Church, in *Autoxidation in Food and Biological Systems*, M. G. Mimic and M. Karel, Eds. (Plenum, New York, in press).
12. Z. M. Iqbal, K. Dahl, S. S. Epstein, *Science* **207**, 1475 (1980).
13. N. J. Parks and K. A. Krohn, *Int. J. Appl. Radiat. Isot.* **29**, 754 (1978).
14. M. W. McElfresh, J. C. Meeks, N. J. Parks, *J. Radioanal. Chem.* **53**, 337 (1979).
15. The concentration of ^{13}N from the target was ~ 10 mCi/ml as NO_3^- and variable amounts of NO_2^- and ammonium (NH_4^+) radiochemical impurities at the end of bombardment. Only radiochemical preparations with > 97 percent $^{13}NO_3^-$ were used for animal experiments. We used a rapid concentration and purification technique with high-performance anion exchange (J. H. Chasko and J. R. Thayer, in preparation) to remove any ammonia (NH_3) formed in the reduction procedure. The technique resulted in solutions with ^{13}N concentration > 150 mCi/ml and a radiochemical purity of > 99 percent.
16. K. A. Krohn and C. A. Mathis, in *Short-Lived Radionuclides in Chemistry and Biology*, K. A. Krohn and J. W. Root, Eds. (American Chemical Society, Washington, D.C., in press).
17. C. Sedgwick and S. Jahn, *Calif. Vet.* **34**, 27 (1980).
18. The time dependence of the distribution data was analyzed with a simple linear least-squares regression model: percent $A(t) = mt + b$, where percent $A(t)$ is the percentage of administered dose in each organ at time t, and m and b are the calculated slope and intercept, respectively. With one exception (IT lung), the slopes for all the various combinations of organs and tracer ions were not significantly different from zero at the 5 percent level in both the IT and IV experiments. For both anions, the IT lung data gave a coefficient of determination indicating that only ~ 18 percent of the dispersion could be associated with time.
19. A. C. Guyton, *Textbook of Medical Physiology* (Saunders, Philadelphia, ed. 4, 1971), p. 380.
20. H. P. Misra and I. Fridovich, *J. Biol. Chem.* **247**, 6960 (1972).
21. G. Cohen, M. Martinez, P. Hochstein, *Biochemistry* **3**, 901 (1964).
22. B. Chance, *J. Biol. Chem.* **182**, 649 (1950).
23. F. L. Rodkey, *Clin. Chem.* (*N.Y.*) **22**, 1986 (1976); H. Kosaka, K. Imaizumi, K. Imai, I. Tyuma, *Biochim. Biophys. Acta* **581**, 184 (1979).
24. We are addressing this question with ^{13}N tracer techniques similar to those described earlier but with logarithmically increasing amounts of added carrier.
25. Research supported by the California Air Resources Board, the National Science Foundation, and the U.S. Department of Energy. We thank G. Vial, M. Al-Bayati, J. C. Meeks, J. R. Thayer, E. Profita, and L. A. Swartz for experimental assistance and H. P. Misra, W. D. Brown, L. S. Rosenblatt, and G. F. Russell for reviewing the manuscript.

24 November 1980

Inherited Primary Hypothyroidism in Mice

Abstract. *A new autosomal recessive mutation that causes hypothyroidism has been identified in mice. The gene, herein named hypothyroid* (hyt), *has been mapped on chromosome 12 approximately 30 units from the centromere. The mutants are characterized by retarded growth, infertility, mild anemia, elevated serum cholesterol, very low to undetectable serum thyroxine, and elevated serum thyroid-stimulating hormone. Thyroid glands are in the normal location but are reduced in size and hypoplastic. Mutant mice respond to thyroid hormone therapy by improved growth and fertility. These findings suggest that the* hyt *mutant gene results in primary hypothyroidism unresponsive to thyroid-stimulating hormone.*

