in Beaumont Formation elevation under the mound and between mounds is a function of mound formation. We suggest that these particular pimple mounds are aggradational features formed from the coarser fraction (sands and silts) reworked out of Beaumont Formation sediments eroded from the intermound areas. Such a process of erosion and redeposition is substantially consistent with previous explanations of pimple mound formation, except that at Pipkin Marsh there is direct evidence for redeposition of eroded materials, evidence lacking or equivocal in previous soil and sediment analyses (*2*). The differential ages of artifacts from sites 26 and 31(B) suggest that these mounds each took about 300 to 500 years to form.

Pimple mounds seem to accumulate by one or more processes until an equilibrium profile is approached, after which net accumulation slows or stops (*10*). Indeed, if most of mound 31(B) accumulated during a 400-year period ending some 1600 years ago, the truncated cone morphology must be an equilibrium form. Storm surges may enhance this local relief and inhibit lateral spreading of mounds by maintaining nick points at their bases.

The diachronic nature of mound initiation in a pimple mound field also implies spatial processes and a mound density equilibrium. Although it is unknown whether this equilibrium has been reached in the Pipkin Marsh area, mound formation there continued at least to the early second millennium A.D. Examination of the geometry of mounds within a field and a search for "juvenile" mounds would aid in understanding the processes by which they form. The incorporated archeological remains may also assist in reconstructing the environmental conditions, such as climate, under which pimple mounds formed.

The Pipkin Marsh sites indicate that pimple mound terrains should be considered as having archeological potential. In view of the thousands of Gulf Coast mounds, it is necessary to have means to weight the probability of associated archeological deposits.

A 1767 Spanish colonial report (*11*) describes the usefulness of pimple mounds in areas with poor drainage: "Extraordinary features of this plain are flat-topped mounds, from four to six varas in diameter and from one to two in height, which nature has scattered about in great number making it passable. Rising above the surface of the water they serve as a resting-place for the people who often have to carry their goods on their shoulders because the beasts become worn out, or lack food. On these mounds they can keep their feet dry while packing the loads which otherwise would be almost impossible."

Presumably, pimple mound areas likely to have been sites of prehistoric cultural activity would be those adjacent to aquatic habitats whose resources were being intensively exploited, as in Pipkin Marsh. The most probable location for such mounds is along the contact of Beaumont and Prairie formations with Recent marsh near the coast. Conversely, pimple mounds on high bluffs around estuaries or on segments of the Ingleside barrier would be unlikely areas for archeological deposits of the kind described here. Mound fields on upland surfaces of the Beaumont Formation also would have a low probability of associated archeological remains, except perhaps for fields close to small streams or to chains of small lakes in degraded Pleistocene river channels, since such streams and lakes may have helped to determine aboriginal routes of access across the coastal upland prairies.

The Pipkin Marsh data indicate that the genesis of pimple mounds may be much more recent than was previously supported by field evidence. This process continued until at least 700 years ago and may be continuing today as a concomitant of drainage network development on late Pleistocene meander and barrier island ridges. Individual mounds may form rapidly in relation to the overall period of mound field development. This suggests a need to focus less on individual mounds and more on the development of mound fields as an integrated system of physical and biological processes for creating and modifying landforms.

LAWRENCE E. ATEN
Division of State Plans and Grants, National Park Service, Department of the Interior, Washington, D.C. 20243
CHARLES N. BOLLICH
5795 Viking Drive, Beaumont, Texas 77706

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Acetylcholine and Bradykinin Relax Intrapulmonary Arteries by Acting on Endothelial Cells: Role in Lung Vascular Diseases

Abstract. *Acetylcholine and bradykinin produced potent relaxation of isolated canine intrapulmonary arteries contracted by serotonin, norepinephrine, or phenylephrine—provided the endothelium was left intact. Selective mechanical destruction of the endothelium transformed the activity of these substances from vasodilatation to vasoconstriction. Acetylcholine-induced relaxations, in the presence of intact endothelium, could be selectively inhibited competitively by atropine, but could not be inhibited by cyclooxygenase inhibitors, a lipoxygenase inhibitor, adrenergic blocking drugs, or histaminergic antagonists. Relaxations produced by prostacyclin, prostaglandin E_1, isoproterenol, papaverine, or histamine H_2-receptor agonists were not modified, or attenuated, by selective destruction of pulmonary endothelial cells. These observations might provide insight into the etiology of the increased pulmonary resistance observed in pulmonary hypertension and shock lung.*

Rabbit aortic rings contracted with various stimulants will relax when acetylcholine is added if the aortic rings are prepared with their endothelial cells left intact (*1*). We wondered whether selective removal of the endothelium from intrapulmonary arteries would result in a loss of ability of these vessels to relax in response to certain neurohumoral vasodilators and whether the vessels would contract, rather than relax, in response to these agents.

