Reports

Evolutionary Significance of Morphospecies: A Test with Cheilostome Bryozoa

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Much of the controversy concerning the theory of punctuated equilibrium stems from skepticism about the biologic validity of fossil morphospecies, particularly for supposedly simple invertebrate taxa like cheilostome Bryozoa that form the bulk of the fossil record. However, evidence from breeding experiments and protein electrophoresis shows that morphospecific identity of cheilostomes is heritable and that morphospecies are genetically distinct with no indication of morphologically cryptic species. Thus paleontologists can study cheilostome evolution at the species level, and previously demonstrated patterns suggesting punctuated speciation in cheilostomes should be taken at face value.

MANY FOSSIL SPECIES APPEAR IN the record fully differentiated morphologically and persist for millions of years with little or no indication of transitional morphologies (*1, 2*). But are fossil species really biologic species, and to what extent can we recognize biologic species using only morphology, especially that restricted to preservable skeletal features (*3–5*)? A single morphologically defined species (morphospecies) might comprise two or more morphologically indistinguishable (cryptic) species (*3, 5–7*) or, alternatively, several morphospecies might be defined from a single, morphologically variable, interbreeding population (*8*). In either case, morphology would not be sufficient to discriminate species, and evolutionary stasis would be more apparent than real. We present evidence from breeding experiments and protein electrophoresis showing that skeletal characters of the kinds typically available in fossil material are indeed sufficient to discriminate biologic species of living cheilostome Bryozoa. Thus paleontologists can describe the tempo and infer the mode of evolutionary change at the species level in this group.

Cheilostomes are a diverse group of predominantly sessile invertebrates that have been abundant on the sea floor for about 100 million years (*9*). Cheilostomes grow by budding minute modules termed zooids that together constitute a colony. As conventionally distinguished, species of cheilostomes are morphospecies based on skeletal characters of ordinary feeding zooids and, where present, of specialized polymorphs (*9, 10*). Despite their asexual origin, zooids within the same colony typically vary morphologically. Variation within colonies may be as great as that between zooids of different colonies, producing overlapping ranges of variation from colony to colony. This variation provides the basis for a statistically rigorous approach to morphospecies discrimination that is applicable to both living and fossil cheilostomes (*2*).

To explore the degree to which morphospecies correspond to biologic species, we studied three distantly related genera of Panamanian cheilostomes (*11*). *Parasmittina* is morphologically complex, with nearly 40 quantifiable skeletal characters, whereas *Steginoporella* and *Stylopoma* are less complex, each with approximately 20 characters (Fig.

Table 1. Summary of morphometric analyses for three genera of Panamanian cheilostome Bryozoa. $\bar{x}$, mean.

Parameter	*Steginoporella*	*Stylopoma*	*Parasmittina*
Species distinguished morphometrically	3	4	15
Morphologic characters used			
All characters*	20	20	37
Feeding zooids†	11	12	32
Overall morphologic differences‡			
Between species			
All characters	31–103 ($\bar{x}$ = 77)	32–53 ($\bar{x}$ = 41)	14–94 ($\bar{x}$ = 58)
Feeding zooids	10–14 ($\bar{x}$ = 13)	8–10 ($\bar{x}$ = 9)	7–38 ($\bar{x}$ = 26)
Within species (feeding zooids only)			
Total§	4.9 ± 0.2‖	4.3 ± 0.2	6.0 ± 0.1
Within colonies	3.0 ± 0.3	3.5 ± 0.3	4.1 ± 0.2

*Includes vicarious avicularia and ovicelled zooids, if present. †Includes adventitious avicularia, if present. ‡Statistical distance, square root of Mahalanobis D^2. §Includes both within- and between-colonies statistical distances. ‖Mean ± standard error.

Table 2. Heritability of morphospecies identities for three genera of Panamanian cheilostomes. Offspring are considered identical to maternal parent if probability of correct identification is >0.99, averaged for three zooids per colony. Data for both parental and offspring colonies entered as "ungrouped" in third discriminant analysis (*14*). Discrepant results for nine F_1 colonies of *Stylopoma* excluded (*18*). $\bar{x}$, mean.

