where Δ_{xen} is the Bijvoet difference ($|F^+| - |F^-|$).
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12 April 1985; accepted 22 January 1986

Separation of Drug Stereoisomers by the Formation of β-Cyclodextrin Inclusion Complexes

DANIEL W. ARMSTRONG,* TIMOTHY J. WARD,
R. DOUGLAS ARMSTRONG, THOMAS E. BEESLEY

For many drugs, only racemic mixtures are available for clinical use. Because different stereoisomers of drugs often cause different physiological responses, the use of pure isomers could elicit more exact therapeutic effects. Differential complexation of a variety of drug stereoisomers by immobilized β-cyclodextrin was investigated. Chiral recognition and racemic resolution were observed with a number of compounds from such clinically useful classes as β-blockers, calcium-channel blockers, sedative hypnotics, antihistamines, anticonvulsants, diuretics, and synthetic opiates. Separation of the diastereomers of the cardioactive and antimalarial cinchona alkaloids and of two antiestrogens was demonstrated as well. Three dimensional projections of β-cyclodextrin complexes of propanolol, which is resolved by this technique, and warfarin, which is not, are compared. These studies have improved the understanding and application of the chiral interactions of β-cyclodextrin, and they have demonstrated a means to measure optical purity and to isolate or produce pure enantiomers of drugs. In addition, this highly specific technique could also be used in the pharmacological evaluation of enantiomeric drugs.

CHIRAL DISCRIMINATION HAS BEEN a long-standing problem in the development, use, and action of pharmaceutical agents. Numerous examples exist where the undesired effects of one isomer limit the overall effectiveness of the active species because of host toxicities, biodistribution problems, altered metabolism, and unwanted drug interactions. This problem is illustrated by the prototype β-blocker propranolol. *d*-Propranolol is approximately 40 times more potent than *l*-propranolol and appears to mediate the antiarrhythmic and antihypertensive activity of the racemic mixture, whereas only *l*-propranolol appears to be beneficial in treating angina pectoris (*1*). A similar situation occurs with synthetic opiates such as methadone, for which there may be three to five stereoselective opiate receptors, each of which triggers a different physiological response (*2*). The use of specific isomers could allow one to elicit more exact therapeutic effects. Unfortunately, as is true for approximately 25 percent of pharmaceutical products, only racemic mixtures of propranolol and methadone are available for clinical use. This is a direct result of the difficult (and thus expensive) traditional methods for resolving enantiomers or for completing stereoselective syntheses.

Recently, a number of highly specific liquid chromatographic techniques were developed to separate certain enantiomers (*3–7*). However, only a few compounds of clinical interest were resolved (*5, 8–13*), and these were restricted to a few pharmaceutical classes. There was evidence that stable, high-coverage, β-cyclodextrin–bonded media could be employed for stereoselective drug separations (*5, 8, 14–17*). Indeed, it had been shown that cyclodextrins are useful as biomimetic models in studies of substrate binding (*18–20*), enzymatic catalysis (*18–22*), and membrane transport (*19*) and as novel reaction media (*23–25*). We now show that β-cyclodextrin–bonded media separate stereoisomers of a wide variety of clinically relevant cyclic and heterocyclic drugs.

The two types of separations investigated included that of enantiomeric drugs (Table 1) and of diastereomeric drugs (Table 2). Although no chiral stationary phase could be universally effective for the resolution of enantiomers, the variety of compounds resolved by inclusion-complex formation was encouraging. Resolution values (R_S) greater than 1.0 were obtained for the drugs propranolol, chlorthalidone, mephenytoin, phensuximide, nimodipene, triazoline, ketoprofen, chlorpheniramine, methylphenidate and the barbiturates hexobarbital and mephobarbital. Slightly lower, but satisfactory, resolution was obtained for methadone, verapamil, metoprolol, aminoglutethamide, and nisolidipene. These compounds were