There is at present no agreement on the etiology of pulmonary hypertension, although various mechanisms have been proposed (*2*). During the past 10 years,

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evidence has accumulated suggesting that circulatory shock induced by such factors as septicemia, trauma, and hypovolemia often results in a condition known as shock lung (*2, 3*). The mortality in humans with this syndrome is around 50 percent.

Shock lung syndrome is characterized by several pathologic findings that include massive vascular congestion, interstitial and intra-alveolar edema and hemorrhage, hyaline membrane formation, and—if the patient survives—pulmonary fibrosis. The pulmonary hypertension and shock lung syndromes both involve elevations in pulmonary vascular resistance and pulmonary congestion of unknown etiology. On autopsy, both of these syndromes reveal destruction of intrapulmonary arterial endothelial cells (*2, 3*).

If intrapulmonary arteries and arterioles in situ, which are normally very distensible, develop constrictor responses to circulating neurohumoral agents that normally induce vasodilatation in the lung, such as acetylcholine, kinins, and prostanoids, this mechanism could aid in explaining the etiology of shock lung and pulmonary hypertension.

We now report that acetylcholine and bradykinin produce potent concentration-dependent relaxation of isolated intrapulmonary arteries, provided the endothelium is left intact. Selective removal of the endothelium causes a transformation in these effects so that only contractile actions are exerted on the intrapulmonary arteries.

Mongrel dogs of either sex, weighing 15 to 20 kg, were anesthetized with pentobarbital sodium (30 mg/kg). After thoracotomy, the lungs were rapidly removed and intrapulmonary arteries (outer diameter, 2 to 4 mm) were excised. Helical strips, cut from segments of intrapulmonary arteries, were 30 to 40 mm long and 3 to 4 mm wide (*4*); they were prepared with extreme care so as not to injure the endothelium. They were then suspended isometrically under 2 g of tension and incubated in 10-ml muscle chambers containing Krebs-Ringer bicarbonate solution (composition in millimoles per liter: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; glucose, 10; and $NaHCO_3$, 25) at 37°C through which a mixture of O_2 (95 percent) and CO_2 (5 percent) was bubbled (*5*). The force of contractions was measured with force-displacement transducers (Grass FT-03C) and recorded on a polygraph (Grass model 7). Two hours after the preparations were incubated, under tension, the effects of 80 m*M* potassium chloride were ascertained. The tissues were then exposed to one of three stimulants, serotonin (10^{-8} to $10^{-7}M$), norepinephrine (1.5×10^{-8} to $3 \times 10^{-7}M$), or phenylephrine (5×10^{-7} to $1.2 \times 10^{-6}M$), in order to produce sustained contractions (1.6 ± 0.5 g, $N = 275$). Agonists to be tested—acetylcholine, bradykinin, isoproterenol, papaverine, prostacyclin (PGI_2), prostaglandin E_1 (PGE_1), dimaprit, and impromidine—were then added to the contracted tissues either in cumulative or single doses. Additional experiments were undertaken in the presence of the cyclooxygenase inhibitors indomethacin and 5,8,11,14-eicosatetraynoic acid (ETYA), a lipoxygenase inhibitor (BW 755C), and atropine, metiamide, propranolol, phentolamine, hypoxia, and elevated potassium.

Addition of acetylcholine or bradykinin to the physiologic salt solution bathing the contracted blood vessels resulted in potent concentration-dependent relaxation in the intrapulmonary arteries with intact endothelium (Figs. 1 and 2). Although there was considerable variability in the concentrations required to exert minimal threshold relaxations (Table 1), most of the arteries responded to these vasodilators in the range of 10^{-11} to $10^{-9}M$, and the maximum concentration (10^{-7} to $10^{-6}M$) produced complete relaxation. Relaxations induced by acetylcholine were antagonized competitively and

Table 1. Comparison of the relaxant actions of acetylcholine and bradykinin with those of other vasodilator agents on canine intrapulmonary arteries (with intact endothelium) contracted with serotonin, norepinephrine, or phenylephrine. The data are presented as means ± standard error. Three intrapulmonary arteries responded to minimal effective concentrations of bradykinin in the range 10^{-13} to $10^{-12}M$, whereas five intrapulmonary arteries responded to concentrations of acetylcholine as small as $10^{-12}M$. Relaxation responses induced by impromidine and isoproterenol, but not any other agonist, could be antagonized competitively with metiamide and propranolol, respectively.