Morphospecies of maternal colony	Maternal colonies (n)	Probability of correct assignment ($\bar{x}$)	F_1 colonies (n)	Identical to maternal colony (%)	F_2 colonies (n)	Identical to maternal colony (%)
Steginoporella						
buskii	1	1.0000	4	100.00	0	
connexa	1	1.0000	1	100.00	0	
Stylopoma						
Species 1	39	0.9998	111	91.89	12	75.00
Species 2	45	0.9996	215	100.00	15	93.33
Parasmittina						
Species 1	1	1.0000	1	100.00	0	
Species 6	42	0.9921	115	100.00	21	100.00
Species 7	2	1.0000	3	100.00	0	
Total	131	0.9973	450	98.00	48	91.67

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1). A range of morphologic complexity was chosen to test whether ability to discriminate biologically significant taxa depends strongly on the number of available characters (*3*).

Each genus was considered separately. To minimize effects of nonheritable variation on comparisons, the first step in distinguishing morphospecies used only characters that could be measured, counted, or coded on two replicate sets of five feeding zooids in each colony (Fig. 1) (*12*). Colonies were clustered by the unweighted pair-group method with the use of arithmetic averages of intercolony statistical distances (*13*). The resulting clusters were tested for stability in a series of discriminant analyses, using first the characters of feeding zooids and then all characters, including those of polymorphs (Fig. 1) (*14*).

Results of the morphologic analyses are summarized in Table 1. Most of the difference within morphospecies (approximately 70%) is due to variation within colonies and therefore unlikely to be genetically based. Differences between morphospecies average at least two times greater than differences within species, even when only characters of feeding zooids are used. Despite previously expressed doubts (*3*), differences between morphospecies within the different genera appear unrelated to either the total numbers of characters or the numbers of species per genus.

Heritability of morphospecies identity was studied by raising offspring of colonies from different populations in a common garden experiment (*15*). Offspring colonies were grown from embryos derived from known maternal colonies but of unknown paternity (*16*). Two generations of offspring, F_1 and F_2, were raised to test for

