12. The reference heparin, heparan sulfate, and the dermatan sulfate were supplied by M. B. Mathews and J. A. Cifonelli, University of Chicago, under contract with NIH.
13. To be certain that the presence of heparin did not affect the quantitation of fibronectin by electroimmunoassay, we ran a control test as follows. To a phosphate buffer extract of tissue which contained fibronectin, we added enough heparin to bring the concentration of heparin to 10 mg/ml. Two aliquots of this mixture were run side by side with two aliquots of phosphate buffer extract to which no heparin was added. The analytical values for fibronectin were not affected by the addition of heparin; these values were 0.051 and 0.045 mg/ml for the phosphate buffer extract and 0.051 and 0.045 for the phosphate buffer extract containing heparin. Similar comparisons showed no effect when heparan sulfate or dermatan sulfate was used in place of heparin. However, the dextran sulfates of 5000 and 8000 molecular weight showed a slight effect toward increasing the height of the precipitin peaks and thus the fibronectin values of these same phosphate buffer extracts.
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17. Normal lung tissue was obtained at autopsy from a 32-year-old female and was stored at −70°C. Dissected parenchyma, consisting mostly of alveolar-capillary tissue, was washed free of blood with 0.15*M* NaCl containing 2 m*M* PMSF. A term placenta was perfused immediately after birth with phosphate-buffered, citrated saline until it was free of blood. It was then perfused with a mixture of 1 m*M* PMSF, 1 m*M* iodoacetamide, 100 m*M* ϵ-aminocaproic acid, 5 m*M* benzamidine hydrochloride, 10 m*M* disodium ethylenediaminetetraacetic acid, and soybean trypsin inhibitor (50 μg/ml) at physiological *p*H and ionic strength. The perfused cotyledons were teased off and stored frozen.
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20. We thank Anita Hersh for technical assistance and Dr. Karl Meyer for review of the manuscript. Supported by NIH grant HL 15832. A preliminary report of the work was presented at the FASEB meeting in Atlanta, 1981 (*16*).

15 May 1981; revised 26 August 1981

Lamellar Twinning Explains the Nearly Racemic Composition of Chiral, Single Crystals of Hexahelicene

Abstract. *Solvent etching of single crystals of hexahelicene grown from a racemic solution reveals an unusual layer-like pattern in which pure (+)- and pure (−)-layers alternate through the crystal; this arrangement results in a nearly racemic composition although the crystal is ostensibly chiral, space group* $P2_12_12_1$. *Etched crystals of enantiomerically pure hexahelicene display no such pattern. The two kinds of crystal are indistinguishable by x-ray diffraction.*

Crystals of hexahelicene grown from racemic solution show an unusual behavior: although the crystals are apparently chiral ["spontaneously resolved"; enantiomorphic space group $P2_12_12_1$ (*1*), structure refined to $R = 4$ percent (*2*)], dissolved single crystals display optical rotations corresponding to enantiomeric excesses of only ~ 2 percent instead of 100 percent expected for chiral, single crystals. Similar results have also been obtained with other, related materials (*3*). This puzzling phenomenon is relevant to the general problem of spontaneous resolution (*4*) and to the use of crystal-state reactions for asymmetric synthesis (*5*) and may be of fundamental importance for understanding the nature of chiral interactions. We have therefore examined the situation in more detail.

Substitutional solid solutions of (+)- and (−)-enantiomers seemed unlikely on the basis of the packing arrangement of the molecules (*2*), their marked steric differences (Fig. 1), and the significant difference in the melting points of racemic (231° to 233°C) and optically pure (265° to 267°C) material (*6*). The x-ray study showed no evidence of molecular disorder.

Ordinary twinning (*7*) along a growth direction was initially considered but rejected on the basis of the following experiment. A large single crystal grown from a racemic solution was cleaved in two, each half was further cleaved in two, and the four fragments were allowed to partially dissolve in *n*-hexane until small crystals, each ~ 10 percent of the original crystal, remained. Each of these crystals contained the same, ~ 2 percent, enantiomeric excess and had the same sign of rotation as the original crystal (*8*). Had the fragments contained opposite enantiomers in excess, ordinary twinning along a growth direction would have been indicated.

