the female was always the heterozygous parent (*Rb5Bnr* +/+ *hyt*). It is of interest that of the 141 offspring analyzed, only 48 inherited the *Rb5Bnr* translocation. This is a significant departure from the assumed 1:1 segregation ($\chi^2 \pm 14.4$; d.f. = 1; $P < .005$). In a study involving several mouse Robertsonian translocations, B. M. Cattanach [*Cytogenet. Cell Genet.* **20**, 264 (1978)] noted that he often obtained a deficiency of progeny carrying a metacentric chromosome, especially when the female was the heterozygous parent.

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Gene for Neuraminidase Activity on Mouse Chromosome 17 Near H-2: Pleiotropic Effects on Multiple Hydrolases

Abstract. *The low activity of liver neuraminidase that is characteristic of mouse strain SM/J is inherited as a single gene on chromosome 17, near the major histocompatibility complex. This gene, neuraminidase-1* (Neu-1), *is represented by the low activity allele* Neu-1^s *in SM/J and the high activity allele* Neu-1^b *in C57BL/6J and most other strains. Previously described variations in the posttranslational processing of acid phosphatase, α-mannosidase, arylsulfatase-B, and α-glucosidase are attributed to pleiotropic effects of this gene.*

Much of the total genetic variation affecting the phenotypic expression of mammalian enzyme activity involves some form of posttranslational modification (*1*). Several such genes controlling the sialylation of acid hydrolases have been described and mapped on mouse chromosome 17, near the major histocompatibility (H-2) complex (*2–6*). The presence in SM/J mice of a specific allele for excessive sialylation was implied in each of these studies because differences in the electrophoretic mobilities of the enzymes were neutralized by incubation in vitro with bacterial neuraminidase. In this report we demonstrate that liver neuraminidase deficiency (*7*) is characteristic of mice of strain SM/J and is inherited as a single gene on chromosome 17, near H-2. Genetic recombination with the "processing genes" for liver acid phosphatase (*Apl*) and α-mannosidase (*Map-2*) did not occur in the production of backcross and F_2 generations or in the development of the congenic strain B10.SM(70NS)/Sn (*8*). We propose that neuraminidase deficiency is a primary genetic lesion with pleiotropic effects on the posttranslational processing of these and possibly other acid hydrolases on the basis of (i) the lack of genetic recombination between neuraminidase activity and processing genes, (ii) the prefect correlation among strains of neuraminidase deficiency and hypersialylated hydrolases, and (iii) the observation that incubation in vitro with bacterial neuraminidase equalizes the hydrolases of SM/J and other strains (*2–4*).

We have assayed liver neuraminidase activity in 20 inbred strains, in F_1 and F_2 offspring generated from a cross of SM/J × C57BL/6J, in backcross offspring from (SM/J × C57BL/6J)F_1 × SM/J, and in the congenic strain B10.SM(70NS)/Sn (*8*). F_1, F_2, backcross, and B10.SM(70NS)/Sn animals were also tested for *Apl* and *Map-2* by electrophoretic methods (*3, 6*).

Strain SM/J is unique among the inbred strains tested for a marked deficiency in liver neuraminidase (Table 1). The difference in enzyme activity between SM/J and C57BL/6J segregates as a single Mendelian gene with codominant alleles (Fig. 1). The enzyme activity of (SM/J × C57BL/6J) F_1 animals is intermediate to that of parental strains, whereas the enzyme activities of F_2 animals clearly fall into the three classes expected from segregation of two codominant alleles at a single locus (Fig. 1). When F_1 animals are backcrossed to SM/J, two activity classes are obtained, one intermediate and one low. In both crosses, liver neuraminidase activity segregates independently of sex. These data demonstrate the presence of an autosomal locus controlling liver neuraminidase activity in mice. We have designated the locus neuraminidase-1 (*Neu-1*) with an allele *Neu-1*^{s} responsible for reduced activity in strain SM/J. The high enzyme activity in strain C57BL/6J is attributed to the alternative *Neu-1*^{b} allele. Other strains with high activity have either the *Neu-1*^{b} allele or alleles responsible for comparable levels of neuraminidase activity.

Liver neuraminidase activity in the congenic strain B10.SM(70NS)/Sn is indistinguishable from that of strain SM/J (Table 1). The *Neu-1*^{b} allele has apparently been retained, along with the SM/J haplotype for H-2 on chromosome 17, in the construction of this congenic strain.