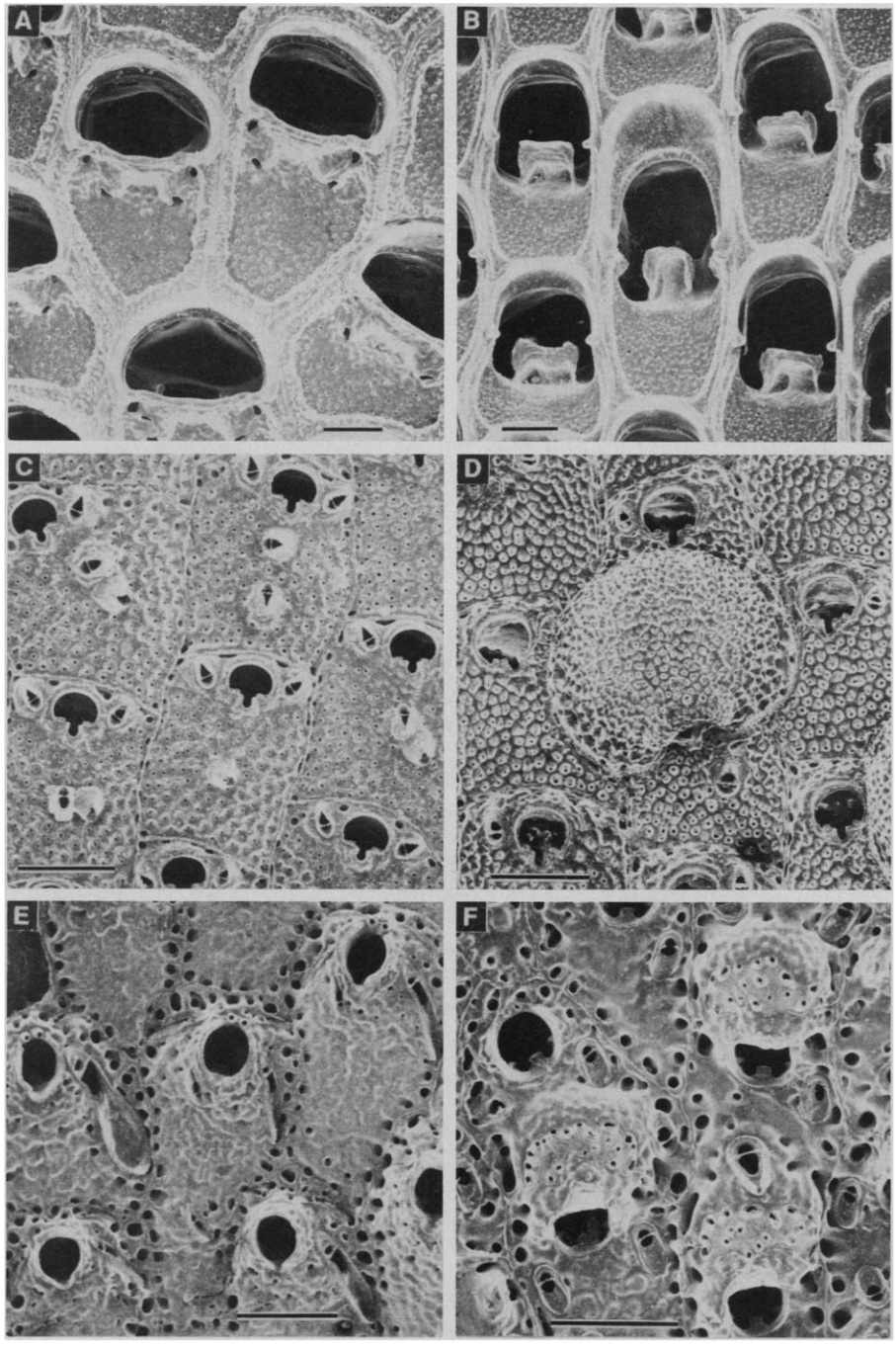

Fig. 1. Skeletal morphology of ordinary feeding zooids (**A, C,** and **E**) and polymorphs (**B, D,** and **F**) in representative species of three Panamanian cheilostome genera. Skeletons bleached in commercial sodium hypochlorite and carbon coated for scanning electron microscopy (SEM). (A and B) *Steginoporella connexa, S. magnilabris*; (C and D) *Stylopoma* species 3, *Stylopoma* species 1; (E and F) *Parasmittina* species 6, *Parasmittina* species 1; scale bars, 0.25 mm. Characters of feeding zooids: length and width of zooid, length and width of orifice (all genera); length and width of cryptocystal perforate area, length and width of polypide tube, length and width of opesiule slit, distance between adjacent opesiules (*Steginoporella*); number, size, position, and orientation of lateral-oral adventitious avicularia, number and size of proximal adventitious avicularia (*Stylopoma, Parasmittina*); frontal pore density, length and width of oral sinus (*Stylopoma*); number and disposition of frontal areolae, ornamentation of frontal surface, number of oral spines, length and width of lyrule, length and shape of oral condyles, intercondyle distance, origin of peristome, shape of lateral-oral adventitious avicularia, number and size of distal adventitious avicularia (*Parasmittina*). Characters of polymorphs: characters of B-zooids as for ordinary feeding zooids (*Steginoporella*); length and width of ovicell, number and size of adventitious avicularia on ovicells (*Stylopoma, Parasmittina*); size and shape of vicarious avicularia (*Stylopoma*); size and pattern of ovicell perforations (*Parasmittina*).

maternal effects on development, such as could result from maturation of embryos within maternal colonies in their natural environment (*17*). A total of 507 offspring of seven species were grown to a size sufficient to classify by skeletal characters.

All but nine of the offspring in all three genera were classified by discriminant analysis in the same morphospecies as their maternal parents (Table 2); the few exceptions we attribute to mixing of colonies that had fallen off their rack during a storm (*18*). In *Parasmittina* and *Steginoporella*, all offspring colonies were virtually identical to their parents ($P > 0.99$), despite having been raised in an unnatural environment. Moreover, congruence of results for the F_1 and F_2 generations of *Parasmittina* species 6 indicates an absence of maternal effects on skeletal development in this species. Slightly different results were obtained in *Stylopoma*. In both species, the probability of assignment of several F_2 colonies to their parental morphospecies dropped below 0.99 (Table 2), apparently reflecting sensitivity to the unnatural conditions of the experiment. A similar effect on the development of F_1 colonies is evident in *Stylopoma* species 1.

Horizontal starch gel electrophoresis was used to study enzyme variation in 402 colonies in three species each of *Steginoporella* and *Stylopoma* and two of *Parasmittina*. Each species was collected from one to five populations at depths of 10 to 20 m (*19*). Colonies were assigned to morphospecies by visual inspection, and identifications were checked for 10 to 20% of the colonies by the same procedure used to establish morphospecies identities in the heritability experiment. These checks confirmed the initial taxonomic assignment in all cases, at probabilities averaging more than 0.99 and in no case less than 0.92. We scanned more than 30 enzymes using conventional methods and staining (*20*). Only seven loci can be consistently resolved and scored, but all are polymorphic and vary within and between morphospecies (Table 3).