Congenital hypothyroidism is a recognized cause of retarded somatic and neurological development in mammals. Among human beings, this glandular malfunction occurs once in every 3684 births (*1*). Approximately two-thirds of affected infants have primary hypothyroidism, indicated by findings of depressed serum thyroxine (T_4), elevated serum thyroid-stimulating hormone (TSH), and hypoplastic or, more rarely, ectopic thyroid glands (*2*). The resulting cretinism can be prevented only by immediate hormone replacement therapy. Although the explanation for the triad of low serum T_4, elevated serum TSH, and hypoplastic thyroids is not conclusively established, three reports provide important clues to one possible mechanism: Stanbury *et al.* (*3*) reported on an 8-year-old boy with congenital hypothyroidism in the absence of goiter, who was unresponsive to exogenous TSH; Medeiros-Neto *et al.* (*4*) described a 19-year-old male with identical symptoms and showed that neither thyroidal radioiodine uptake nor function of adenosine 3′, 5′-monophosphate increased with exogenously administered TSH; Codaccioni *et al.* (*5*) discovered similar features in a 17-year-old male and showed that TSH receptors were present, but activation of thyroid adenylate cyclase was deficient. These observations support the idea that thyroid hypoplasia and hypofunction in some patients may result from failure of thyrofollicular cells to respond to endogenous TSH. That such a situation may arise from a genetic event has been suggested in reports of nongoitrogenic hypothyroidism in siblings or in parents and their offspring (*6*). We now report a new recessive mutation in laboratory mice (named hypothyroid, gene symbol *hyt*) that causes hypothyroidism in the absence of goiter.

The mutant phenotype was first observed in a sib-mated pair of RF/J mice (Animal Resources colonies, the Jackson Laboratory) when the female failed to grow or reproduce. A small research colony was established by mating the normal male parent to normal RF/J females, followed by backcrossing female offspring to the sire and by sibling matings of the original pair. In addition, we began the transfer of the mutant gene by outcrossing to BALB/cByJ mice to circumvent the poor reproductive performance of RF/J mice and to test the effects of the mutant gene on a heterogeneous background.

The hypothyroid phenotype was due to an autosomal recessive mutation, because the mating of a normal individual to an affected female or male whose fertility had been restored (see below) resulted in all normal young and the intercrossing of these young produced approximately 25 percent affected individuals (1047 normal and 325 mutant). Positive genetic linkage was found with the metacentric Robertsonian chromosomal translocation *Rb(8.12)5Bnr* (hereafter *Rb5Bnr*). First filial generation (*Rb5Bnr* +/+ *hyt*) females were mated to *hyt*/*hyt* males, and the resultant offspring were classified visually for the *hyt* phenotype and chromosomally for the *Rb5Bnr* translocation (*7*). Of 141 offspring, 102 were of a parental phenotype (31, *Rb5Bnr* +; 71, + *hyt*) and 39 were of a crossover phenotype (17, *Rb5Bnr hyt*; 22, + +). Therefore *hyt* is linked to the *Rb5Bnr* translocation and carried at the proximal end of chromosome 8 or 12 at a distance of 27.7 ± 3.8 map units from a centromere (*8*). Since *hyt* did not show linkage to the *Os* gene located on chromosome 8 approximately 25 map units from its centromere, we conclude that *hyt* is located on chromosome 12.

Retarded growth was consistently observed in RF/J and BALB/cByJ-N1F2 outcross mutant mice at weaning (3 weeks of age). This deficiency became more pronounced during the rapid growth rate of the pubertal period. Weekly body weight data obtained on 18 BALB/cByJ-N1F2 litters, toe-clipped at birth for later genotype identification, revealed that *hyt*/*hyt* mice weighed significantly less than their normal littermates by 22 days of age (Fig. 1). A study of *hyt*/*hyt* mice and their normal littermates was conducted to determine whether a diet supplemented with desiccated thyroid powder could initiate growth and restore fertility, as has been demonstrated with Snell's dwarf (*dw*/*dw*) mice (*9*). Weekly body weight data obtained from weanling N1F2-*hyt*/*hyt* and +/− littermate mice fed either 0.025 percent desiccated thryoid powder or control diet (Fig. 1) showed that (i) thyroid replacement induced rapid catch-up growth in *hyt*/*hyt* mice, (ii) *hyt*/*hyt* mice displayed a very low rate of growth without thyroid therapy, and (iii) normal mice were not affected by the treatment. After 4 to 5 weeks of treatment with desiccated thyroid, *hyt*/*hyt* mice were phenotypically similar to +/− controls,

0036-8075/81/0403-0061$00.50/0

although not quite as large. Furthermore, thyroid replacement restored fertility in both male and female *hyt/hyt* mice.