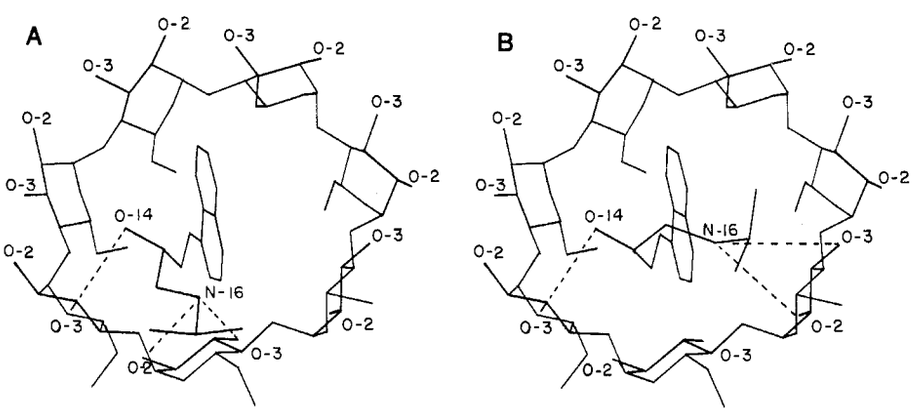


Fig. 1. Computer projections of inclusion complexes of (A) *d*-propranolol and (B) *l*-propranolol in β-cyclodextrin, from x-ray crystallographic data. Dotted lines represent potential hydrogen bonds (distances noted in the text). The configurations shown represent the optimal orientation of each isomer on the basis of the highest degree of hydrogen bonding and complexation.

D. W. Armstrong and T. J. Ward, Department of Chemistry, Texas Tech University, Lubbock, TX 79409.
R. D. Armstrong, Cancer Research Institute and Department of Pharmacology, University of California, San Francisco, CA 94143.
T. E. Beesley, Advanced Separation Technologies, Whippany, NJ 07981.

*To whom correspondence should be addressed.

resolved by hydro-organic isocratic separations or gradients; however, more exotic mobile phases could have been used. The separation profiles were reproducible during the 4 months that the columns were used. Analogous preparative- and semipreparative-scale separations are feasible (*26*).

The proficiency of this technique is even more pronounced when it is applied to separations of diastereomers (Table 2). Diastereomeric pairs of cardioactive and antimalarial cinchona alkaloids (quinidine, quinine and cinchonidine, cinchonine) were easily separated, and separation of the geometric (*cis* and *trans*) isomers of the antiestrogens tamoxifen and clomiphene was equally facile.

There are a number of requirements for chiral recognition by cyclodextrins. For example, an inclusion complex must be formed, and there must be a relatively tight fit between the complexed moiety and the β-cyclodextrin. In addition, the chiral center or one substituent of the chiral center must be near and interact with the mouth of the cyclodextrin cavity. The unidirectional 2- and 3-hydroxyl groups located at the mouth of the cyclodextrin cavity are thought to be particularly important in chiral recognition. This is apparent in Fig. 1, which shows computer-generated projections of the lowest free energy inclusion complexes of *d*- and *l*-propranolol with β-cyclodextrin. In this configuration, *d*- and *l*-propranolol are placed identically within the cyclodextrin cavity, and the structures are overlaid exactly to the point of the chiral carbon. The hydroxyl group attached to the chiral carbon is in the same position for the *d* and *l* compounds, placed for optimal hydrogen bonding to a 3-hydroxyl group of the cyclodextrin. Important differences are observed between the complexes of the *d* and *l* isomers with respect to their secondary amine group. In the *d*-propranolol complex, this nitrogen is ideally placed for hydrogen bonding to both a 2- and 3-hydroxyl group of the cyclodextrin, with respective bond distances of 3.3 and 2.8 Å. The amine in the *l*-propranolol complex is positioned less favorably for hydrogen bonding; the bond distances to the closest 2- and 3-hydroxyls of the cyclodextrin are 3.8 and 4.5 Å, respectively. This suggests that the *d* isomer would preferentially interact with the β-cyclodextrin and thereby be retained longer. Regardless of the bond rotations of this aliphatic chain, our studies demonstrate that this difference is maintained and sometimes increased. Thus, in its most stable configuration, *d*-propranolol can hydrogen bond to the cyclodextrin in a manner that *l*-propranolol cannot. Figure 2 illustrates *d*-propranolol in the β-cyclodextrin cavity with van der Waals radii shown. Both rings of the naphthyl group fit into the cavity for optimal complexation, and the side chain lays directly over the lip of the β-cyclodextrin.