However, when single crystals were allowed to dissolve only slightly in *n*-hexane or in carbon tetrachloride, an interesting lamellar pattern was readily discerned under the light microscope (Fig. 2). The layers, 10 to 30 μm thick, could be carefully cleaved from the crystal, and individual layers were now found to display optical purities of ~ 100 percent (*9*). When adjacent layers in the same crystal were examined, the signs of rotation alternated. Although all crystals grown from racemic hexahelicene dis-

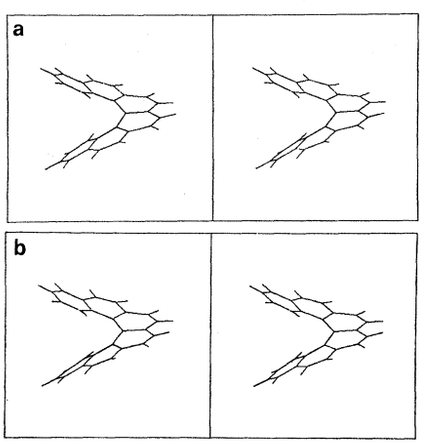

Fig. 1 (left). Stereodrawings of enantiomers of hexahelicene: (a) P-(+)-hexahelicene; (b) M-(−)-hexahelicene.

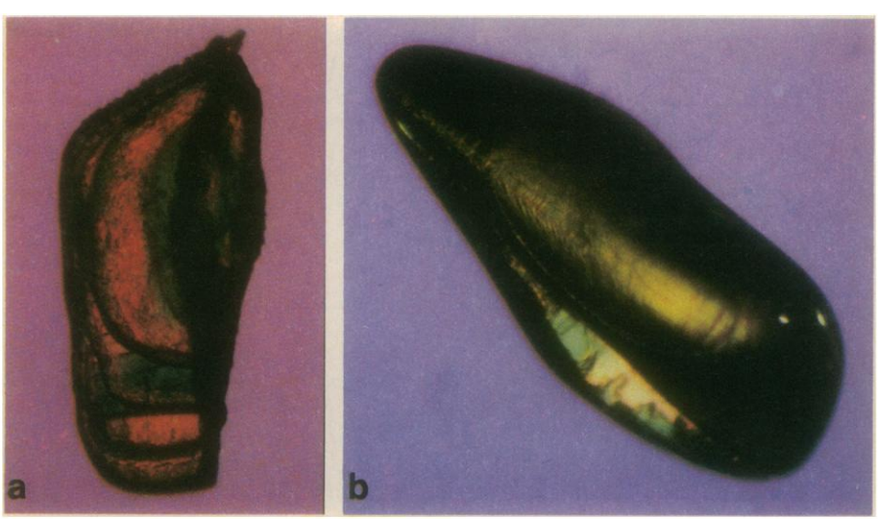

Fig. 2 (right). (a) Crystal of nearly racemic hexahelicene (measured enantiomeric excess 2 percent), grown from an ether–*n*-hexane solution of racemic hexahelicene, after partial solution (etching) in *n*-hexane. (b) Crystal of optically pure hexahelicene (measured enantiomeric excess > 99 percent) grown from ether-*n*-hexane solution after etching in *n*-hexane.

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played this layer structure on etching, when single crystals of optically pure hexahelicene were similarly etched no such pattern was observed (Fig. 2). Thus, the observed nearly racemic composition of ostensibly chiral hexahelicene crystals is due to "lamellar twinning," an alternation of layers of optically pure material of opposite sign.