These data suggest the location of *Neu-1* on chromosome 17, near the H-2 region. Furthermore, we observed no recombination between *Neu-1*, *Apl*, and

Table 1. Specific activity of liver neuraminidase, expressed as micromoles of 4-methylumbelliferone released per hour per gram of protein, in 20 inbred strains of mice, one F_1 hybrid, and one congenic strain. Two assays were conducted per mouse. The neuraminidase assay methods are described by Potier *et al.* (*7*). The methods used for *Apl* and *Map-2* are from Lalley and Shows (*2*) and Dizik and Elliot (*3*).

Strain	Number of mice tested	Activity	*Apl* allele	*Map-2* allele
MOL/Wo	6	4.2 ± 0.9	b	b
C57BR/cdJ	4	4.1 ± 1.0	b	b
SWR/J	4	4.1 ± 1.1	b	b
KK/Wo	6	4.0 ± 1.1	b	b
CE/J	4	3.8 ± 1.0	b	b
SJL/J	4	3.7 ± 1.0	b	b
C57BL/6J	17	3.5 ± 0.2	b	b
C57L/J	4	3.5 ± 0.8	b	b
DBA/2J	4	3.5 ± 0.9	b	b
SK/Cam	6	3.5 ± 1.1	b	b
A/J	4	3.4 ± 0.9	b	b
C57BL/10J	4	3.4 ± 0.6	b	b
BALB/cJ	4	3.3 ± 0.9	b	b
P/J	4	3.3 ± 0.6	b	b
AKR/J	4	3.2 ± 0.7	b	b
RF/J	4	3.2 ± 0.7	b	b
C3H/HeJ	4	3.2 ± 0.8	b	b
PL/J	4	3.0 ± 0.6	b	b
CBA/J	4	3.0 ± 0.8	b	b
(SM/J × C57BL/6J)F_1	19	1.7 ± 0.3*	ab	ab
B10.SM(70NS)/Sn	4	0.6 ± 0.3†	a	a
SM/J	15	0.6 ± 0.1†	a	a

*Significantly different from all other means at $P < .01$. †Significantly different from all other means at $P < .01$ but not different from each other.

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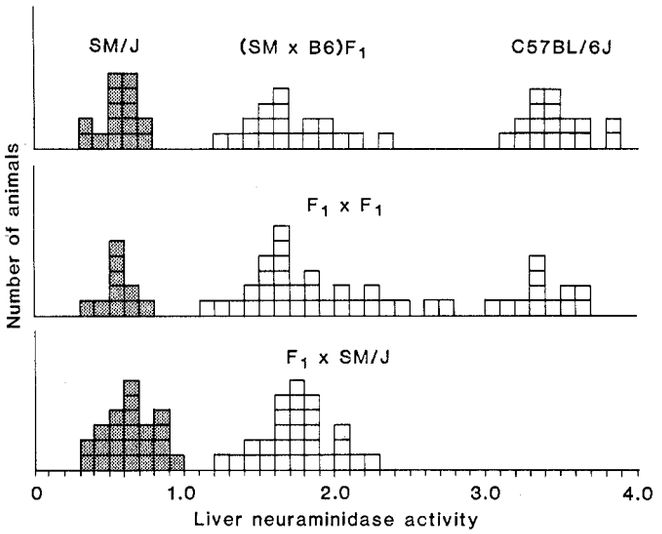

Fig. 1. Activity of liver neuraminidase, expressed as micromoles of 4-methylumbelliferone released per hour per gram of protein, in SM/J, C57BL/6J, F_1 and F_2 mice, and backcross offspring. All animals were also tested for *Apl* and *Map-2*; the hatched squares represent animals that were homozygous for both Apl^a and *Map-2*[a]. There were no recombinants between *Apl* and *Map-2*.

Map-2 in 52 F_2 and 51 backcross offspring (Fig. 1). Every animal with liver neuraminidase activity of less than 1 μmole of 4-methylumbelliferone per hour per gram of protein was also homozygous for the SM/J allele at both *Apl* and *Map-2*. Since *Apl* and *Map-2* are located on chromosome 17 (*3*, *6*), *Neu-1* can be assigned to a position very near or identical to that for *Apl* and *Map-2* on chromosome 17.