Our results also suggested that, for chiral interaction with β-cyclodextrin, a compound needed at least one aromatic ring, although two appeared to be of greater benefit. In addition, the proximity of the ring moieties to the chiral center of the compound appeared to improve chiral resolution, perhaps as a result of less bond rotation than could occur with aliphatic side groups. Heterocyclic drugs such as mephenytoin and the barbiturates, which had chiral centers within ring groups, were optimal candidates for chiral separation (Table 1). However, a number of other enantiomers were tested and failed to be resolved in spite of their apparent structural similarity to the resolved compounds listed in Table 1. Drugs that were not effectively resolved by the formation of β-cyclodextrin inclusion complexes included: doxylamine, ketamine,

Table 1. Separation of enantiomeric drugs. The chromatographic data in this table are the mean of three identical runs. The coefficient of variation for the retention times is 2%. Definitions: k', capacity factor of the first eluted isomer; α, separation factor; R_S, resolution value. Mobile phase ratios show the relative volume of methanol to 1% aqueous triethylammonium acetate (*p*H 4.1) unless otherwise indicated. Flow rates were 1.0 ml/min.

Drug	k'	α	R_S	Mobile phase	Column
β-Adrenergic blockers					
Propranolol	2.78	1.04	1.40	25:75	‡
Metoprolol	3.51	1.03	0.90	32:68	‡
Antihistamine					
Chlorpheniramine	5.86	1.07	1.51	15:85*	§
Calcium channel blockers					
Verapamil	2.94	1.03	0.71	†	§
Nisolidipene	4.13	1.04	0.87	30:70	‡
Nimodipene	5.09	1.05	1.10	30:70	‡
Diuretic					
Chlorthalidone	0.50	1.44	1.95	30:70	§
Sedative-anticonvulsants					
Hexobarbital	9.39	1.14	1.51	15:85	‖
Mephobarbital	14.80	1.14	1.60	20:80	‖
Mephenytoin	0.48	1.33	1.83	40:60	§
Triazoline	5.00	1.15	1.50	40:60	§
Phensuximide	1.97	1.15	1.54	10:90*	§
Anticorticosteroid					
Aminoglutethimide	7.49	1.03	0.91	†	§
Nonsteroidal anti-inflammatory agent					
Ketoprofen	7.67	1.06	1.24	27:73	‡
Narcotic-analgesic					
Methadone	2.38	1.04	0.81	†	§
Central nervous system stimulant					
Methylphenidate	1.17	1.14	1.57	10:90*	‡

*Acetonitrile was used as the organic modifier in place of methanol. †Separation was done with a gradient of acetonitrile and 1% aqueous triethylammonium acetate that changed from 10:90 to 20:80 in 20 minutes. ‡Two 25-cm β-cyclodextrin columns were used in series. §One 25-cm β-cyclodextrin column was used. ‖One 10-cm β-cyclodextrin column was used.

Table 2. Separation of diastereomeric drugs. The chromatographic data in this table are the mean of three identical runs. The coefficient of variation for the retention times is 2%. Definitions: k', capacity factor of isomer; α, separation factor; R_S, resolution value. Mobile phase ratios show the relative volume of acetonitrile to 1% aqueous triethylammonium acetate (*p*H 4.1) unless otherwise indicated. Flow rates were 1.0 ml/min.

Drug	k'	α	R_S	Mobile phase	Column
Cinchona alkaloids					
Quinidine	2.16	1.21	1.76	10:90	‡
Quinine	1.78				
Cinchonidine	1.62	1.31	1.86	10:90	‡
Cinchonine	2.12				
Antiestrogens					
Tamoxifen	0.41*	2.73	2.60	25:75	‡
Clomiphene	3.60*	1.50	2.00	65:35†	‡

*Capacity factor of the first eluted isomer. †Methanol was used as the organic modifier in place of acetonitrile. ‡One 25-cm β-cyclodextrin column was used.