There were no diagnostic alleles at any locus between any pair of populations of the same morphospecies (*21*). Moreover, values of Nei's unbiased genetic distance D between any two local populations of the same morphospecies were always less than 0.08, with average values per morphospecies pair ranging from 0.00 to 0.03 (*22*). These averages are similar to those reported for local population differences and considerably lower than those for cryptic species or subspecies in other animals (*6, 23*).

Gene frequency data for each species with all populations combined are presented in Table 3. There are diagnostic alleles between each of the pairs of morphospecies within all three genera. In addition, genetic distances between morphospecies are consistently much higher than between populations of the same morphospecies (*24*). Thus problems of false species distinctions can be discounted at least for these cheilostome morphospecies.

Our results show that the identity of quantitatively defined morphospecies of cheilostome bryozoans is both heritable and unambiguously distinct genetically. The im-

Table 3. Allele frequencies in eight morphospecies of Panamanian cheilostomes, all local populations combined, where n is the number of individuals (colonies) per species per locus. GPI, glucosephosphate isomerase; TPI, triosephosphate isomerase; PEP, peptidase; EST, esterase; and MDH, malate dehydrogenase. The alleles identified for each locus are represented by *a* to *h*. Alleles are presented in order of mobility on the gel, *a*, representing slowest, *h* representing fastest. Allele designations for GPI locus in *Parasmittina* were not compatible with other genera because of different gel buffers. The probability of assigning correctly an individual to one of two species based only on a single locus is explained in the footnote to this table [see also (*21*)].

Locus	*Steginoporella*			*Stylopoma* species			*Parasmittina* species	
	buskii	*magnilabris*	*connexa*	1	2	3	1	6
GPI								
(*n*)	(41)	(77)	(17)	(36)	(85)	(9)	(13)	(12)
a					0.188			1.000
b				0.139	0.812	0.889	1.000	
c		0.019		0.583		0.111		
d		0.097		0.278				
e		0.481						
f	0.524	0.403						
g	0.476		1.000					
TPI								
(*n*)	(35)	(44)	(15)					
a	1.000							
b		0.920						
c		0.080	1.000					
PEP I								
(*n*)	(18)	(78)	(16)					
a	0.278	1.000						
b	0.722		1.000					
PEP II								
(*n*)	(42)	(102)	(61)	(63)	(90)	(10)	(9)	
a				0.286				
b				0.714	0.922	1.000		
c					0.078			
d							1.000	
e	0.024	1.000						
f			1.000					
g	0.929							
h	0.048							
PEP III								
(*n*)		(102)	(61)	(64)	(90)	(10)	(13)	(12)
a								1.000
b				0.016			0.962	
c		0.015	0.926			1.000	0.038	
d		0.985	0.074		1.000			
e				0.984				
EST II								
(*n*)	(43)	(88)	(50)	(57)	(89)	(9)		
a				0.026	0.011			
b				0.833	0.096			
c				0.140	0.893	1.000		
d	1.000							
e		0.602	0.680					
f		0.369	0.160					
g		0.028	0.160					
MDH								
(*n*)							(13)	(12)
a								0.042
b								0.958
c							1.000	

Diagnostic loci between morphospecies,** $P > 0.999$; *$P > 0.99$. Between species of *Steginoporella: buskii-magnilabris*, TPI**, PEP II**, EST II**; *buskii-connexa*, TPI**, PEP II**, EST II**; *magnilabris-connexa*, GPI**, PEP I**, PEP II**. Between species of *Stylopoma*: species 1 and 2, GPI*, PEP III**; species 1 and 3, PEP III**, EST II*; species 2 and 3, PEP III**. Between species of *Parasmittina:* species 1 and 6, GPI**, PEP III**, MDH**.

portance of rigorous quantitative analysis was underlined by our discovery of three species of *Stylopoma* previously classified as one, a separation subsequently confirmed genetically. The widely supposed lack of correspondence between morphospecies and biospecies (*3, 5*) may result as much out of uncritical acceptance of outdated, subjectively defined taxa as from any fundamental biologic differences between the two kinds of species.

