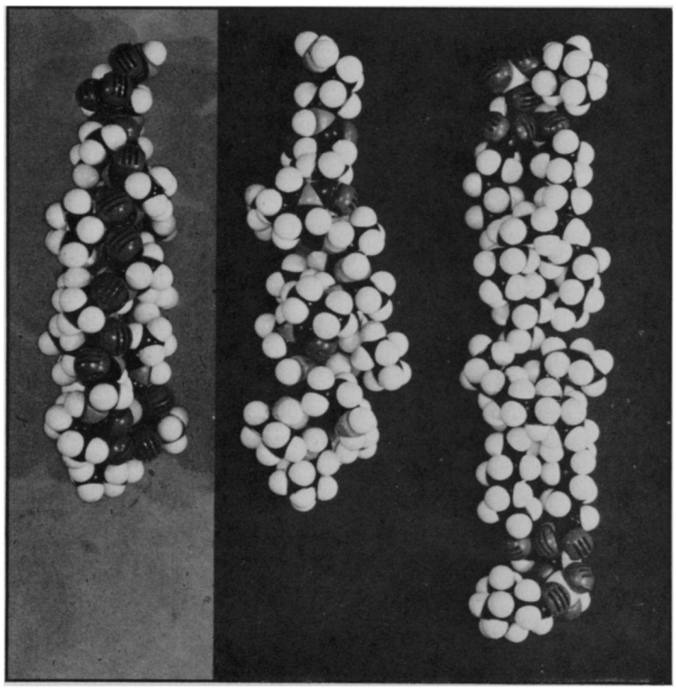
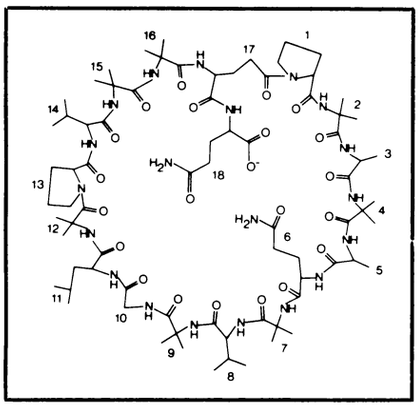
Exploiting Synthetic Membranes

Synthetic cell membranes are being used to explore nerve firing, the operation of drugs at the molecular level and other membrane phenomena

by Joan Arehart-Treichel

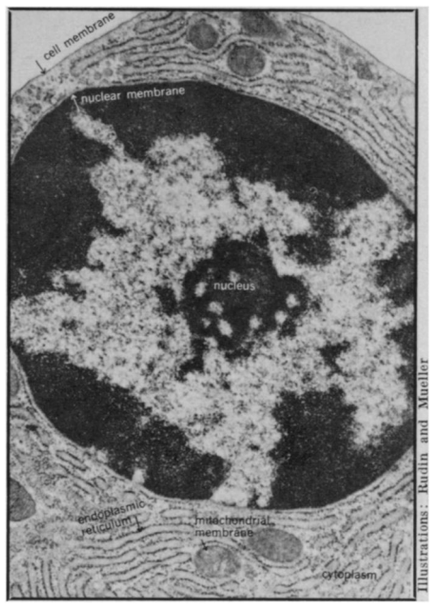


Some of the chemical molecules, in three dimensions, that make up the synthetic membrane (photo at right). At the left and in the middle of the photo are two side views of the alamethicin molecule. At right is a pair of lipid molecules, thousands of which stack up on each other to make a membrane. The chemical configuration of alamethicin in two dimensions (photo below).



During the 1940's and 1950's, biologists made spectacular progress in understanding the basis of life—cells and their molecular machinery. Then during the 1960's biologists turned from probing cells and their subunits to synthesizing them. Two molecular biologists who were foremost in the synthesis of the membrane that surrounds the cell, the so-called plasma membrane, were Donald O. Rudin and Paul Mueller of the Eastern Pennsylvania Psychiatric Institute.

During the past 15 years, Rudin, Mueller and their colleagues have indeed managed to synthesize a plasma membrane. Now they are using their artificial membrane to learn more about the role of the membrane in nerve cell firing, the way drugs work at the molecular level and whether membrane defects might underlie schizophrenia. They also hope to synthesize membranes that reside within the cell.