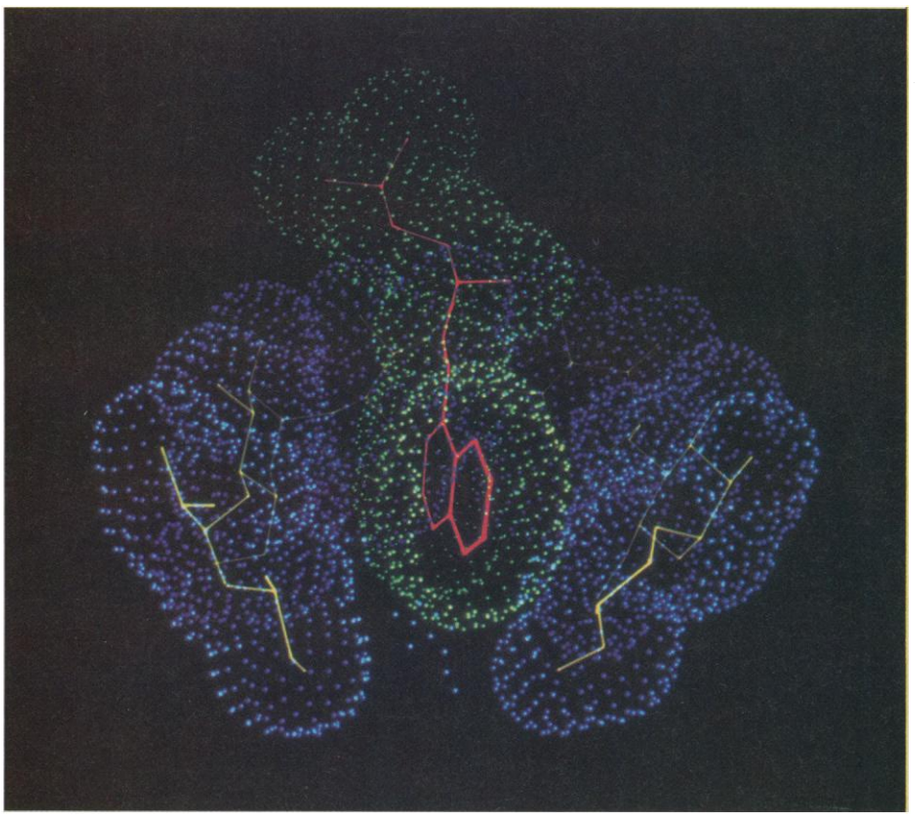


Fig. 2. Computer-graphic image of the inclusion complex of *d*-propranolol in β-cyclodextrin. Compounds are illustrated with van der Waals radii to demonstrate the fit of *d*-propranolol within the β-cyclodextrin cavity and the interaction of the side chain (see text) with the lip of β-cyclodextrin.

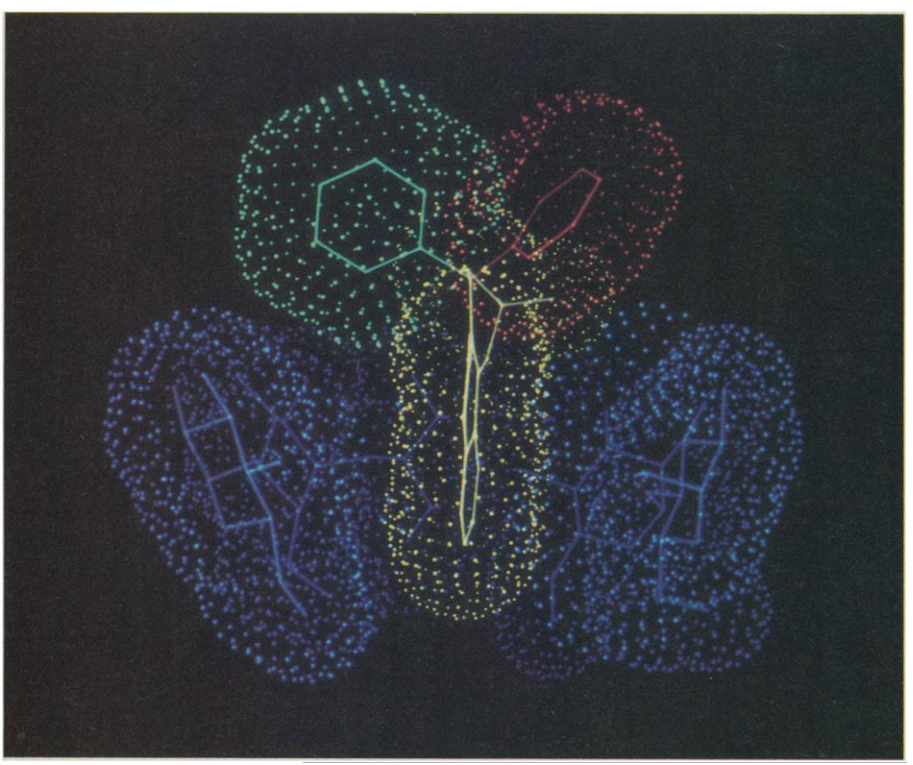
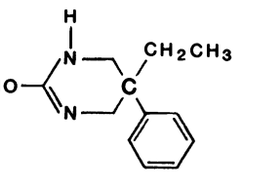