Strain SM/J is reported to express unique processing genes on chromosome 17 for at least four liver lysosomal hydrolases (*2*–*5*). The electrophoretic pattern expressed by SM/J liver homogenates in each case was converted to a pattern characteristic of liver homogenates of other mouse strains by incubation of the homogenate with bacterial neuraminidase. It seems unlikely that strain SM/J carries four independent mutations, each unique among mouse strains, and each resulting in excessive sialylation of different lysosomal enzymes. A more plausible hypothesis is that strain SM/J carries a mutation at a single locus with pleiotropic effects on the electrophoretic patterns of several hydrolases. This locus might code for a sialyltransferase or other enzyme conveying a greater capacity for complete sialylation of certain lysosomal enzymes to animals possessing the SM/J allele. Alternatively, SM/J may carry an allele for neuraminidase deficiency rendering specific cell types incapable of complete cleavage of sialic acid residues.

The second hypothesis became testable with the synthesis of 4-methylumbelliferyl-α-D-*N*-acetylneuraminate, an artificial substrate of neuraminidase (*9*). Utilizing this sensitive substrate we have shown that liver homogenate of strain SM/J mice contains only 15 percent of the activity of that of C57BL/6J mice (*7*). A labile component that comprises 82 to 87 percent of the total neuraminidase activity of C57BL/6J mice is absent in SM/J, whereas a stable component (about 15 percent of total activity in C57BL/6J mice) appears to be unaffected in SM/J mice (*7*). Thus, deficiency of the labile component may be the primary lesion responsible for multiple hypersialylated hydrolases.

A model in which neuraminidase deficiency accounts for known genetic variation of four different hydrolases has several requirements: (i) the distribution of neuraminidase deficiency among strains must parallel the strain distribution of the previously described variation, (ii) neuraminidase deficiency must be inherited as a single gene on chromosome 17, and (iii) there should be no genetic recombination between neuraminidase deficiency and the altered electrophoretic mobilities of the lysosomal hydrolases on which it supposedly acts. These conditions appear to be met.

We propose that *Neu-1*, *Apl*, and *Map-2* are one and the same gene. Variation in liver arylsulfatase-B (*4*) and α-glucosidase (*5*) are likely manifestations of this gene as well. This hypothesis can be disproved by demonstrating recombination between variant phenotypes, either in the distribution of alleles among inbred strains (or wild mice), or in the progeny of crosses involving SM/J. Although Daniel *et al.* (*4*) found apparent rare recombinants between *Apl* and arylsulfatase-B patterns, the suspected recombinant animals were not bred for genetic verification. Dizik and Elliot (*3*) found no recombinants between *Map-2* and *Apl* among 88 progeny chromosomes and we found none in this study. In total, there have been 0/149 recombinants between *Map-2* and *Apl*, and 0/61 recombinants between *Map-2*, *Apl*, and *Neu-1*.

The conversion of each of the four hydrolases to "wild-type" by treatment in vitro with neuraminidase (*2*–*4*) implies that *Neu-1* is the site of a primary lesion and the altered electrophoretic profiles of four lysosomal hydrolases are secondary effects. Although the codominant expression of alleles at *Neu-1* is suggestive of a structural gene or a *cis*-acting proximate regulatory locus (*1*), neither would explain the liver-specific expression of differential neuraminidase activity (*7*). It is possible that *Neu-1* is a gene required for the secondary modification of liver neuraminidase and in turn the processing of several acid hydrolases. Needleman *et al.* (*10*) have suggested that neuraminidase cleaves sialic acid residues on several lysosomal enzymes in the lysosome or during transport of hydrolases into lysosomes. Variation in the extent of sialylation in Golgi processing, in the degree of desialylation in lysosomes, or in recognition factors for lysosome packaging as in human I-cell disease (*11*) may explain the allelic differences at *Neu-1*.

Interest in mammalian neuraminidases has increased with the discovery that a number of inherited human diseases, including mucolipidoses I, II, and III (*12*) and cherry-red spot myoclonus syndrome with or without dementia (*13*), are associated with neuraminidase deficiency. It is unclear how the biochemical peculiarities of healthy SM/J mice relate to these diseases. However, the *Neu-1* mutation will probably be useful for defining the role of neuraminidase in enzyme processing and for experimental enzyme replacement therapy.