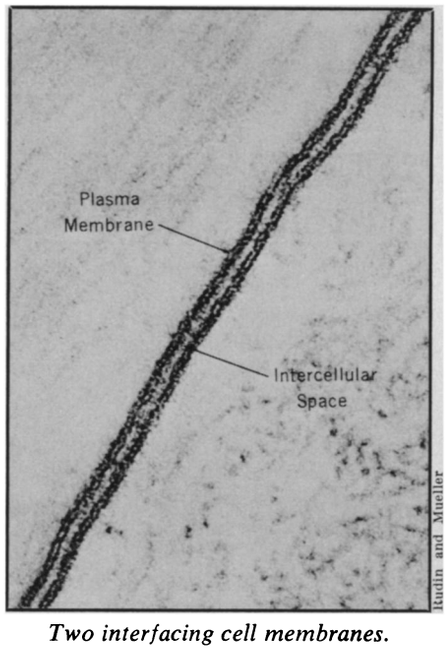


A cell magnified 10 million times.

Biologists in the 1950's and 1960's learned a lot about plasma membrane structure and function. The membrane appeared to consist of a double layer of lipid molecules (specifically of hydrocarbon chains). Interspersed throughout the lipids were proteins that serve as channels for molecules and ions. These channels allow molecules and ions to slip in and out of a cell. Rudin and Mueller were closely attuned to these discoveries and decided to try to synthesize a plasma membrane. But how should they go about it? They found their inspiration in the chemistry of soap film.

A soap film stretched across a metal ring, as for soap bubble blowing, has much in common with a plasma membrane. The film will undergo a thinning process until it is a double layer of lipid molecules, specifically of hydrocarbon chains. It is then 50 angstroms in thickness, precisely that of a plasma membrane. Rudin and Mueller took lipid molecules from a plasma membrane and dissolved them in organic solvent and spread them over a metal ring under water. The lipids that spread across the ring looked like a soap film across a ring. They shimmered in rainbow colors. If scientists blew with water at the lipid film, it formed a bubble, just as soap film would. The artificial membrane that the Philadelphia molecular biologists had created, however, was several hundred thousand times more impermeable to various substances than a natural membrane.

Then they tackled the next challenge. They put proteins into their membrane to make it permeable—to serve as channels for the passage of ions and molecules. They found a protein called alamethicin that, if added to the solution containing the membrane, would incorporate itself in the membrane like a channel. The investigators attached an electrode to the water phase on each side of the channel and applied electric current. The current could open and close the channel and thus control passage of potassium ions. Potassium is one of the ions that passes most often through the plasma membrane. Then



Two interfacing cell membranes.

they found another protein, protamine, that charged the alamethicin channel, thus permitting only chloride ions to pass through the membrane. Thus they incorporated both alamethicin and protamine-alamethicin channels into the membrane, and the membrane had two kinds of ion gates.

Now the artificial membrane was, for all practical purposes, a synthetic membrane, both in structure and in function. What's more, it could produce nerve impulses indistinguishable from those of living nerve cells.

Rudin, Mueller and their co-workers have used their synthetic membrane to confirm the well-established theory of the nerve impulse, proposed by Nobel laureates Alan L. Hodgkin and Andrew F. Huxley of Cambridge University. The theory states that the nerve impulse—action potential—consists of the transport of ions of opposite charges through the nerve cell membrane, and this transport is controlled by electric current.

Ordinarily there are more positive potassium ions inside a cell than outside. Thus the potassium ions tend to leak through the membrane. This leakage of positive potassium ions produces the resting potential of the membrane. The same situation exists for the synthetic membrane of the Philadelphia team. Then the team applied current to their membrane. The current caused the faster chloride channel in the membrane to open. Negative chloride ions roared through the channel and outside the membrane. Now the outside of the membrane was more negative than positive, producing the peak of the nerve impulse (action potential). But the excess negative ions now lured more positive potassium ions out of the membrane, thus returning the membrane to its resting potential, and a closing of the ion gates.

