to males of the same kind and to *I. amiskwiensis*.

From these tests it is clear that specificity of sex pheromones tends to prevent natural hybridization between *I. amiskwiensis* and *I. borealis* or *I. pilifrons*. Comparisons of laboratory and field tests with *I. amiskwiensis* suggest that specificity is greatest under field conditions and that greater discrimination is exercised in entering nuptial chambers (log tests) than in flying to the attractant (trap tests). However, natural hybrids which might occur could readily breed among themselves, or assimilate with either parent species.

Pheromones and pheromone receptors of hybrids theoretically could be new, the same as those of the parent species (either singly or in combination), or a combination of new and parent types. Receptors of pheromones in some insects have been shown to be quite specific; even isomers of the same compound often fail to evoke equivalent response (*9*). It is therefore unlikely that the genetic condition of hybrids results in new receptors which are spontaneously keyed to new pheromones. Rather, the parent pheromones and receptors are probably present in proportions similar to the degree of heterosis. If the olfactory response is a function of the number of individual receptors stimulated (*10*), the F_1 *a-p* females should be expected to respond highest to F_1 *a-p* males because the mixed pheromones of those males would excite the maximum number of the mixed receptors of the females. The preference of backcross females for the pheromone of the pure (backcross) species would also be predicted (*11*). Lack of discrimination by *I. amiskwiensis* between pheromones produced by male *p* (*a-p*) and *a* (*a-p*) and the partial breakdown in response specificity of this species in the presence of the F_1 *a-p* pheromone may be associated phenomena—the cause of which could come to light when the chemistry of the component pheromones is known.

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1. G. R. Hopping, *Can. Entomol.* **97**, 159 (1965); *ibid.*, p. 193.
2. Based on recent collections by the author.
3. The male *Ips* selects the host tree, initiates the attack, and constructs a nuptial chamber in the phloem-cambium region. In so doing it discharges a pheromone which attracts both sexes, stimulates mass attack on the host selected, and induces females to enter the nuptial chambers and mate [R. F. Anderson, *J. Econ. Entomol.* **41**, 596 (1948); D. L. Wood and J. P. Vité, *Contrib. Boyce Thompson Inst.* **21**, 79 (1961)]. Cross attractiveness of sex pheromones has been demonstrated for several closely related allopatric bark beetles [J. P. Vité, R. I. Gara, H. D. von Scheller, *ibid.* **22**, 461 (1964); D. L. Wood and G. N. Lanier, unpublished data]. However, sympatric species are generally not cross attractive [R. C. Wilkinson, *Fla. Entomol.* **47**, 57 (1964); J. P. Vité, *Naturwissenschaften* **52**, 267 (1965)].
4. *Picea engelmannii*, 40 cm long and 10 to 20 cm in diameter.
5. Crown Zellerbach Ltd.
6. J. A. Chapman, *Can. Entomol.* **98**, 50 (1966); ——— and J. M. Kinghorn, *ibid.* **87**, 46 (1955).
7. Attraction index of *b* to *a*, for example, is calculated as follows: *b* per *a* nuptial chamber divided by *a* per *a* nuptial chamber times 100.
8. *I. amiskwiensis, a; I. pilifrons, p; I. borealis, b*; the progeny resulting from backcross (B_1) of *I. amiskwiensis–I. pilifrons* hybrids to *I. pilifrons* are designated *p* (*a-p*).
9. M. Jacobson, *Insect Sex Attractants* (Interscience, New York, 1967).
10. D. Schneider, *J. Insect Physiol.* **8**, 15 (1962).
11. The simplest genetic situation possible is single loci for pheromone and pheromone receptor type. Thus, the genotype for receptors of the F_1 *amiskwiensis-pilifrons* would be *ap* and those of the backcross to *pilifrons* would be ½ *ap* and ½ *pp*. The response of backcross females with the *ap* genotype is expected to be similar to that of the F_1. Those with the *pp* genotype should respond in a manner similar to *I. pilifrons*. If the response levels of the two genotypes predicted by the attraction indices in Table 1 are summed, it is clear that the *I. pilifrons* pheromone will provide the greatest aggregate attraction to backcross females. If more than one locus is responsible for determining receptor type, this preference of the B_1 to the backcross species should be accentuated. These approximations could be further complicated if more than one compound differs in the respective pheromones. The sex pheromone of *Ips confusus* (LeConte) consists of three compounds which act synergistically [R. M. Silverstein, J. O. Rodin, D. L. Wood, *Science* **154**, 509 (1966); D. L. Wood, R. W. Stark, R. M. Silverstein, J. O. Rodin, *Nature* **215**, 206 (1967)]. *Ips latidens* (LeConte), a primitive species in my judgment, was attracted to a combination of two of these compounds, but response was inhibited by addition of the third compound [D. L. Wood *et al.*, cited above].
12. I thank Dr. J. A. Chapman for review of the manuscript and G. A. Shofer for technical assistance.