Absence of cryptic species is not as well established because of the limited number of consistently resolvable loci. Nevertheless, high concurrence of shared common alleles and absence of diagnostic alleles among local intraspecific populations suggest that cryptic species are rare. Thus cheilostome morphospecies appear to be good biologic species, at least within local populations and between those up to 100 km apart. Even this distance should be sufficient to restrict gene flow severely between cheilostome populations. This is because larval dispersal by cheilostomes is typically very short (*9*), a pattern that might be expected to bias against our results. Nevertheless, the possibility of geographically segregated cryptic species needs testing on a larger scale.

Similarly good correspondence between genetically and morphologically defined species is apparent for scattered examples of marine organisms with preservable skeletons, the land snail *Cerion*, and various vertebrates and amphipods (*25*). In contrast, cryptic species are common among soft-bodied ctenostome bryozoans and sea anemones, the marine snail *Crepidula*, freshwater mollusks, salamanders, and butterflies (*4, 7, 26*), so that each major taxon clearly needs to be examined case by case. Such diversity is expected given differences in ecology, behavior, limits to distribution, and potential isolating mechanisms that exist between taxa and environments. Regardless of problems in other groups, however, the consistency of our results across three distantly related cheilostome genera suggests that previously documented patterns of morphologic stasis punctuated by relatively sudden appearances of new morphospecies in the cheilostome *Metrarabdotos* (*2*) do indeed reflect patterns of evolution at the species level. This is consistent with the punctuated equilibrium model.

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11. Collections were made with scuba and by dredging along the Caribbean coast between the Holandes Cays in the San Blas Archipelago and Portobelo, and in the Gulfs of Panama and Chiriqui on the Pacific coast. Colonies from both oceans were included in the morphometric analyses for discrimination of morphospecies, but subsequent studies of heritability and electrophoresis were restricted for logistic reasons to common Caribbean species.
12. Colonies were not preassigned to species; instead, the numbers of species and their morphologic limits were developed empirically from the morphometric data. In order to maximize the sensitivity of morphologic discrimination, morphospecies were distinguished on the smallest statistically significant intercolony differences in zooid morphology. Two independently varying sets of feeding zooids were measured in most colonies to provide a check against oversplitting.
13. All calculations were made with the Statistical Package for the Social Sciences (SPSS/PC+) Advanced Statistics. Statistical distances between colonies were calculated as the square root of Mahalanobis D^2. The level of clustering was chosen by a modified jackknife procedure in which the replicate within-colony sets were successively dropped out and statistical distances and clustering recalculated. The final level separated clusters in such a way that all colonies in any cluster were distinct from those in others at $P < 0.001$, and replicate sets of zooids from the same colony were placed in the same cluster at $P > 0.999$.
14. In the first discriminant analysis, in which we used zooid data from the replicate within-colony sets, the percent of zooids "correctly classified" in clusters was 100 in *Steginoporella*, 98.52 in *Stylopoma*, and 99.60 in *Parasmittina*. Similar results were obtained in two subsequent discriminant analyses, first using data for three zooids each from a new group of colonies, not used in determining the original clusters, and then the combined zooid data from all colonies. In a fourth and final discriminant analysis, zooid data were averaged to form colony means and mean values for characters of polymorphs were added; the percent of colonies "correctly classified" then was 100 in all three genera.
15. Experimental design modified from F. J. S. Maturo, in *Living and Fossil Bryozoa*, G. P. Larwood, Ed. (Academic Press, London, 1973), pp. 577–584. The experiment was done at the Smithsonian field station just east of San Blas Point. Bryozoans were collected from one to five sites, depending on species, between Holandes Cays and Isla Grande in depths of <1 to >40 m and were maintained in running sea water for usually no more than 1 day before use.
16. Substrata with bryozoans containing embryos were cleaned of other organisms and isolated in delivery chambers made from plastic food containers with sides cut open and replaced by plankton net. Filtered sea water entered each chamber from above and exited through the net walls. One maternal colony was placed in each chamber under pieces of bare coral substratum, which were removed after 5 to 10 days. Substrata with newly settled F_1 bryozoans were placed in the sea attached to concrete blocks on a sandy bottom at ~0.5 m below low water. A monthly census was made to record the condition of colonies and to clean the substrata of other organisms.
17. F_1 colonies with embryos were returned to the delivery chambers to obtain F_2 recruits, after which the process was repeated. Outbreak of an infestation of planaria and nearby passage of a hurricane forced early termination of the experiment in late October 1988 when most F_2 colonies had not reached reproductive maturity.
18. Five F_1 colonies of *Stylopoma* species 1 and four of species 2 differed in morphospecies assignment from their parents, all with $P = 1.0000$. Some mix-ups apparently occurred in returning fallen colonies to their positions after a storm. Substrata were distinguished by their distinctive shapes and position on the blocks, but were not marked.
19. Collecting sites: Palina West, Isla Grande (IG); Ulagsukun west and east (UW, UE), on the southeast coast of San Blas Point; Aguadargana, southwest and northeast (ASW, ANE), 0.5 km east of STRI station. Locations of these reefs are illustrated in H. A. Lessios *et al.* [*Coral Reefs* **3**, 173 (1984)] and J. B. C. Jackson *et al.* [*Science* **243**, 37 (1989)]. Sample sizes for each species on each reef: *Steginoporella buskii* (25 UW, 18 UE); *S. magnilabris* (23 IG, 27 UW, 21 UE, 20 ANE, 16 ASW); *S. connexa* (25 UW, 27 UE, 9 ASW); *Stylopoma* species 1 (13 IG, 26 UW, 25 UE, 2 ASW); *Stylopoma* species 2 (19 IG, 26 UW, 23 UE, 11 ASW, 11 ANE), *Stylopoma* species 3 (6 IG, 2 ASW, 2 ANE); *Parasmittina* species 1 (13 IG); *Parasmittina* species 6 (3 ASW, 9 ANE).
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27. J. Sanner visually sorted, prepared, and measured specimens using computer programs developed by R. Chapman and J. Russo. A. Herrera collected specimens and ran the heritability experiment; in this she was assisted by J. Jara and H. Ramos. L. Weigt did the electrophoresis. S. Braden prepared the scanning electron photomicrographs. L. Castell, I. Gonzalez, H. M. Guzman, G. Iturrado, G. Jacome, C. Jimenez, E. Padron, A. Rodaniche, A. Velarde, and L. Wiskowski provided additional