One might have expected that the x-ray patterns would differentiate crystals containing optically pure material from those displaying lamellar twinning, or that this twinning would complicate the crystal structure determination. This is not the case; because of the absence of observable anomalous dispersion for hexahelicene with the use of Cu Kα radiation, the x-ray patterns of enantiomorphic crystals are identical. In the x-ray diffraction experiment the entire crystal is sampled, but, as long as the adjacent lamellae are aligned to the precision of a usual mosaic crystal, there is no distinction between such a racemic crystal and an enantiomerically pure one. It has been noted (*10*) that in space group $P2_12_12_1$ such conservation of orientation among enantiomeric crystals can occur. Indeed, in the case of hexahelicene, x-ray rotation or Weissenberg photographs of optically pure and nearly racemic crystals were found to be indistinguishable.

A rationale for this behavior was sought in the molecular packing of hexahelicene. When the structure is examined for possible twin planes, which would involve minimal change in intermolecular contacts, one sees that planes perpendicular to the *a*-axis may be drawn which divide the crystal but induce no disorder (Fig. 3, a and b). Other planes through the structure invariably pass through zones of high electron density and thus "cut through" hexahelicene molecules. The (1,0,0) plane is thus the natural candidate for a twin plane which will allow P and M molecules to coexist in a crystal (Fig. 3c). This structure will have the same density as an optically pure crystal. Of course, intermolecular contacts between heterochiral molecules across this plane differ slightly from those between homochiral species, but these differences are not major (Table 1). The only atom-to-atom separation that is less than the normal van der Waals distance (*11*) in the optically pure crystal (C_{12} . . H_8, 2.71 Å) remains almost unchanged in the twinned crystal (2.69 Å). No additional shorter-than-normal van der Waals contacts are present in the twinned crystal. This analysis was corroborated by the observation that the lamellae of an etched, racemic hexahelicene crystal were indeed oriented perpendicular to the *a*-axis of the crystal (*12, 13*).

We cannot yet present a quantitative explanation for this twinning phenomenon, but it is evidently a consequence of the weak "enantiomer discrimination" across the twin planes and the resulting low-energy difference between an optically pure crystal and a twinned one; as the crystal grows, the probability of chiral turnover is relatively high (*14*). The phenomenon may also occur in other helicene-type materials (*3*) and may be

Table 1. Intermolecular contacts in optically pure and twinned hexahelicene crystals through a plane perpendicular to a at $x = ½$. Distances are given which, in either of the two crystals, are less than the sum of the van der Waals radii plus 0.2 Å. Atom 1 belongs to an M-(−)-molecule in the reference asymmetric unit; atom 2 belongs to an M-(−)-molecule in the optically pure crystal or a P-(+)-molecule in the twinned crystal. Symmetry operations that relate atom 2 to the corresponding atom in the reference asymmetric unit are identified by the following code: (1) $1 - x, ½ + y, ½ - z$; (2) $½ + x, ½ - y, 1 - z$; (3) $1 - x, -½ + y, ½ - z$; (4) $1 - x, ½ + y, ½ + z$; (5) $½ + x, ½ - y, z$; (6) $1 - x, -½ + y, ½ + z$; and (7) $1 - x, ½ - y, ½ - z$. Atoms are numbered as in (*2*).

Atom 1	Atom 2	Symmetry in optically pure crystal	Symmetry in twinned crystal	Distance in optically pure crystal (Å)	Distance in twinned crystal (Å)
C_8	C_{12}	(1)	(4)	3.62	3.64
H_8	C_{12}	(1)	(4)	2.71	2.69
C_9	C_3	(2)	(5)	4.14	3.66
C_9	H_3	(2)	(5)	3.72	2.93
C_9	C_{13}	(1)	(4)	3.56	4.73
C_9	C_{14}	(1)	(7)	3.64	4.59
C_{12}	C_8	(3)	(6)	3.62	3.64
C_{12}	H_8	(3)	(6)	2.71	2.69
C_{13}	C_9	(3)	(6)	3.56	4.73
C_{14}	C_9	(3)	(6)	3.64	4.59
C_{22}	H_3	(2)	(5)	3.21	2.81
C_{22}	C_3	(2)	(5)	3.90	3.68