The efficacy of the thyroid replacement therapy suggested that the mutant gene was involved with thyroid function. Gross inspection of the *hyt/hyt* thyroid glands in situ revealed a very small bilobed gland normally situated adjacent to the caudal border of the cricoid cartilage and beside the first and second tracheal rings. The relative reduction in the *hyt/hyt* thyroid gland mass became evident when thyroid glands from adult mutant and normal littermate pairs of both inbred and heterogeneous backgrounds (four or five pairs in each group) were weighed (RF/J: *hyt/hyt*, 0.67 ± 0.17 mg and +/−, 2.48 ± 0.09 mg; N1F2: *hyt/hyt*, 1.78 ± 0.17 mg and +/−, 4.70 ± 0.49 mg). Although the hybrid vigor of N1F2 mice is reflected in the increased mass of thyroid tissue, the reduced mass of *hyt/hyt* thyroid as compared with +/− thyroid is obvious. When histological sections of normal and mutant thyroid tissue were examined (Fig. 2), the hypoplastic nature of the *hyt/hyt* gland became even more apparent. The tissue was clearly recognizable as thyroid, but there were often regions deep in the gland that were not organized into follicles. The follicles that were present were smaller and fewer in number than those in the normal thyroid gland. Thyrofollicular cells were flat and contained very little cytoplasm. Colloid was present but lightly stained. Lymphocyte invasion and proliferation were not observed in *hyt/hyt* thyroids.

Additional evidence of hypothyroidism in these mutant mice was found in blood measurements (*10*). Mild anemia and elevated serum cholesterol are characteristic of thyroid insufficiency within the clinical or experimental setting. Hematocrit values were significantly lower (*hyt/hyt*, 45.6 ± 0.7 percent; +/−, 51.8 ± 0.9 percent) and serum cholesterol levels were significantly higher (*hyt/hyt*, 1.86 ± 0.10 mg/ml; +/−, 1.40 ± 0.08 mg/ml) in five adult RF/J mutants than the corresponding values in normal littermate controls. When serums from mutant mice were assayed for T_4 levels, the hormone was undetectable (less than 2.0 μg/dl), whereas in normal littermates, the hormone levels were 5.8 ± 0.5 μg/dl. Virtually identical data were recorded for N1F2 mutant and normal mice.

Hypothyroidism can arise from (i) disturbed follicular cell processes responsible for synthesis and release of thyroid hormones (primary hypothyroidism), (ii) pituitary TSH deficiency or molecular abnormality (secondary hypothyroidism), or (iii) hypothalamic thyrotropin-releasing hormone deficiency or abnormality (tertiary hypothyroidism). We believe that the *hyt/hyt* mutant mouse most clearly fits within the classification of primary hypothyroidism. With respect to morphology, classic primary hypothyroidism is often typified by TSH-induced goiter; however, it is also possible for this disorder to result from a hypoplastic thyroid, unresponsive to TSH (*3–5*). The *hyt/hyt* thyroid is of the hypoplastic type and is similar to thyroids from *dw/dw* mice (*9*), a genetic mutant with known secondary hypothyroidism. With respect to function, *dw/dw* mice are genetically deficient in TSH and have thyroids capable of responding to exogenous TSH treatment with iodine uptake (*11*), whereas *hyt/hyt* mice have tenfold or greater elevation in serum TSH and have thyroids incapable of responding to exogenous TSH treatment with iodine uptake (*12*). Thus, morphological and functional features of thyroids in *hyt/hyt* mice are indicative of primary hypothyroidism resulting from failure to respond to TSH stimulation.

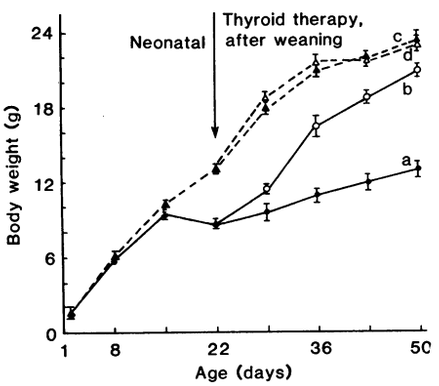

Fig. 1. Growth of female hypothyroid (*hyt/hyt*) and normal +/− (+/+ or +/*hyt*) littermate mice during the neonatal and postweaning period when they were fed desiccated thyroid powder. Males exhibited growth patterns identical to those in females. Weekly body weights were obtained throughout the experiment. At weaning, the mice in each litter were distributed into two groups by genotype; one group received the control diet (curve a: *hyt/hyt*, $N = 6$; curve c: +/−, $N = 17$) while the other received the diet supplemented with desiccated thyroid powder (curve b: *hyt/hyt*, $N = 9$; curve d: +/−, $N = 21$). Powdered diet (Old Guilford diet 96, Emory Morse, Guilford, Conn.) was supplemented each week with 25 mg of desiccated thyroid powder (Sigma Chemical, T-1251) per kilogram of diet. Each value represents the mean body weights (± standard error) in grams. The *hyt/hyt* mice weighed significantly less than the corresponding +/− controls at 22 days. The mutant mice fed the thyroid-supplemented diet weighed significantly more than the mutant mice fed the control diet after 1 week (*t*-test at $P < .01$).