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Scheie and Hurler Syndromes: Apparent Identity of the Biochemical Defect

Abstract. *Fibroblasts cultured from the skin of Scheie and Hurler patients are deficient in the same specific factor required for normal mucopolysaccharide metabolism.*

The Scheie syndrome, a rare genetic disorder of mucopolysaccharide (MPS) metabolism, resembles in some ways the better-known Hurler syndrome (*1, 2, 3*). Both diseases are characterized by increased amounts of two MPS's, chondroitin sulfate B and heparitin sulfate, in the urine (*4*). The patient's corneas are cloudy, and there are pathological changes of the skin and cardiac valves. However, the two disorders vary greatly in severity. Hurler patients have stunted growth and mental capacity and severe skeletal deformities; they rarely survive beyond age 10. By contrast, individuals affected with the Scheie syndrome have normal stature and lifespan, skeletal problems in the extremities only, and normal or superior intelligence.

A technique developed in this laboratory has made it possible to examine the biochemical relationships between the Hurler syndrome and other mucopolysaccharidoses. Because of an impairment in the mechanism of degradation of MPS, fibroblasts cultured from the skin of Hurler patients accumulate excessive amounts of radioactive chondroitin sulfate B when supplied with $^{35}SO_4^{2-}$ (*5*). Their characteristic patterns of ^{35}MPS accumulation and turnover can be converted to normal, however, if they are mixed with fibroblasts of genotype other than Hurler, or supplied with secretions of such fibroblasts (*6, 7*). The corrective "factor" in these preparations is a heat-labile macromolecular substance, probably a protein. Among the fibroblasts tested, those found to correct the defect of Hurler fibroblasts include cells derived from normal individuals, from patients with two closely related mucopolysaccharidoses (the Hunter and Sanfilippo syndromes), and from patients with several other genetic diseases. On the other hand, fibroblasts derived from Hurler patients do not correct each others' abnormal patterns. Thus, absence of a factor required for normal MPS metabolism is characteristic of Hurler cells and is the most specific defect reported so far in that disorder (*8*). Cells of the Hunter genotype likewise lack a specific factor, different from that in which Hurler cells are deficient (*7, 9*); lack of yet two other genotype-specific factors occurs in the Sanfilippo syndrome (*10*).

Fibroblasts derived from Scheie individuals resemble those from Hurler patients in that they do not secrete the factor required to correct the defect in

Hurler fibroblasts. In addition, the defect in Scheie fibroblasts is not corrected by secretions of Hurler fibroblasts, although it is correctable by secretions from fibroblasts derived from normal individuals or from patients with other disorders. Thus, in spite of the clinical differences between Scheie and Hurler patients, their fibroblasts share a deficiency of the same corrective factor.