Fig. 3. Computer-graphic image of the inclusion complex of warfarin in β-cyclodextrin. (*R*)-(+)- and (*S*)-(−)-warfarin are overlaid in their optimal orientation within the β-cyclodextrin. The red and green areas represent (*R*)-(+)- and (*S*)-(−)-warfarin, respectively. The yellow area is the superimposed portion of the warfarin isomers, and the blue region is the β-cyclodextrin. Warfarin easily forms a hemiketal ring by cyclization of its keto side chain with the 4-phenolic group of its coumarin ring system (*27*); this form was used to model the inclusion complex.

warfarin, chlorcyclizine, bupivacaine, glycopyrrolate, and leucovorin. The chemical structures of three drugs that varied in ease of resolution from excellent (mephobarbital, **1**) to moderate (propranolol, **2**) to no resolution (warfarin, **3**) are shown below. Eval-



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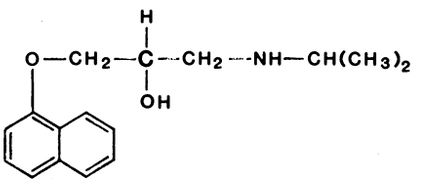

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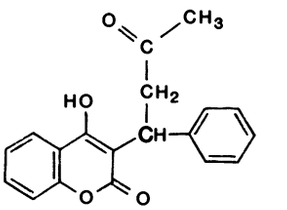

3

uation of enantiomers that show no chiral recognition, such as (*S*)-(−)- and (*R*)-(+)-warfarin, also can be useful in understanding this system. A computer model of the superimposed *d* and *l* isomers of warfarin in β-cyclodextrin is shown in Fig. 3. Even under conditions of optimal complex formation, the phenyl group is sufficiently far from the cyclodextrin to preclude any significant interactions. Thus, it is difficult for β-cyclodextrin to discriminate between the enantiomers even though there is a difference in orientation (Fig. 3). This is in contrast to the *d*-propranolol complex in Fig. 2, where the side group is positioned favorably for hydrogen bonding with β-cyclodextrin. Our results suggest that the absence of differential interaction at the mouth of the cyclodextrin cavity may preclude chiral recognition in some cases. It may be possible to circumvent this problem by forming derivatives of the 2- or 3-hydroxyl groups of the cyclodextrins. For example, stereoisomers of (±)-norgestrel can be separated with an acetylated β-cyclodextrin column but not with an un-derivatized one (*16*).

The use of inclusion-complex formation with β-cyclodextrins to resolve many classes of racemic drugs offers new avenues for pharmacological research and development. In addition to their projected role in nontoxic drug delivery systems, cyclodextrins may facilitate the replacement of racemic

drugs with their more selective and often safer enantiomers as well as provide a rapid, specific technique for pharmacological evaluation of racemic drugs. Computer modeling of x-ray crystal structures coupled with energy minimization calculations is a powerful technique for evaluating and understanding chiral interactions. Cyclodextrins are particularly amenable to this approach because of their well-defined and relatively static structure. However, we believe this method may be equally valuable in the evaluation and understanding of a variety of other chiral-separation systems (*3–17*).

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26. A liquid chromatograph (Shimadzu LC-4A) with a variable wavelength detector (set at 254 nanometers) was used for all separations. High-coverage, stable β-cyclodextrin–bonded packing was prepared as reported (*8, 14*); columns were packed by Advanced Separation Technologies, Inc. Circular dichroism spectra were measured in a spectropolarimeter (Jasco 500A; cell pathlength, 1 cm).
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28. Supported by Department of Energy grant DE-AS0584ER13159 and American Cancer Society grant CH-239. We thank N. Pattabiraman of the Computer Graphics Laboratory, University of California, San Francisco (R. Langridge, director; supported by NIH grant RR1081), for assistance with the computer graphics analysis and the figures.