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Malignant Potential of Murine Stromal Cells After Transplantation of Human Tumors into Nude Mice

Abstract. *Human malignant cancer tumors grafted into nude mice produce tumors containing both human cancer cells and the host's stromal cells. After short-term propagation of these tumors in vitro, the murine mesenchymal cells appear transformed and are tumorigenic in nude mice. However, established human cancer cell lines fail to similarly alter adjacent murine stromal cells when used to produce tumors in nude mice. These experiments suggest that cancer cells may recruit normal cells to become malignant, qualifying the view of the clonal (unicellular) origin of cancer.*

The development of a malignant tumor is generally attributed to the selection and growth of a single clone originating in one or more mutational events (*1*). The heterogeneity of cells in a tumor can be explained by the subsequent selection of mutated variants, especially among a karyologically unstable population of cells (*1*). This heterogeneity is particularly pronounced in tumors composed of histogenetically mixed cell types, which have been recorded since the beginnings of histopathology (*2*).

Another explanation for the occurrence of mixed cell types and different cell populations in a tumor is the horizontal induction of malignancy in differentiated normal host cells by adjacent malignant cells, a process that may be mediated by oncogenic viruses, cell products, cell fusion, or as yet unknown mechanisms. This would imply that neoplasia is a continuing process whereby cancer cells transfer the malignant genotype to nonmalignant cells. The experiments described here support this hypothesis. They involve the transplantation of human tumors into nude mice, the cultivation of these tumors in vitro, the separation of murine stromal cells showing evidence of transformation, and the production of murine fibrosarcomas by the stromal cells (*3*).

A freshly excised adenocarcinoma of the colon of a 53-year-old man was minced and suspended in 0.9 percent sterile NaCl (50 percent tumor cell suspension, weight to volume) containing penicillin (200 U/ml) and streptomycin (50 μg/ml). One milliliter of this suspension was injected subcutaneously into the flanks of each of two nude mice (*nu/nu*-BALB/c). There were tumors in both animals after about 2 weeks, and after 4 weeks a tumor measuring 10 by 23 mm was excised from one mouse and used for tissue culture, histological evaluation, and retransplantation into three other mice. The microscopic appearance of the tumor grafts was similar to that of the original tumor (Fig. 1, a to c). The portion used for in vitro propagation was washed in Hanks balanced salt solution (Gibco) containing streptomycin and penicillin at five times the previous concentration. The minced tumor was incubated with 0.25 percent trypsin (Gibco) for 1.5 hours and the resulting cell suspension was cultured in a 75-cm^2 plastic flask containing Liebovitz L-15 medium and 15 percent fetal bovine serum, penicillin (200 U/ml), and streptomycin (50 μg/ml). Twenty-four hours later, the unattached cells were poured off, centrifuged, and cultured in a second 75-cm^2 flask. After 3 weeks colonies of epithelioid tumor cells were observed surrounded by transformed fibroblastoid cells showing loss of the inhibition that normal cells have upon contact in monolayer culture (Fig. 1d).

The second-passage transplants grew in all three mice. After 2 weeks a tumor measuring 16 by 15 mm (Fig. 1c) was excised for propagation in vitro and in vivo. After 24 hours the unattached cells in culture were removed and subcultured in another flask until confluence was achieved (2 weeks) (Fig. 1e). The fibroblastoid cells demonstrated an ability to detach and grow in suspension (anchorage independence), could be subcultured in continuous passage, and had a murine karyotype and a modal chromosome number of 77 (Fig. 2). Subsequent inoculation of nude mice with 1.0×10^7 cells resulted in fibrosarcomas (Fig. 1f), confirming the tumorigenic potential of the

Table 1. Findings for nude mouse passages of the GW-985 human colon adenocarcinoma in vitro; N.A., not available.

Number of passages	Earliest transformation in vitro (weeks)	Morphology		Modal chromosome number	
		Epithelioid	Fibroblastoid	Human	Murine
1	3	Yes	Yes	N.A.	77
2	2	Yes	Yes	N.A.	66; 69
5	N.A.	Yes	No	50	N.A.
17	10	Yes	Yes	N.A.	76
19	8	Yes	Yes	N.A.	N.A.
20	5	Yes	Yes	N.A.	N.A.

Table 2. Results of in vitro cultivation of human tumors that were transplanted into nude mice. The number of passages in vivo is given by the number in parentheses; N.A., not available.

Tumor number	Locus of adenocarcinoma	Earliest in vivo passage explanted (weeks)	Earliest appearance of transformation in vitro (weeks)	Modal chromosome number of tumor explants	
				Human	Murine
GW-869	Colon	6	2	50 (12)	76 (10)
GW-870	Ovary	5	<1	N.A.	60 to 70 (18)
GW-875	Colon	5	<1	85; > 100 (1)	70 to 74 (7)
GW-927	Ovary	1	3	N.A.	67 (1)
GW-985	Colon	1	3	50 (5)	77 (1)

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