"So," Rudin says, "we have synthesized nerve cell firing in our synthetic membrane. The firings can beat spontaneously like heart beats or we can control the frequency of the firings."

Only recently have the Philadelphia molecular biologists started looking at the effects of drugs on their synthetic membranes. They have found that the local anesthetic procaine blocks the nerve impulse in a synthetic membrane by blocking the chloride channel. On the other hand, one of the major tranquillizers used to treat schizophrenia—chloropromazine—reduces the nerve impulse in the membrane by blocking the potassium channel. Such action may explain how the drug helps schizophrenics. Nerve impulses in the brain lead to the production of the nerve transmitter dopamine, and an excess of dopamine, the result of a genetic defect, has been linked to schizophrenia.

Such discoveries, Rudin, Mueller and their colleagues believe, represent a first step toward molecular pharmacology—understanding how drugs work at the molecular level, and thus tailoring drug therapy accordingly.

The molecular biologists suspect that one or several ion channels in the membranes of the brains of schizophrenics may be faulty and hence trigger or at least exacerbate the disease. Thus they hope to use a synthetic membrane to probe for such defects. They would isolate and purify some of the ion channels in the membranes of the brains of schizophrenics. They would insert the channels into their synthetic membrane to see whether the channels work normally or not. "Extracting and purifying such channels will be tough business, though," Rudin admits. "To do so we'll have to devise some new techniques in lipid phase biochemistry."

The Philadelphia molecular biologists are also interested in synthesizing membranes that reside inside the cell instead of around it. These membranes include the nuclear membrane (the membrane that surrounds the nucleus), the mitochondrial membrane (the membrane that surrounds the mitochondrion) and the endoplasmic reticulum (a tubular membrane network that contains ribosomes, on which proteins are packaged).

On the whole, the Philadelphia biologists' research is helping set the stage for two new fields—molecular pharmacology and molecular psychiatry. It is also helping set the stage for a new era—biological synthesis. "The age of biological synthesis is in its infancy, but it is clearly discernible," James Danielli, a theoretical biologist with the State University of New York, has declared. □

. . . Gene Conference

nique allows more time for experiments and less time doing tedious extractions or slowly assembling individual nucleotides or amino acids.

Researchers plan to use recombinant DNA to modify normal organisms and correct defective ones. Plant physiologists envision the day when staple food crops can be fitted with all of the most efficient plant equipment—genes for fixing nitrogen, for resisting diseases, for producing essential amino acids and for stepped-up carbohydrate production. And medical researchers would like someday to correct genetic errors that cause human and animal diseases by excising defective genes and inserting operative ones.

Perhaps the most immediate application, and a very appropriate one, is the development of a safety vector. Brenner, like a fast-talking salesman, dazzles the ear with descriptions of recombinant vehicles with "self-destruction capability" and the "built-in assurance of add-on safety devices." Brenner and others are developing safe vectors now. Those planning moderate- and high-risk experiments are cheering from the sidelines, since they cannot proceed under the guidelines without such vectors.

Brenner says the danger of an unnatural, recombined organism escaping the laboratory (with unforeseen consequences) can be slashed to perhaps one chance in 10^{60} if enough safety devices are added to make it impossible for the organism to live outside of highly artificial conditions. One could, for example, take a bacterium that has lost the ability, through mutation or genetic manipulation, to manufacture an amino acid essential to the growth and repair of its cell wall. The amino acid would be available only if provided in the culture medium. If such an organism were to escape containment, it would "self destruct" since the researcher would no longer be providing the missing amino acid. One such safety feature might reduce the chances of escape to one in a million, Brenner says, so adding 10 features would make the chances incredibly remote.

Commenting on the conference, Berg voiced pleasure that guidelines were reached. It appeared up until the last session that some members might not agree to the general guidelines or even to the need for any self-regulation. Berg feared that such a position might appear self-serving to legislators and bring swift controls upon the research. A by-product as beneficial as the guidelines, he says, was that the conference "raised the level of discussion in the field." No one will go into this field without thinking about the risks and benefits "and I couldn't have said that eight months ago." □