15 October 1985; accepted 3 March 1986

Neutralization of HTLV-III/LAV Replication by Antiserum to Thymosin α_1

PREM S. SARIN, DAISY K. SUN, ARTHUR H. THORNTON, PAUL H. NAYLOR, ALLAN L. GOLDSTEIN

An antiserum prepared against thymosin α_1, a hormone secreted by the thymus gland, effectively neutralized the AIDS-associated virus [HTLV-III/LAV(clone BH-10)] and blocked its replication in H9 cells. Reverse transcriptase activity and expression of the HTLV-III/LAV antigens p15 and p24 were inhibited by purified immunoglobulin G preparations of antisera to thymosin α_1. The antiviral activity of the antiserum was found to be due to a region of homology between thymosin α_1 and p17, a product of the *gag* gene of HTLV-III/LAV. Comparison of the primary sequences of thymosin α_1 and the *gag* protein revealed a 44% to 50% homology in an 18–amino acid region, between positions 11 and 28 on thymosin α_1 and 92 and 109 on the *gag* protein. The effectiveness of the thymosin α_1 antiserum and of immunoglobulin G–enriched preparations in blocking replication of HTLV-III(BH-10) in H9 cells suggests a novel approach to the development of an AIDS vaccine. A vaccine directed against the *gag* protein might overcome the problem of genetic drift in the envelope region of the virus and be useful against all genetic variants of HTLV-III/LAV.

THE ACQUIRED IMMUNE DEFICIENCY syndrome (AIDS) is characterized by a decrease in the ratio of $T4^+$ to $T8^+$ lymphocytes, an increase in the incidence of opportunistic infections, and progressive paralysis of the immune system. The etiologic agent of AIDS has been identified as the human T-lymphotropic retrovirus HTLV-III/LAV (also termed ARV)(*1*). In many respects, the clinical symptoms of AIDS are indistinguishable from symptoms often seen in children with rare primary immunodeficiency diseases (PID's) associated with thymic aplasia or hypoplasia (*2*) and an increased susceptibility to opportunistic infections, including *Pneumocystis carinii* pneumonia. That the thymus gland, which plays a key role in the maturation and function of the lymphoid system, might be involved in the development of AIDS was first suggested by the detection of increased concentrations of peptides similar to thymosin α_1 in the blood of individuals with AIDS or belonging to the AIDS risk group (*3*).

Thymosin α_1 ($T\alpha_1$) was the first thymic hormone purified to homogeneity and sequenced from the partially purified thymosin fraction 5 (TF5) (*4*). It is an acidic polypeptide (*p*I 4.2) with a molecular weight of 3108, has many of the biological activities of TF5, and is a potent immunomodulator in vivo and in vitro (*5*). It acts primarily on helper T cells, and in humans and other animals enhances the expression of T-cell markers, stimulates the production of a number of lymphokines, including interleukin-2 (IL-2) and γ-interferon, and restores immune function and tumor immunity. $T\alpha_1$ has been found to restore helper T-cell function and prolong survival in phase II clinical trials in patients with lung cancer treated by radiotherapy (*6*).

Because of the clinical similarities between AIDS and PID's in children, we expected that $T\alpha_1$ levels would be depressed in patients with AIDS or AIDS-related complex (ARC). When we measured $T\alpha_1$ by radioimmunoassay (RIA) (*7*), however, we were surprised to find markedly elevated levels in the blood of patients with AIDS and ARC (*3*). For example, 44 of 72 individuals with Kaposi's sarcoma (61%) and 12 of 22 individuals with *P. carinii* pneumonia (55%) had $T\alpha_1$ levels above two standard deviations from the mean of normal individuals (*3*). Subsequent studies confirmed the initial findings for a nationwide sampling of patients with AIDS that included homosexuals and bisexuals, intravenous drug abusers, Haitians, and homosexuals with lymphadenopathy (*3*).

Three alternatives have been proposed to account for the elevation of $T\alpha_1$ in AIDS patients: (i) a defect in helper T cells resulting in increased levels of biologically active peptide, that is, end-organ failure; (ii) a viral invasion of the thymus epithelial cells with

P. S. Sarin, D. K. Sun, A. H. Thornton, Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20892.
P. H. Naylor and A. L. Goldstein, Department of Biochemistry, George Washington University School of Medicine and Health Sciences, Washington, DC 20037.