chain acids in the 2-position of the phosphoglycerides (*8*). It appears from these experiments that a functional biomembrane can be formed in which a single fatty acid—elaidic acid—forms at least 97 percent of the total, and that extremely wide variations in fatty-acid composition are possible.

Unlike bacteria, most mycoplasmas (including strain Y) require sterol for growth and incorporate large amounts of sterol into their membranes. It has been postulated that cholesterol stabilizes biological membranes by causing a closer packing of the fatty-acid chains (*9*). The nature of the component fatty acids has a marked influence on the condensing effect of cholesterol (*9*). A phospholipid with a *trans* monoenoic acid in the 2-position may form a condensed layer, and cholesterol may not be needed to perform this condensing function in membranes in which a *trans* acid occupied the 2-position in the phosphoglycerides (*10*). Although nothing is known about the positional distribution of fatty acids in the phospholipids of *Mycoplasma* strain Y when grown with different pairs of fatty acids, the ratio of cholesterol to lipid phosphorus did not vary significantly with these alterations in fatty-acid composition (Table 1, last column).

ALAN RODWELL
Commonwealth Scientific and Industrial Research Organization, Animal Health Research Laboratory, Parkville, Victoria 3052, Australia

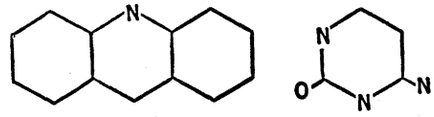
References and Notes

1. S. Razin, *Ann. N.Y. Acad. Sci.* **143**, 115 (1967).
2. J. D. Pollack and M. E. Tourtellotte, *J. Bacteriol.* **93**, 636 (1967).
3. A. W. Rodwell, *Ann. N.Y. Acad. Sci.* **143**, 88 (1967).
4. Lipids were extracted from the cells with 19 volumes of a mixture of chloroform and methanol (2 : 1), and the extracts were washed with 0.1*M* KCl. Methyl esters of fatty acids were prepared by direct alkaline methanolysis [J. L. Foote, R. J. Allen, B. W. Agranoff, *J. Lipid Res.* **6**, 518 (1965)] of the lipids and separated from cholesterol by chromatography on Florisil columns [K. C. Kopaczyk and N. S. Radin, *ibid.*, p. 140 (1965)]. An F and M 810 instrument with flame-ionization detector was used for gas-liquid chromatography. Columns were packed with 15 percent ethylene glycol succinate (EGS) or 10 percent Apiezon L on Diatoport S and were calibrated with National Heart Institute (N.H.I.) standard mixtures. Methyl pentadecanoate was the internal standard. Cholesterol was eluted from the Florisil (activated magnesium silicate preparation manufactured by Floridin Co., Tallahassee, Florida) columns with a chloroform-methanol system (3 : 1) and was determined colorimetrically [H. D. Wycoff and J. Parsons, *Science* **125**, 347 (1957)]. Lipid phosphorus was determined in the water-soluble products of alkaline methanolysis.
5. R. F. Chen, *J. Biol. Chem.* **242**, 173 (1967).
6. The fatty-acid methyl esters were prepared, and were separated from cholesterol as described in (*4*). They were chromatographed on thin layers of silica gel G impregnated with silver nitrate and developed in a diethyl ether–light petroleum system (5 : 95) [L. J. Morris, in *New Biochemical Separations*, A. T. James and L. J. Morris, Eds. (Van Nostrand, London, 1964), p. 296]. Spots were located by spraying with sulfuric acid and charring.
7. M. G. MacFarlane, *Biochem. J.* **79**, 4P (1961); J. H. Law, H. Zalkin, T. Kaneshiro, *Biochim. Biophys. Acta* **70**, 143 (1963).
8. F. Meyer and K. Bloch, *J. Biol. Chem.* **238**, 2654 (1963).
9. L. L. M. van Deenen, *Phospholipids and Biomembranes* (Pergamon, Oxford, 1965), pp. 12–15; R. A. Demel, L. L. M. van Deenen, B. A. Pethica, *Biochim. Biophys. Acta* **135**, 11 (1967).
10. D. Chapman, N. F. Owens, D. A. Walker, *Biochim. Biophys. Acta* **120**, 148 (1966).
11. I thank B. Zwolak for technical assistance.
26 February 1968

Crystal Structure of a Complex of Acridine, Cytosine, and Water (1 : 1 : 1)

Abstract. *X-ray structural analysis of a crystalline complex between acridine $C_{13}H_9N$, cytosine $C_4H_5N_3O$, and water (1 : 1 : 1) has been completed. The cytosine and water molecules form a sheet-like structure through a series of hydrogen bonds. The acridine molecules are bound to this layer through a hydrogen bridge from the water to the ring nitrogen. The acridine molecules stack in a parallel fashion normal to the cytosine-water sheets, with an average interplanar spacing of 3.5 angstroms.*

Many members of the acridine family elicit mutagenic effects through their interaction with DNA. Lerman (*1*) has proposed from a wide variety of experimental evidence that the acridine dyes bind by intercalation between adjacent hydrogen-bonded base pairs in the helix of DNA. Optical studies (*2*) with various dyes have indicated that electrostatic forces may be of primary importance. Recently, Pritchard *et al.* (*3*) presented a modified version of the Lerman model to account for the electrostatic interactions. However, the exact mode and site of the acridine interaction is uncertain. In order to provide more definitive information about these interactions, I have made a crystallographic study of a complex of acridine (I), cytosine (II), and water (1 : 1 : 1).