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Anesthesia Cutoff Phenomenon: Interfacial Hydrogen Bonding

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Anesthesia "cutoff" refers to the phenomenon of loss of anesthetic potency in a homologous series of alkanes and their derivatives when their sizes become too large. In this study, hydrogen bonding of 1-alkanol series (ethanol to eicosanol) to dipalmitoyl-L-α-phosphatidylcholine (DPPC) was studied by Fourier transform infrared spectroscopy (FTIR) in DPPC-D_2O-in-CCl_4 reversed micelles. The alkanols formed hydrogen bonds with the phosphate moiety of DPPC and released the DPPC-bound deuterated water, evidenced by increases in the bound O–H stretching signal of the alkanol-DPPC complex and also in the free O–D stretching band of unbound D_2O. These effects increased according to the elongation of the carbon chain of 1-alkanols from ethanol (C_2) to 1-decanol (C_{10}), but suddenly almost disappeared at 1-tetradecanol (C_{14}). Anesthetic potencies of these alkanols, estimated by the activity of brine shrimps, were linearly related to hydrogen bond–breaking activities below C_{10} and agreed with the FTIR data in the cutoff at C_{10}.

THE ANESTHETIC POTENCY OF 1-ALkanols increases with elongation of their carbon chains, but the potency suddenly disappears when the carbon-chain length exceeds about C_{12} (*1*). No anesthetic potency is observed above C_{14}. This phenomenon is known as "cutoff" and several explanations have been proposed, such as the size of the anesthetic binding site is not large enough to accommodate longer alcohols, or the low water solubility of longer 1-alkanols limits their access to the action sites (*2*). With DPPC vesicle membranes, the main transition temperature between the liquid-crystalline and solid-gel phases decreases by the addition of short-chain 1-alkanols, but increases by long-chain 1-alkanols (*3*). The switchover from depression to elevation of the transition temperature occurs at the same carbon-chain length as the cutoff point. This suggests that the disordering effect of anesthetics on the hydrophobic core of the membrane determines the cutoff phenomenon.

The above result on the phase transition of lipid membranes supports the lipid theory of anesthesia: anesthetics disorder and expand the lipid membrane, and the increased lateral pressure interferes with the electrogenic ionic flow through the ion channels ensuing anesthesia (*4*). Against the lipid theory, which assumes nonspecificity of anesthetic actions, there are proponents for the protein theory who advocate that anesthetics interact with specific receptors of certain proteins (*5*).

