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This Week's Citation Classic

Huang C. Studies on phosphatidylcholine vesicles. Formation and physical characteristics. *Biochemistry* 8:344-51, 1969.
[Department of Biochemistry, University of Virginia, School of Medicine, Charlottesville, VA]

This paper describes a method to prepare spherical unilamellar phospholipid bilayer vesicles and the application of various physical techniques to characterize these vesicles as model bilayer membranes. [The *SCI*® indicates that this paper has been cited over 595 times since 1969.]

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"After working on planar bilayer membranes at the Johns Hopkins Medical School with Thomas E. Thompson for three years, I joined Manfred Eigen's group in 1965 as a fresh postdoctoral fellow in Göttingen, Federal Republic of Germany. I planned to learn the chemical relaxation technique and to apply it to studying the dynamic properties of phospholipid bilayers. It became apparent very quickly that the chemical relaxation technique cannot practically be applied to the planar bilayer membrane, since any rapid perturbation such as temperature jump will break the planar bilayer at once. Consequently, I decided to prepare a new phospholipid model membrane system which could survive the rapid perturbation. As the only lipid biochemist at Göttingen then, and the lowest on the totem pole among all the eminent scientists there, I had trouble assembling the necessary chemicals and supplies to begin the long investigation. In fact, by the end of my two-year stay at Göttingen, I did not have enough data to write even a brief communication. Nevertheless, I knew that it was only a matter of time before I finished the work and I had a good, albeit slow, start. Looking back, it was quite a sharp contrast that scientists at Göttingen at the time were making many significant contributions to our understanding of allosteric behavior of multisubunit enzymes by chemical relaxation technique, while I was slowly plowing the furrow.

"I spent my last year as a research fellow of the Helen Hay Whitney Foundation in the biochemistry department, University of Virginia School of Medicine, Charlottesville, Virginia. I knew from my ultracentrifugation work at Göttingen that small unilamellar phospholipid vesicles can be generated by subjecting the phospholipids in aqueous solution to ultrasonic irradiation. I was not satisfied with the ultrasonic irradiation, however, because the vesicles prepared are extremely heterogeneous both in size and in slope. Sepharose-4B, a gel-filtration column material for separating large macromolecules, became available commercially in 1967-1968. Quickly, I used the column material and subjected the ultrasonic irradiated lipid dispersion to the Sepharose-4B column. It was quite a day at Charlottesville when the clear separation of large liposomes from the small vesicles was seen. The elution pattern of the gel-filtration step became the first figure used in the 1969 publication. I consider this gel-filtration step as the most crucial step for the preparation of homogeneous phospholipid vesicles.

"I believe that my first independent publication after graduate school became a *Citation Classic* due to the nature of the work. It describes a simple method for the preparation of a new model membrane system and, in addition, it contains a large number of physical characteristics of the system. Our current understanding of the lipid dynamics in bilayer membranes is, to some degree, promoted by the availability of various membrane model systems.[1] This publication can be credited for providing a simple model system. A more complete description of the small unilamellar vesicle was published much later in *Proceedings of the National Academy of Sciences of the USA*.[2] This paper was communicated by Eigen in October 1977, ten years after the original idea was conceived in his laboratory in Göttingen."

1. **Thompson T E & Huang C.** Dynamics of lipids in biomembranes. (Andreoli T E, Hoffman J F & Fanestil D D, eds.) *Physiology of membrane disorder*. New York: Plenum, 1978. p. 27-48.
2. **Huang C & Mason J T.** Geometric packing constraints in egg phosphatidylcholine vesicles. *Proc. Nat. Acad. Sci. US* 75:308-10, 1978.