Agonist	*N*	Minimal effective concentration (*M*)	Maximum relaxation (mg)
Bradykinin	27	$9.2 \times 10^{-11} \pm 4.7 \times 10^{-11}$	1160 ± 300
Acetylcholine	39	$6.5 \times 10^{-10} \pm 5.2 \times 10^{-10}$	1260 ± 210
Isoproterenol	8	$2.5 \times 10^{-9} \pm 1.9 \times 10^{-9}$	700 ± 150
Prostacyclin	15	$1.7 \times 10^{-8} \pm 1.5 \times 10^{-8}$	1280 ± 305
Impromidine	7	$8.5 \times 10^{-8} \pm 2.4 \times 10^{-8}$	1040 ± 250
Prostaglandin E_1	5	$2.7 \times 10^{-7} \pm 1.8 \times 10^{-7}$	990 ± 260
Papaverine	17	$9.6 \times 10^{-7} \pm 2.4 \times 10^{-7}$	1720 ± 270

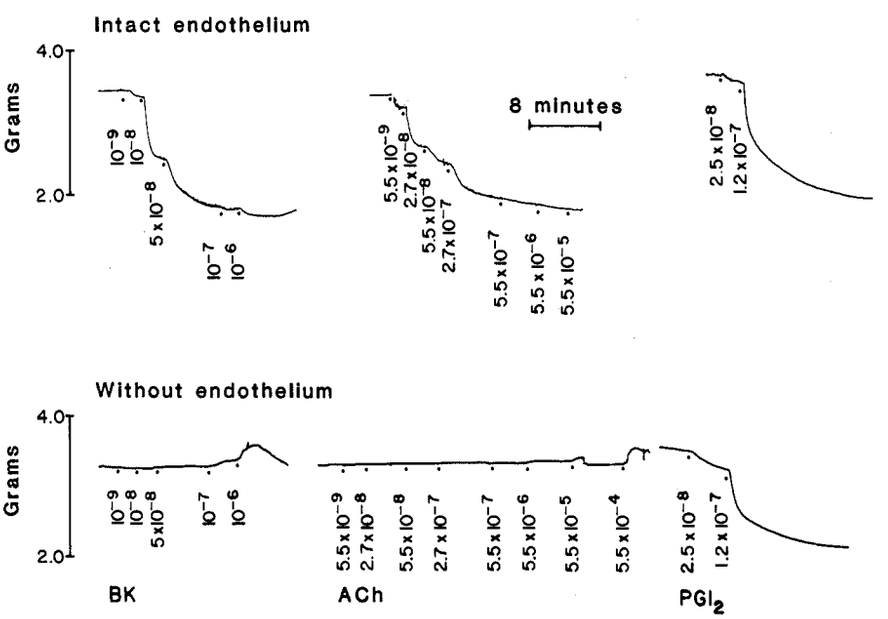

Fig. 1. Responses of canine intrapulmonary arteries contracted with phenylephrine to cumulative concentrations (moles per liter) of bradykinin (*BK*), acetylcholine (*ACh*), and prostacyclin (PGI_2). (A) All three agents induce concentration-dependent relaxation in the presence of intact endothelial cells. (B) Selective, mechanical destruction of the endothelium results in the obliteration of relaxant responses to bradykinin and acetylcholine but not to prostacyclin. The vertical bars on the left represent tension; time marker, 8 minutes. Dots indicate points at which cumulative concentrations of agonists were added.