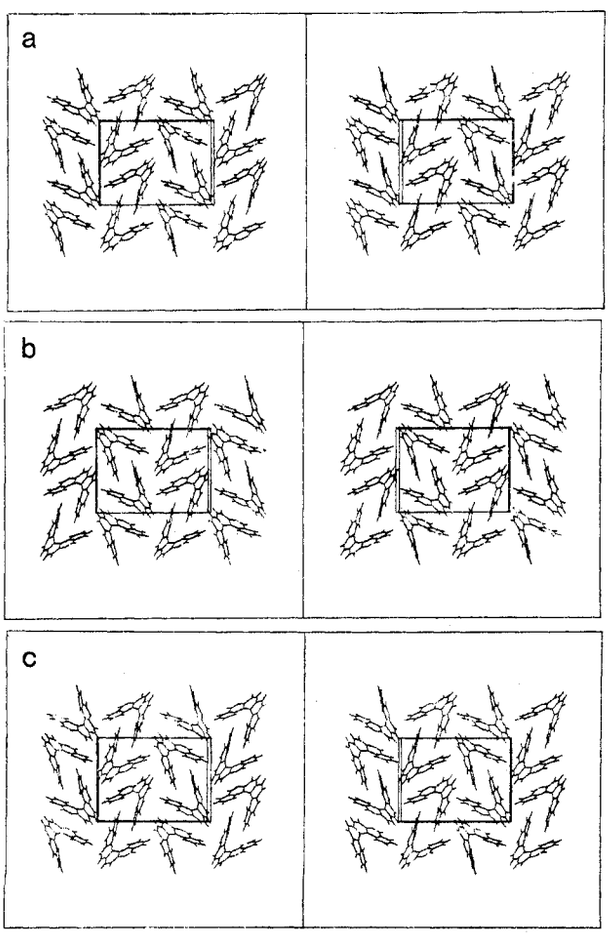

Fig. 3. Stereodrawings of the packing of optically pure and twinned hexahelicene crystals. The unit cell is plotted. The axial directions are $a \rightarrow$, $b \uparrow$, and c out of the plane of the paper. (a) Packing of M-(−)-hexahelicene; (b) packing of P-(+)-hexahelicene; (c) twinned crystal; the two left-hand columns of molecules are M-(−)-hexahelicene as in (a); the two right-hand columns are P-(+)-hexahelicene, but here the two middle columns of P-(+)-hexahelicene in (b) have been translated by (½,0,½). Note the similarity in projection between the chiral structure (a) and the twinned crystal (c).

dependent on crystallization conditions.

Layered crystals were only obtained from racemic, or close to racemic, solutions. Solutions that contained an appreciable enantiomeric excess, ~ 20 percent, deposited optically pure hexahelicene crystals. This result is due to the well-documented (*15*) solubility difference between pure enantiomers and racemic mixtures of conglomerates, a difference that is of practical use in the preparative resolution of hexahelicene by repeated crystallization after use of TAPA (*6*).

It has usually been taken for granted that crystallization in a chiral space group is sufficient to allow resolution of enantiomers if the crystals are sorted according to a chirality observation such as sign of optical rotation. The hexahelicene case exemplifies a situation that may have been overlooked in other systems as well, where crystals grow as nearly racemic mixtures of enantiomers and are indistinguishable from genuinely chiral material as measured by standard x-ray diffractometry.