The two Scheie patients from whom biopsies were obtained for this study are a brother and sister described by Scheie [cases 1 and 2 in (*1*)], and by McKusick [cases 11 and 12 in (*2*)]; analysis of their urinary MPS was performed by Kaplan [cases 40 and 41 in (*4*)]. They are now 39 and 37 years old, respectively. Fibroblasts derived from the biopsies were maintained in culture as described (*5*, *7*). They are markedly metachromatic when stained with toluidine blue O, in agreement with the report of Danes and Bearn (*11*). When supplied with radiosulfate, the Scheie fibroblasts accumulate intracellular radioactive MPS in great excess over the normal and turn it over relatively slowly, in patterns resembling closely those published for a line of Hurler fibroblasts (*5*). The half-life of the pool of radioactive MPS, calculated from a pulse-chase experiment, is 4 days for Scheie fibroblasts; by contrast, most of the ^{35}MPS pool of normal cells has a half-life of only 8 hours, and a minor component has a half-life of 3 to 4 days. The ^{35}MPS accumulated by Scheie cells is chondroitin sulfate B, as determined by electrophoresis in $ZnSO_4$ (*12*); this is the MPS accumulated by fibroblasts from patients with the Hurler syndrome as well as other MPS-storage disorders (*13*).

Table 1. Effect of factors prepared from Scheie medium on Hurler, Hunter, and Sanfilippo fibroblasts. Cells were incubated for 2 days in 5 ml of medium, containing 5×10^6 count/min ml^{-1} $^{35}SO_4^{2-}$, mixed with 1 ml of factor prepared from medium that had been in contact with cells of the type indicated.

Genotype of cells	Source of factor	MPS accumulation (count/min mg^{-1} protein)
Hurler	Hurler	41,900
	Scheie	40,600
Hunter	Hunter	48,100
	Scheie	19,400
Sanfilippo	Sanfilippo	34,100
	Scheie	16,500

Cross-correction by cells of different genotypes may be demonstrated in two ways. One is by mixing the fibroblasts from two individuals before performing labeling experiments; correction occurs by transfer of factors through the medium, and perhaps also by direct transfer from cell to cell (Fig. 1).

Accumulation of radioactive MPS by a mixture of Scheie and Hunter fibroblasts is markedly lower than by either one alone. By contrast, accumulation of a mixture of Scheie and Hurler cells is the precise average of the accumulation by the two lines separately, as is the accumulation of a mixture of the two Scheie lines. Whereas the data plotted in the left and middle panels are from experiments performed with fibroblasts derived from the brother (designated Scheie 1), results obtained with his sister's cells (Scheie 2) were essentially identical.

Alternatively, correction may be studied by supplying the mutant cells with secretions from other cell lines. These are concentrated by precipitation of previously incubated medium with ammonium sulfate, followed by extensive dialysis of the precipitated proteins, as described (*6*). Similar concentrates of fresh medium are used for control. As shown in Fig. 2, preparations of the factor from medium previously incubated with fibroblasts of normal individuals and of patients with cystic fibrosis or the Hunter and Sanfilippo syndromes decreased the accumulation of radioactive MPS by Scheie fibroblasts, whereas preparations from medium previously incubated with Hurler cells did not; nor did concentrates of medium incubated beforehand with fibroblasts of the same individual (Scheie 1) (*14*). Preparations from medium that had been in contact with his sister's fibroblasts (Scheie 2) gave slight lowering suggestive of some residual activity of the factor but not sufficient to exclude experimental error.

In converse experiments, preparations from medium incubated previously with Scheie fibroblasts reduced accumulation in Hunter and Sanfilippo fibroblasts, but not in Hurler fibroblasts (Table 1).

Deficiency of an essential enzyme does not necessarily result in the full range of clinical problems (*15*). Where clinical variability exists, careful measurements have usually shown some residual activity of the enzyme in the less severely affected individuals (*16*).