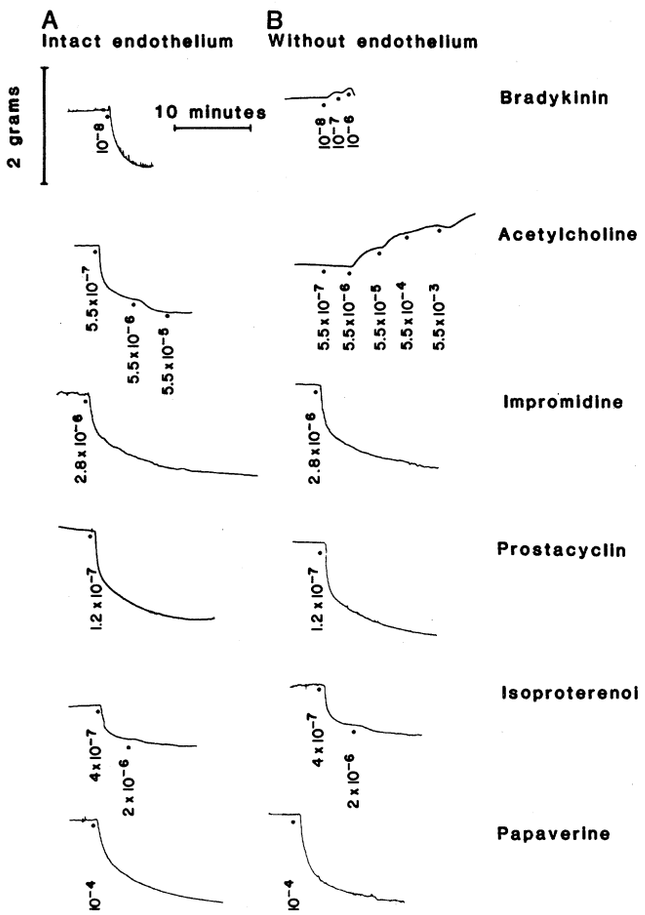

Fig. 2. Comparative responses of canine intrapulmonary arteries contracted with serotonin (2.6×10^{-8} to $1.3 \times 10^{-7}M$) to single doses of bradykinin, acetylcholine, impromidine, PGI_2, isoproterenol, and papaverine (A) in the presence and (B) in the absence of endothelium. Dots indicate points at which cumulative concentrations (moles per liter) of agonists were added. Selective removal of endothelium (B) results in a complete loss of relaxant responses to acetylcholine and bradykinin; whereas relaxant responses to the other agonists are not altered by selective destruction of endothelium.

selectively by atropine (1.4×10^{-8} to $7 \times 10^{-8}M$, producing a 50- to 100-fold parallel shift of the dose-response curves to the right), an indication that this neurohumoral agent acts through muscarinic receptors on the endothelial cells. Acetylcholine-induced relaxations were not modified by indomethacin ($2.8 \times 10^{-6}M$), ETYA ($1 \times 10^{-5}M$), or BW 755C (4×10^{-5} to $8 \times 10^{-5}M$). Nor were they modified by the β-adrenergic blocking agent propranolol ($3.5 \times 10^{-6}M$), the α-adrenergic blocking agent phentolamine ($1.8 \times 10^{-6}M$), or the H_2-histamine receptor blocking agent metiamide ($5 \times 10^{-5}M$). These observations exclude the possible mediation of acetylcholine-induced vasodilation by adrenergic and histaminergic receptors and eliminates mediation by prostaglandins, and, possibly, lipoxygenase products. Relaxations induced by a median effective concentration (EC_{50}) or by higher concentrations of bradykinin were not inhibited by addition of indomethacin or any of the other pharmacologic antagonists.

When the endothelial surface of the pulmonary arteries was rubbed on filter paper (Whatman, No. 4) for 30 to 60 seconds, the relaxation responses to acetylcholine ($N = 47$) and bradykinin ($N = 39$) were completely lost. Electron microscopy of the tissues shows that only endothelial cells are removed by this technique; the underlying structures, including the vascular smooth muscle cells, are not damaged (Fig. 3).

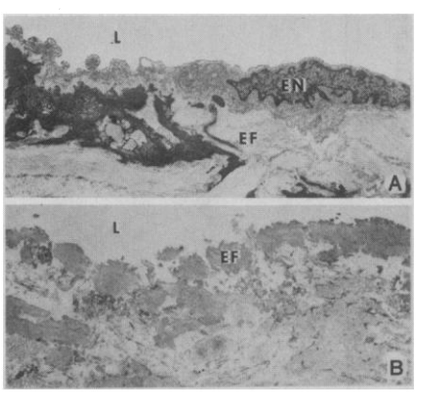

Fig. 3. Electron microscopy of intrapulmonary arteries (A) before and (B) after removal of the endothelium. The intrapulmonary arteries were carefully dissected from the lungs, and some had their luminal surfaces rubbed with filter paper (B) as described. Both control and rubbed vessels were fixed and processed for transmission electron microscopy (*7*). In control preparations (A) the lumen (*L*) of the artery is clearly lined with intact endothelial cells (*EN*). The rubbed preparations (B) lack endothelium, and only the underlying elastic fibers (*EF*) are observed at the vessel lumen (*L*) surface (×5000).

