*), showing deficient activity of the two major enzyme forms, A and B, in a cat with GM_2 gangliosidosis. Lane 1: 10 percent extract of normal liver, 5 μl. Lanes 2, 3, and 4: 30 percent extract of liver from affected cat, 5 μl, 10 μl, and 20 μl, respectively. To demonstrate the residual β-hexosaminidase activity (approximately 1 percent of normal) in lanes 2 to 4, that section of the gel was photographed with a 30-fold longer exposure than that used for lane 1. A minor β-hexosaminidase C band was also present in the normal sample (lane 1), but could not be photographically recorded with the exposure time optimal for β-hexosaminidase A and B.

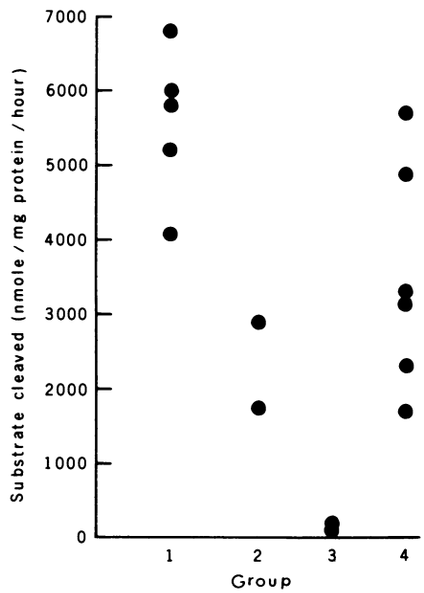

Fig. 4. The activity of β-D-*N*-acetylhexosaminidase in cultured skin fibroblasts. Group 1, normal unrelated cats; group 2, parents of diseased kittens (Nos. 3 and 5); group 3, diseased kittens (Nos. 10 and 18); and group 4, related cats (Nos. 1, 13, 14, 15, 16, 17). The other cats shown in the pedigree (Fig. 1) were not available for study.

Table 1. Gangliosides in brain of normal cats and cats with feline gangliosidoses. Values are expressed as micrograms of *N*-acetylneuraminic acid per gram (wet weight) of brain.

Source	Total gangliosides	Distribution of gangliosides			
		GM_2	GM_1	GD_{1A}	Other GD_{1B} and GT_1
Normal cat	925	0	235	265	425
Feline GM_2					
Kitten 10	1806	688	61	299	740
Kitten 18	1629	733	140	419	342
Feline GM_1	1844	0	1,255	275	263

Table 2. Lysosomal hydrolase activity in tissues of normal cats and cats with feline gangliosidoses. Values are expressed as nanomoles of substrate cleaved per milligram of protein per hour (ND, no data). The substrates were 3-methylumbelliferyl-2-acetamido-2-deoxy-β-glucopyranoside for β-hexosaminidase, and 4-methylumbelliferyl-β-Δ-galactopyranoside for β-galactosidase.

Source	Brain		Liver		Fibroblasts	
	β-Hexosaminidase	β-Galactosidase	β-Hexosaminidase	β-Galactosidase	β-Hexosaminidase	β-Galactosidase
Normal cat	537.0	50.5	994.0	69.6	5664.5	394.4
Feline GM_2						
Kitten 10	3.6	38.6	ND	ND	70.0	ND
Kitten 18	3.7	78.2	4.0	147.7	168.0	ND
Feline GM_1	844	3.9	4,817	4.9	5101.0	8.8

Electrophoresis on cellulose acetate gel (*11*) of liver extracts from diseased animals showed that the two major forms, A and B, of β-hexosaminidase present in normal tissues were inactive (Fig. 3). The residual enzymatic activity could be attributed to a more electronegative, minor enzyme form, C, also present in normal tissues. An analogous electrophoretic pattern is observed in extracts of liver and other organs from human patients with GM_2 gangliosidosis type 2 (*12*).

Cultured fibroblasts from skin biopsies of the affected kittens, their parents, granddam, siblings, and five normal unrelated cats were assayed for β-D-*N*-acetyl hexosaminidase according to the method of Okada *et al.* (*13*) (Fig. 4). Hexosaminidase activity in fibroblast homogenate from the parents was 31 and 51 percent of mean normal activity. Three siblings and the maternal granddam had fibroblast hexosaminidase activity less than 60 percent of normal.

Feline GM_2 gangliosidosis appears to be inherited as an autosomal recessive trait because (i) the parents of the kittens used in our experiments were phenotypically normal, (ii) the parents were consanguineous, (iii) both sexes were affected, (iv) the activity of the pivotal lysosomal hydrolase in tissues from parents was intermediate between unrelated normal and diseased cats, and (v) the ratio of affected to phenotypically normal individuals appeared consistent with that expected for an autosomal recessive inheritance (*14*).

Feline GM_2 gangliosidosis has several features in common with the analogous human disease including (i) progressive nervous system deterioration leading to death, (ii) accumulation of GM_2 ganglioside in brain, (iii) the presence of membrane-bounded inclusions in the nervous system and viscera, (iv) a marked decrease in β-D-*N*-acetyl hexosaminidase activity in brain, liver, and skin fibroblasts, (v) reduction of β-D-*N*-acetyl hexosaminidase activity in skin fibroblasts of parents, a grandparent, and some siblings, and (vi) the apparent autosomal recessive mode of inheritance.

Although a definitive comparison of the feline disease with specific clinical and biomedical subtypes of human ganglioside storage diseases must await a more complete understanding of β-hexosaminidases in tissues from normal and

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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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 ($P > .05$).