I II

Small needles of the complex were obtained from a solution of water and alcohol containing equal molar quantities of acridine and cytosine. These crystals were determined to be orthorhombic (space group *Pbca*), having cell dimensions of *a* equal to 19.95 Å, *b* equal to 9.327 Å, and *c* equal to 16.908 Å. In attempts to measure the density of the crystals in nonaqueous solvents, it was found that the crystals rapidly changed to anhydrous cytosine. The intensity data were collected on a GE XRD-6 diffractometer equipped with a single-crystal orienter. These intensities were measured by the stationary counter-stationary crystal technique with balanced filters used for the Cu$K\alpha$ radiation. A marked drop in the number of measurable reflections at two theta values greater than 100°, as a result of the thermal motion of the complex, made it feasible to only collect data to this value. Of the 1614 independent reflections measured, 1037 had values significantly greater than their respective background count. The intensities were converted to structure factors (*F*'s) and normalized structure factors (*E*'s) in the usual manner.

The phases of 230 reflections having *E* greater than 1.4 were determined by use of the Sayre relationships (*4*). A program written by Long (*5*) was used for this purpose. An *E* map calculated with the most consistent set of signs determined for the 230 planes revealed the positions of all the nonhydrogen atoms. The structure was then refined by the least-squares method using a block diagonal approximation to the normal equations. At the present stage of refinement, the *R* value (usual discrepancy index) is 0.112 for the observed data and with all the nonhydrogen atoms having anisotropic temperature factors. Because of the high temperature factor of the complex (average isotropic B approximately 8 Å^2), difficulty in obtaining accurate hydrogen positions is being experienced. It is through analysis of bond lengths and angles that the hydrogen bonding scheme has been proposed.

The bond lengths and angles obtained for the acridine and cytosine molecules agree well with those found in x-ray analyses of crystals of these

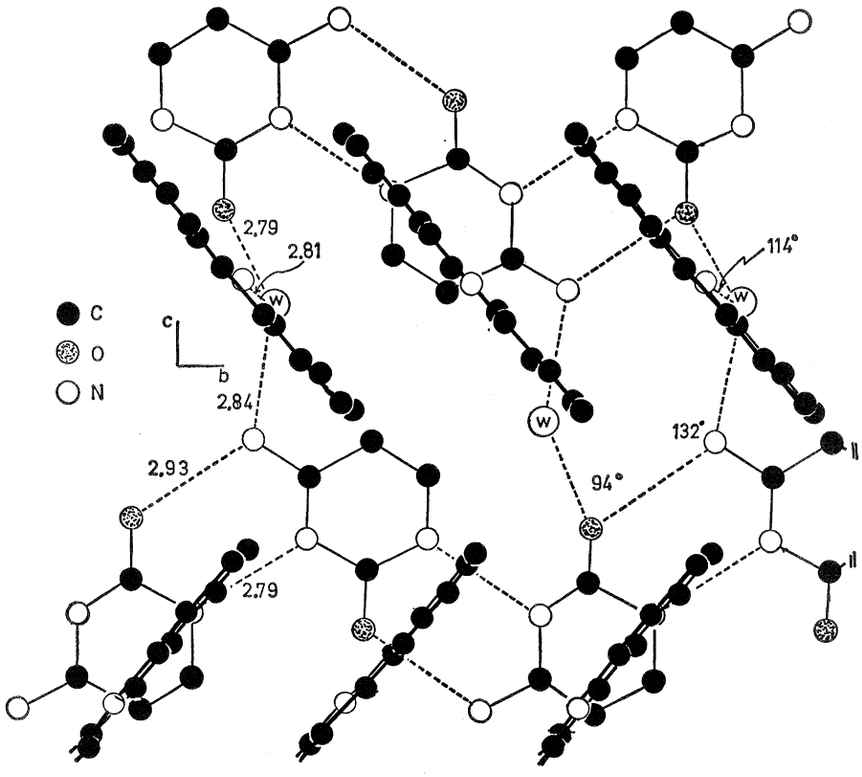


Fig. 1. Projection of molecules down the *a*-axis, showing hydrogen bonds.

respective compounds (*6*, *7*). The acridine molecule appears to be symmetric about the middle ring with none of the differences between the two halves exceeding 1 estimated standard deviation. The acridine and cytosine molecules are essentially planar as the root-mean-square deviations for the respective least-squares planes are 0.003 and 0.005.