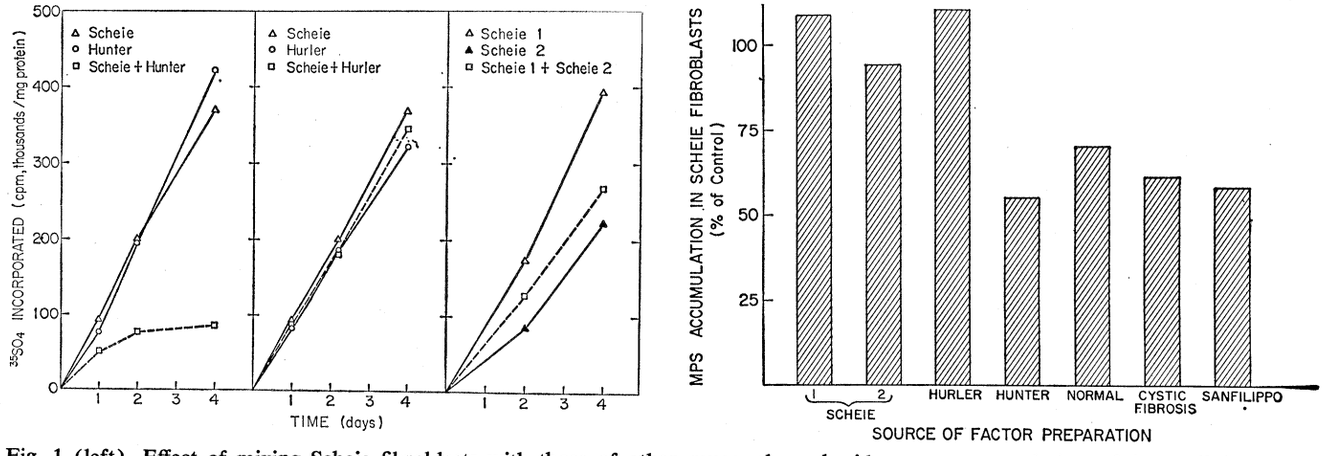

Fig. 1 (left). Effect of mixing Scheie fibroblasts with those of other mucopolysaccharidoses on accumulation of intracellular MPS. The cells were plated singly or in the specified combinations 5 days before administration of labeled medium (8 ml, containing 14×10^6 count/min ml^{-1} of $^{35}SO_4^{2-}$). Fig. 2 (right). Effect of factor preparations from different sources on intracellular MPS accumulation by fibroblasts from patients with Scheie syndrome. Factors were prepared by precipitation with ammonium sulfate of medium that had been in contact with cells of the indicated genotype for 3 days. Accumulation was measured after 2 days of incubation in 5 ml of medium, containing 15×10^6 count/min ml^{-1}, mixed with 1 ml of factor concentrate.

Whether such is also the case in the Scheie syndrome could not be determined, for precise determination of low activity of the factor is difficult. Alternatively, the Scheie individuals may have compensatory mechanisms which prevent accumulation of MPS in certain tissues in spite of the absence of factor. Or the loss of activity of the factor may be of late onset, after some critical stage in growth and mental development has been passed. Discrimination between these and yet other possibilities must await studies of the structure and function of the normal factor and of its presumably altered counterparts in the Scheie and Hurler syndromes.

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13. R. Matalon and A. Dorfman, *Lancet* **1969-II**, 838 (1969).
14. The extent of correction by factor preparations is considerably less than that obtained by cell mixing. We attribute this difference to the relative inefficiency of a single administration, compared to the constant supply when cells are mixed with each other, as well as to some loss of factor activity during preparation.
15. A. G. Knudson, Jr., *Annu. Rev. Genet.* **3**, 1 (1969).
16. Phenotypic variations have been correlated with varying degrees of deficiency of the following enzymes: phenylalanine hydroxylase (phenylketonuria), branched amino acid decarboxylase (maple syrup urine disease), glucocerebrosidase (Gaucher's disease), sphingomyelinase (Niemann-Pick disease), glucose-6-phosphate dehydrogenase and hypoxanthine-guanine phosphoribosyl transferase (*14*).
17. We thank Dr. V. McKusick for biopsies of the Scheie patients and Dr. J. Derge for identification of the radioactive MPS.
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Absence of Cystathionase in Human Fetal Liver: Is Cystine Essential?