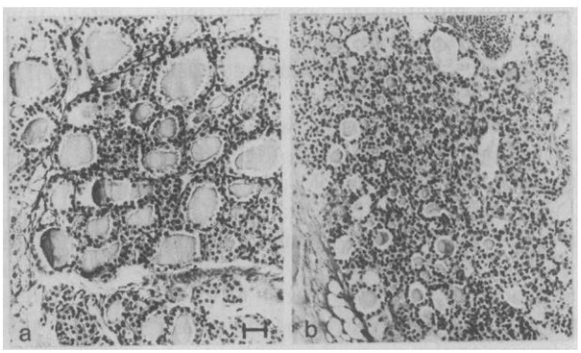

Fig. 2. Photomicrographs of sections from thyroid glands of normal (+/−) and hypothyroid (*hyt/hyt*) mice (×125). Thyroid glands of 8-week-old RF/J mice were fixed in Bouin's solution, embedded in paraffin, sectioned at 8 μm, and stained with hematoxylin and eosin. (a) Normal thyroid showed typical follicular structure, ample colloid within follicles, and thyrofollicular cells with abundant cytoplasm. Only a moderate portion of the section from the normal thyroid gland could be presented for comparison with the section from the thyroid of the *hyt/hyt* mouse (scale bar, 500 μm). (b) Mutant thyroid gland at same magnification showed small sparse follicles, areas not clearly organized into follicles, and thyrofollicular cells poorly endowed with cytoplasm.

WESLEY G. BEAMER
EVA M. EICHER
LOIS J. MALTAIS
JANICE L. SOUTHARD

Jackson Laboratory, Bar Harbor, Maine 04609

References and Notes

1. D. A. Fisher *et al.*, *J. Pediatr.* **94**, 700 (1979).
2. S. H. Ingbar and K. A. Woeber, in *Textbook of Endocrinology*, R. H. Williams, Ed. (Saunders, Philadelphia, 1974), p. 95.
3. J. B. Stanbury, P. Rocmans, U. K. Buhler, Y. Ochi, *N. Engl. J. Med.* **279**, 1132 (1968).
4. G. A. Medeiros-Neto, M. Knobel, M. D. Bronstein, J. Simonetti, F. F. Filho, E. Matlar, *Acta Endocrinol. (Copenhagen)* **92**, 62 (1979).
5. J. L. Codaccioni, P. Carayon, M. Michel-Bechet, F. Foucault, G. Lefort, H. Pierron, *J. Clin. Endocrinol. Metab.* **50**, 932 (1980).
6. W. R. Greig, A. S. Henderson, J. A. Boyle, E. M. McGirr, J. H. Hutchison, *J. Clin. Endocrinol.* **26**, 1309 (1966); H. E. Kulin, P. O. Kohler, B. W. O'Malley, W. D. Odell, *J. Pediatr.* **17**, 714 (1967); H. E. Cross, C. S. Hollander, D. L. Rimoin, V. A. McKusick, *Pediatrics* **41**, 413 (1968); E. Orti, S. Castells, G. H. Qazi, *J. Pediatr.* **78**, 675 (1971); J. M. Jackson, *Clin. Perinatol.* **3**, 221 (1976); M. Kaplan, R. Kauli, U. Raviv, E. Lubi, Z. Laron, *Am. J. Dis. Child.* **131**, 1264 (1977).
7. Chromosome preparations were made from bone marrow according to E. M. Eicher and L. L. Washburn [*Proc. Natl. Acad. Sci. U.S.A.* **75**, 946 (1980)].
8. In the matings segregating for *Rb5Bnr* and *hyt*,

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.

9. A. Bartke, *Gen. Comp. Endocrinol.* **5**, 418 (1965).
10. Serum thyroxine levels were measured with radioimmunoassay kits obtained from Radioassay Systems Laboratories, Inc., Carson, Calif. 90746. Serum cholesterol levels were measured by the method of A. Zlatkis, B. Zak, and A. J. Boyle [*Clin. Med.* **41**, 486 (1953)].
11. O. Wegelius, *Proc. Soc. Exp. Biol. Med.* **101**, 225 (1959).
12. W. G. Beamer and L. J. Maltais, in preparation.
13. We thank R. Coombs for discovery of the original mutant mouse and L. Washburn for analyses of chromosome preparations. Supported by NIH grants AM-17947, RR-01138, NS-09378, and GM-20919.

27 October 1980; revised 16 December 1980

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.

0036-8075/81/0403-0063$00.50/0