Relaxation responses induced by PGI_2, PGE_1, dimaprit and impromidine (H_2-receptor agonists), isoproterenol, and papaverine were not inhibited, attenuated, or altered by selective destruction of the pulmonary arterial endothelium (Fig. 2).

Incubation of pulmonary arteries with intact endothelium under hypoxic conditions [partial pressure of oxygen (PO_2) = 35 mmHg, as opposed to 550 mmHg] for up to 3 hours did not significantly reduce the relaxations induced by acetylcholine ($N = 6$). However, elevating the K^+ concentration of the bathing solution from $5.9M$ to $80M$ (isosmotic substitution of $74.1M$ NaCl) resulted in more than 95 percent inhibition of these relaxation responses ($N = 6$). Since the higher concentration of K^+ would probably induce a marked depolarization of the endothelial cells, acetylcholine, and possibly bradykinin, may hyperpolarize the endothelial cell membrane to elicit the relaxation responses; myo-endothelial junctions could transfer the hyperpolarizing current to the underlying vascular smooth muscle cells and thereby produce relaxation.

Furchgott *et al.* (*1*) proposed, for rabbit aortic smooth muscle, that acetylcholine induces relaxation through vascular endothelial cells by release of some unknown vasoactive mediator. Such a mechanism may not be operative in pulmonary arterial tissues for several reasons. (i) Acetylcholine-induced relaxation is inhibited only by atropine and elevated extracellular K^+ concentration ($[K^+]_o$). (ii) Bradykinin-induced relaxation is inhibited only by elevated $[K^+]_o$; (iii) No other known vasodilator so far tested (including PGE_1, β-adrenergic relaxants, H_2-histamine agonists, and papaverine) requires the presence of endothelial cells for the expression of its relaxant response on intrapulmonary arteries. (iv) A marked reduction in PO_2 or the use of ETYA ($1 \times 10^{-5}M$) does not inhibit the relaxant responses induced by acetylcholine or bradykinin, unlike the result in rabbit aorta (*1*).

Therapeutic administration of acetylcholine can reduce primary pulmonary hypertension and congenital pulmonary hypertension in humans (*2*) by producing pulmonary arterial vasodilation, but often the patient either does not respond or becomes refractory to the injection of acetylcholine. Such perplexing findings could be explained by the present observations; if the pulmonary arterial and arteriolar endothelium is damaged in these syndromes, one would not expect to be able to produce a consistent pulmonary dilatation with injection of acetyl-

choline. Our findings could also explain why, in shock lung syndrome, patients do not always respond to therapy with certain vasodilator drugs (*3*).

Irrespective of the exact mechanism, selective removal of endothelium from intrapulmonary arteries can transform two important circulating dilator agents into pulmonary vasoconstrictors. It is thus conceivable that damage in vivo to intrapulmonary arterial and arteriolar endothelial cells or destruction of pulmonary arterial endothelial cells by a number of agencies acting alone or in concert (for example, chronic hypoxia, release of hydrolytic enzymes, leukocyte platelet–endothelial cell interactions resulting in release of lysosomal enzymes, microaggregation of formed elements adjacent to these endothelial cells) may represent the major pathway in producing pulmonary hypertension or shock lung.

Our data may also explain why, in numerous studies on isolated pulmonary blood vessels—which are often traumatized in preparation—acetylcholine and certain other so-called vasodilators will produce contraction rather than dilation of these vessels (*6*).

NARESH CHAND
BURTON M. ALTURA
Department of Physiology, State University of New York, Downstate Medical Center, Brooklyn 11203

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7. Intrapulmonary arteries were fixed in 2 percent glutaraldehyde and 0.1*M* potassium phosphate (*p*H 7.4) for 60 minutes at 4°C, postfixed in 1 percent O_3O_4 and 0.1*M* potassium phosphate, dehydrated in a graded ethanol series, and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate. These were then examined and photographed in a JEOL 100C electron microscope.
8. Supported in part by PHS research grants NHLBI 18002, NHLBI 18015, and DA 02339 (to B.M.A.). We thank G. K. Ojakian and D. Herzlinger for performing the electron microscopy. Requests for reprints should be addressed to B.M.A.