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12. In the only case, to the best of our knowledge, of lamellar twinning that has been described in the literature (*10*), a similar rationale can be made on the basis of molecular packing. In the crystal structure of β-phenylglyceric acid (*13*), planes parallel to (0,0,1) separate the molecules into stacks; the "chiral interactions" are only between molecules in the same stack. The twin plane is indeed (0,0,1) (*10*). [Different conventions have been chosen in (*10*) and (*13*) to define *a*- and *c*-axes.]
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10 December 1980; revised 11 June 1981

Incorporation of 4-Amino-5-Hydroxymethylpyrimidine into Thiamine by Microorganisms

Abstract. *One possible route for the biosynthesis of the (4-amino-2-methyl-5-pyrimidyl)-methyl moiety of thiamine would involve the formation of a methyl group on the demethylated pyrimidine, 4-amino-5-hydroxymethylpyrimidine, before its incorporation into thiamine. This possibility was tested by preparing the 4-amino-5-hydroxymethylpyrimidine and feeding it to* Escherichia coli, Bacillus subtilis, *and* Saccharomyces cerevisiae. *Analysis of the thiamine produced by these organisms showed that 4-amino-5-hydroxymethylpyrimidine was readily incorporated into thiamine without the addition of a methyl group, and no evidence was found for the conversion of this pyrimidine into normal methylated pyrimidine. Substitution of the demethylated thiamine for thiamine had no effect on the growth rate or the yield of* E. coli *cells. Complete substitution of the thiamine with the (4-amino-5-pyrimidyl)-methyl moiety was possible in an* E. coli *pur I mutant. The extent of incorporation of the demethylated pyrimidine decreased in some organisms and increased in others by the addition of adenine to the growth medium; this difference led to a simple test to separate organisms that use 5-aminoimidazole ribonucleotide for the biosynthesis of thiamine pyrimidine from those that do not.*

Early studies on the incorporation of radioactive precursors into the pyrimidine moiety of thiamine showed that this pyrimidine was formed by a pathway different from that of other pyrimidines (*1*, *2*). Studies of microbial genetics and of certain types of purine toxicity found in microorganisms have shown a connection between purine metabolism and the biosynthesis of thiamine (*3*, *4*).

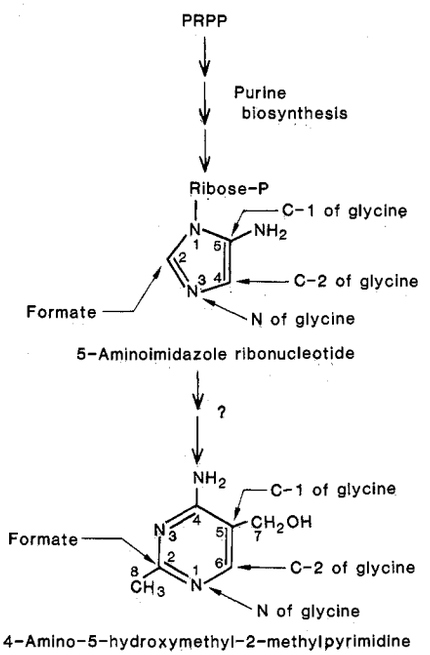

Fig. 1. Biosynthesis of the pyrimidine moiety of thiamine starting from phosphoribosyl pyrophosphate (*PRPP*).

Newell and Tucker (*5*) showed that, in *Salmonella typhimurium*, 5-aminoimidazole ribonucleotide, an intermediate in purine metabolism, is converted into the pyrimidine moiety of thiamine. Laboratory studies with stable isotope–labeled glycine confirmed these results (*6*) and showed how the conversion takes place in enteric bacteria (Fig. 1).

The problem in relation to 4-amino-5-hydroxymethyl-2-methylpyrimidine biosynthesis is to understand how the methyl group is formed at C-2 of the pyrimidine and how the two-carbon unit is inserted into the imidazole ring. One possible pathway is for the imidazole ring to expand to a pyrimidine, with the methyl group introduced as a last step. If this pathway is correct then 4-amino-5-hydroxymethylpyrimidine might function as a precursor; it is clear, however, from other work that if this pathway is correct, the introduced methyl group does not come from methionine (*1*, *7*, *8*). This hypothesis was tested by growing organisms with 4-amino-5-hydroxydideuteromethylpyrimidine. Conversion into the pyrimidine of thiamine was determined by gas chromatographic–mass spectrometric analysis of deuterium

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