From a biological standpoint, the most interesting facet of the structure is the molecular arrangement within the lattice. The packing of the molecules as seen down the *a*-axis is shown in Fig. 1. The cytosine and water molecules form a hydrogen-bonded network perpendicular to *a*. The molecules are held in this sheet-like framework by N1–(H)...N3, N4–(H)...O2, N4–(H)...(H_2), and O–(H)...O2 hydrogen bonds. The acridine molecules are bound to this layer through a hydrogen bridge between the water and the ring nitrogen. The acridine planes are stacked in a regular fashion perpendicular to the cytosine molecules. These stacked planes are very close to being parallel to one another (there is a 3° distortion), and they have an average interplanar separation of 3.5 Å. The dye-dye stacking thus appears to be governed by normal van der Waals forces, with the specific arrangement of the acridine molecules being influenced by the topological nature of the electron cloud of the ring. The dominant force of this association complex is therefore electrostatic in character, that is hydrogen bonding.

Most interesting is the fact that the predominant features of the packing are consistent with the study of Finkelstein and Weinstein (*7*) on the interaction of proflavin with various synthetic nucleic acids. Their findings with polycytidylate indicated that the interaction is primarily "ionic" and that dye-dye stacking was also present.

ELI SHEFTER

Department of Pharmaceutics,
School of Pharmacy,
State University of New York, Buffalo

References and Notes

1. L. S. Lerman, *J. Cell. Comp. Physiol. Suppl. 1* **64**, 1 (1964).
2. D. S. Drummond, V. F. W. Simpson-Gildemeister, A. R. Peacocke, *Biopolymers* **3**, 135 (1965).
3. N. J. Pritchard, A. Blake, A. R. Peacocke, *Nature* **212**, 1360 (1966).
4. D. Sayre, *Acta Cryst.* **5**, 60 (1952).
5. R. E. Long, thesis, University of California, Los Angeles (1965).
6. D. C. Phillips, *Acta Cryst.* **9**, 237 (1956); G. A. Jeffrey and Y. Kinoshite, *ibid.* **16**, 20 (1963); D. L. Barker and R. E. Marsh, *ibid.* **17**, 1581 (1964).
7. T. Finkelstein and I. B. Weinstein, *J. Biol. Chem.* **242**, 3763 (1967).
8. Supported by PHS grant CA-10104. I thank the computing center for a generous allotment of computing time, Mrs. Phyllis Sackman for technical assistance, and Dr. Z. F. Chmielewicz for informative discussions.

19 February 1968

Biosynthesis of Pectic Substance in Germinating Pollen: Labeling with Myoinositol-2-^{14}C

Abstract. *Growing pollen tubes provide a convenient system for study of the biosynthesis of primary cell wall components of higher plants. Pollen of* Lilium longiflorum *(cv. Ace) which was germinated and grown for periods up to 8 hours in a medium that contained pentaerythritol, a metabolically inert substitute for sucrose, removed myoinositol from the medium and utilized this cyclitol as a precursor of galacturonosyl and pentosyl units for the biosynthesis of pectic substance in pollen tube walls.*

Conversion of myoinositol (MI) to uronosyl and pentosyl units of pectic substance and acidic pentosans has been described (*1*). This process appears to be common among higher plants. Efforts to study the biochemical nature of this conversion in detail have been hampered by lack of a suitable system in which a satisfactory rate of conversion can be obtained during a single working day. Germinating pollen holds promise as such a system (*2*). In this report, uptake and incorporation of label from MI-2-^{14}C into pollen tube walls of *Lilium longiflorum* (cv. Ace) are described.

Pollen grains, harvested from greenhouse-grown plants and stored at 2°C for up to 7 weeks, were prewashed and then germinated in Dickinson's pentaerythritol medium (*3*). Dickinson observed that pentaerythritol was not utilized by germinating *L. longiflorum* pollen as a source of metabolizable carbon (*4*). Germination, 60 percent after 2 hours of incubation at 28°C, corresponded to that obtained in medium in which pentaerythritol (0.145*M*) was replaced by sucrose (0.29*M*), but average tube lengths in the latter (1.6 mm in 3 hours) exceeded those in the former (1.2 mm in 3 hours). Average values for percent of pollen germinated and tube length at various times are given in Fig. 1.

In a typical determination, pollen (5 mg) was added to medium (1 ml) containing MI-2-^{14}C (10^{-3} μmole, approximately 30,000 count/min). After incubation, the suspension was filtered in a centrifugal filter holder (Gelman No. 4305) fitted with a type GA-4 membrane. Washed pollen or residue, together with its supporting membrane, was transferred to a liquid scintillation vial that had been prefilled with Cab-O-