Abstract. *Cystathionase activity is not measurable in the livers of 24 human fetuses and 3 premature infants, and the concentration of cystathionine in the liver is higher than that of the brain. The placenta does not subserve the transsulfuration function. Cystine (or cysteine) thus may be an essential amino acid in the immature human.*

In the normal adult human, about 90 percent of ingested methionine is converted to cystine or cysteine (*1*), a nonessential amino acid, via the transsulfuration pathway (Fig. 1). Cystathionine, a key intermediate on this pathway, is normally present in high concentrations in human brain (*2*), is virtually absent from the brain of patients with homocystinuria due to a deficiency of cystathionine synthase activity (*3*), and is present in barely measurable quantities in human liver (*3*).

We have examined the development of the transsulfuration pathway in the human, including in this study: 24 fetuses (2.5 to 20.5 cm, crown-rump length), obtained at therapeutic abortions; 3 premature infants (birth weights of 830, 1060, and 1260 g), who died during the first 24 hours of life; and 1 full-term newborn, who died after 4 hours of life because of an intraabdominal hemorrhage secondary to a catheter accident.

The activities of methionine-activating enzyme (ATP : L-methionine *S*-adenosyltransferase, E.C. 2.5.1.6), cystathionine synthase (*4*), and cystathionase (*4*) were measured by previously described methods (*5*). The concentration of cystathionine was measured by minor modifications of the method of Spackman, Moore, and Stein (*6*).

Results of these determinations in crude extracts of liver from the human fetus, premature infant, and full-term infant are contrasted (Table 1) to previously determined values (*5*) obtained from nine human adults and children (minimum age, 2½ years). The most striking finding is that cystathionase activity is not measurable in the liver of any of the 24 fetuses or 3 premature infants and is only barely measurable in a full-term infant less than 24 hours old. The use of 0 to 4 μmole of cystathionine in the assay (instead of the 2 μmole normally used) or the use of a range of concentrations of pyridoxal 5′-phosphate from 0 to 6.25 μmole (0.125 μmole used normally) failed to elicit a measurable activity of cystathionase in the extracts of fetal liver. The possibility that the lack of cystathionase activity was due to a soluble or loosely bound inhibitor in the crude extracts of fetal liver was tested by incubating these extracts with crude extracts of rat liver; no change in the cystathionase activity of the rat liver extract was found, ruling out the presence of such an inhibitor.

Cystathionine, the substrate of cystathionase, is barely measurable in mature human liver (*3*), but is present in fetal liver in relatively high concentrations (Table 1). The concentrations of cystathionine in the fetal liver are lower than those observed in the adult human brain (Table 2) (*2*, *3*) but are *higher* than in the corresponding immature brains (Table 2). Since there was no apparent relation between concentration of cystathionine in fetal liver or fetal brain and crown-rump length over the period of gestation examined (co-

Table 1. Enzymes of transsulfuration and concentration of cystathionine in developing human liver.

	Methionine-activating enzyme*	Cystathionine synthase*	Cystathionase*	Cystathionine concentration†
Fetus (24)	26 ± 3	21 ± 4	0	14 ± 2
Premature newborn (3)	32 ± 2	17 ± 8	0	13 ± 4
Full-term newborn (1)	10	32	9	32
Mature (9)	86 ± 16	98 ± 19	126 ± 12	0

* Enzymatic activities are expressed as the number of nanomoles of product formed per milligram of soluble protein per hour ± standard error. † Micromoles per 100 g, wet weight.