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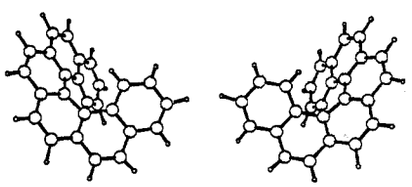
Chiral Recognition by Nucleosides and Nucleotides: Resolution of Helicenes by High-Performance Liquid Chromatography

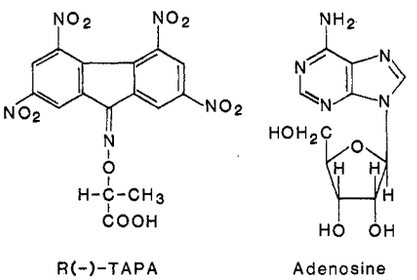
Abstract. *Chiral recognition by nucleosides and nucleotides coated on silica gel was studied by high-performance liquid chromatography. Helicenes, which are chiral polyaromatic hydrocarbons, were used as probes. Stereoselectivity was detected when the nucleobase was a purine (adenosine, deoxyadenosine, adenosine 3′-monophosphate, adenosine 5′-monophosphate, adenosine 3′,5′-monophosphate, and guanosine), but was not detected with the pyrimidine derivative uridine. For a given nucleobase (adenine), all changes in the ribose moiety affected the resolution factors, which ranged between 1.03 and 1.074. These results might be relevant to the enantioselectivity of carcinogenic metabolites of polyaromatic hydrocarbons.*

We recently described enantioselective interactions of riboflavin with chiral ortho-condensed polyaromatic hydrocarbons as manifested in resolutions of optical isomers by high-performance liquid chromatography (HPLC) (*1*). We now report a similar study with nucleosides and nucleotides coated on the column packing material. As in the riboflavin study (*1*), helicenes, which have a helical shape due to overcrowding, were the compounds resolved. Although helicenes do not occur in nature, the results may be of interest for probing the capacity of nucleic acid building blocks for chiral differentiation.



P(+)-Hexahelicene M(−)-Hexahelicene

The compounds tested were **1**, adenosine; **2**, deoxyadenosine; **3**, adenosine 3′-monophosphate (3′-AMP); **4**, adenosine 5′-monophosphate (5′-AMP); **5**, adenosine 3′,5′-monophosphate (cyclic AMP); **6**, guanosine; and **7**, uridine. Purines and pyrimidines form complexes with polyaromatic hydrocarbons. Combination of a purine or pyrimidine with a chiral substituent such as ribose could lead to chiral differentiation of optically active polyaromatic hydrocarbons, in analogy



R(−)-TAPA Adenosine

with the behavior of *R*(−)-2-(2,4,5,7-tetranitrofluorylidene-9-aminooxy)propionic acid (TAPA) (*2*) and of riboflavin (*1*). The molecular interaction of nucleobases with polyaromatic hydrocarbons differs from that of TAPA and riboflavin, since for the nucleobases the contribution of charge transfer is considered to be of little importance as compared with van der Waals forces (*3*).

The nucleosides and nucleotides were coated on 5-μm silica gel (Lichrosorb Si 100, Merck, Darmstadt, Germany) and HPLC columns were prepared by slurry packing. The amount of coated material, determined by elementary analysis, var-

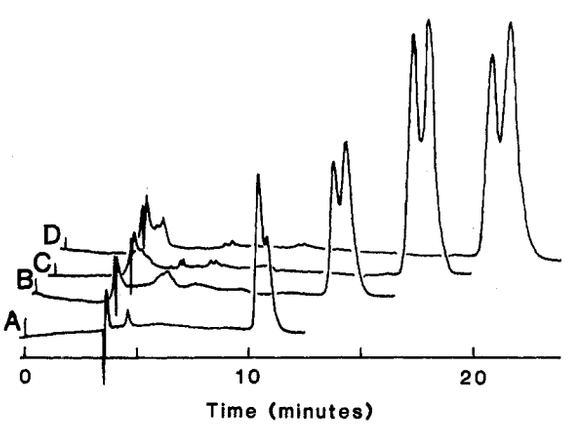


Fig. 1. Resolution of the optical isomers of [10]- to [13]-carbohelicenes on adenosine-coated silica gel. The number in brackets indicates the number of rings in an individual helicene. The chromatographic system consisted of a Waters 6000A pump, a Reodyne 7120 injector with a 20-μl loop, and an LDC ultraviolet monitor set at 254 nm. The column was 20 by 0.46 cm (inside diameter). The eluant was CH_2Cl_2 and *n*-hexane (1:9); the flow rate was 1 ml/min; and the temperature 23° to 25°C. The elution curves correspond to (A) [10]-helicene enriched in the *M*(−)isomer, and (B) [11]-, (C) [12]-, and (D) [13]-helicenes.

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