We contend that anesthetics interact with macromolecules, irrespective of proteins or lipid membranes, and disrupt water-macromolecule associations. These structures are supported by the hydrogen-bonded matrix of water molecules. Only in water, proteins are formed into meaningful structure or optimal conformations for the assigned biological activities, such as enzyme functions or solute transports. Similarly, lipid membranes are assembled by the interaction with water. When anesthetics disengage the macromolecular structure from the constraint of water matrix, these structures become disordered and expand. The relaxed conformations would be suboptimal for their functions and may be accompanied by global attenuation of biological activities leading to anesthesia. In fact, anesthetized states are characterized by generalized depression of all metabolic activities. Eyring and co-workers proposed that the primary action site of anesthetics is the water-macromolecule interface, releasing electrostricted water molecules from the surface charges with disruption of hydrogen bonds (*6*).

Sandorfy and co-workers (*7*) found in a mixture of secondary aliphatic amines and brominated fluorocarbons that the hydrogen-bonded N–H band decreased when the temperature was lowered and disappeared completely at −190°C, while the free N–H stretching band increased. This was unexpected, because lowering the temperature melted ice. They further showed that volatile anesthetics also disrupt hydrogen bonds and that the anesthetic potency correlates to their hydrogen bond–breaking activity. With 1-alkanols in water-in-oil surfactant-reversed micelles, the association constant between alcohols and the hydrophilic group of the surfactant decreased with elongation

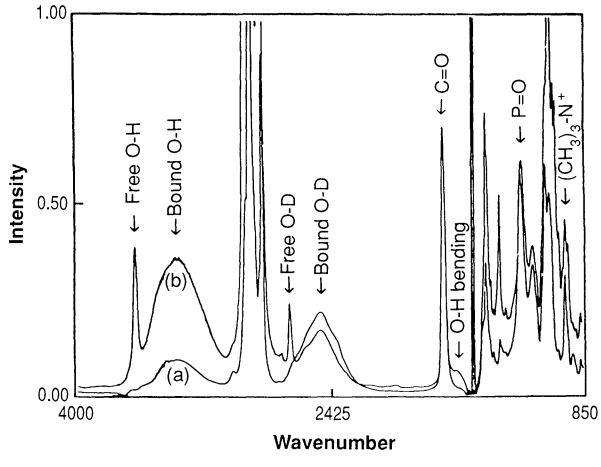

Fig. 1. Difference IR spectra of D_2O-in-CCl_4 reversed micellar systems at 22°C after subtraction of CCl_4 absorption bands: (a) DPPC 0.008*M* in the absence of 1-butanol and (b) DPPC with 0.1*M* of 1-butanol. Synthetic DPPC, D_2O (nominal isotopic purity 100%) and 1-butanol were obtained from Sigma, and spectroscopic-grade CCl_4 from EM Science (Cherry Hill, New Jersey). Carbon tetrachloride was dried by activated aluminum oxide (Fluka) columns. The absence of water was confirmed by infrared spectroscopy. DPPC was kept in a desiccator at reduced pressure until use. D_2O-DPPC-CCl_4 mixtures were sonicated by a Branson ultrasonic disrupter (Danbury, Connecticut) in a cup-horn until clear dispersions were obtained (about 20 min). Each sample was scanned 20 times over the frequency range of 400 to 4000 cm^{-1}. All spectra were acquired at 22.0°C. A Perkin-Elmer model 1750 FTIR spectrophotometer interfaced with a Perkin-Elmer model 7300 computer was used for analysis. The window (FT 04-794) was a fixed-thickness zinc-selenide cell with 1.18-mm pathlength (Spectra-Tech). The cell was calibrated against the benzene band at 845 cm^{-1}. A triglycine sulfate detector was used for all experiments. Assignments of the major peaks are $(CH_3)_3$-N^+ stretching of the choline head at 970 cm^{-1}, P=O stretching at 1238 cm^{-1}, C=O stretching of the ester group at 1734 cm^1, O=D stretching of the free and bound D_2O at 2690 cm^{-1} and 2495 cm^{-1}, respectively, and O–H stretching of the free and bound 1-butanol at 3635 cm^{-1} and 3400 cm^{-1}, respectively. The small absorbance of bound O–H stretching at 3400 cm^{-1} in the control DPPC spectrum (a) may originate from the traces of water contaminating the DPPC and D_2O.

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