This Week's Citation Classic

CC/NUMBER 35
AUGUST 30, 1982

Emmelot P, Bos C J, Benedetti E L & Rümke Ph. Studies on plasma membranes. I. Chemical composition and enzyme content of plasma membranes isolated from rat liver. *Biochim. Biophys. Acta* **90**:126-45, 1964.
[Depts. Biochem., Electron Microscopy, and Immunol., Antoni Van Leeuwenhoek-Huis, The Netherlands Cancer Inst., Amsterdam, The Netherlands]

This paper reported for the first time a method for isolating *pure* liver plasma membranes, accompanied by a comprehensive account of chemical, enzymatic, structural, and immunological properties of the isolated membranes. Marker enzymes were thus established. [The *SCI*® indicates that this paper has been cited in over 695 publications since 1964.]

P. Emmelot
Divisions of Cell Biology and
Chemical Carcinogenesis
Antoni Van Leeuwenhoek-Huis
The Netherlands Cancer Institute
1066 CX Amsterdam
The Netherlands

April 23, 1982

"At the end of the 1950s, methods were available for the isolation of intracellular organelles but a method for plasma membrane isolation was still lacking. Consequently, the biochemistry of the cell surface was virtually a *terra incognita*. About that time, our interest in the plasma membrane was aroused by the finding that transplants of rapidly growing rat liver tumors readily yielded single, metabolically intact cells by suspending the finely divided tissue in a weakly acid phosphate buffer. This procedure did not work with rat liver.[1] The relative ease with which single tumor cells could be obtained was apparently due to a decrease in the mutual adhesiveness of the hepatoma as compared with the liver cells.

"Obviously for me as a biochemical oncologist, cell adhesiveness had a particular ring not only in view of the problem of metastasis but also because of the notions which were emerging at the time about functional relations between cell contact and the biosocial behavior of cells. These relations—pertaining to cell recognition and discrimination, tissue organization, and cell proliferation and differentiation—were mostly of a descriptive type being vague as to the molecular mechanisms concerned. Nevertheless, contact lesions conceptually fitted the asocial behavior of the cancer cell.

"We then set out to find a suitable method for the isolation of plasma membranes from liver and liver tumors of rats and mice. In a number of pilot experiments carried out with fractionated liver homogenates subjected to electron microscopy, we observed that large plasma membrane sheets, preserving intact bile space membranes and being interconnected by junctional complexes—poorly known at the time and qualified as desmosomes and terminal bars—could be observed, mainly as cosediments in the nuclear fraction. It was decided to use these morphological criteria as provisional markers for the isolation of plasma membranes. Early in the course of our work, a paper by Neville[2] describing the isolation of rat liver plasma membranes appeared. The data contained therein, plus our experience then, led to a procedure which was essentially a modification of Neville's method, specifically aiming at the removal of contaminating mitochondria. This method, which has sometimes been quoted as the Neville-Emmelot method, is the one featured in this *Citation Classic*, being our first full paper on the subject. An exhaustive description was provided later.[3]

"From the outset our interest and effort were not so much directed toward the normal plasma membrane per se, as a void area for study, but rather to the elucidation of surface properties which might distinguish tumor from normal cells. The aim of a comparative analysis, however, necessitated a thorough study of the chemical, enzymatic, structural, and immunological properties of the liver plasma membrane of which the first results were contained in our 1964 paper. This and following papers have contributed to the field of *fundamental membranology, besides helping to* elucidate our primary aim.

"The reason that our 1964 paper has been quoted so often is, I think, due to the fact that it presented a body of diversified data—provided by my coauthors in a multidisciplinary approach—which allowed a good appreciation of the method. I also believe that the elaboration of these data and the new findings reported in our subsequent papers have focused attention on the original 1964 paper. Furthermore, I have heard either by letter or personal contact that the method was easily reproducible, also with respect to the quantitative data. Most important, however, I have been informed more than once that the paper has been an impetus for others to study a particular problem at the level of the isolated plasma membrane."

1. **Emmelot P.** Comparative biochemistry of rat hepatomas. *Acta Union Int. Cancer* **20**:902-8, 1964.
2. **Neville D M.** The isolation of a cell membrane fraction from rat liver. *J. Biophys. Biochem. Cytol.* **8**:413-22, 1960.
3. **Emmelot P, Bos C J, Van Hoeven R P & Van Blitterswijk W J.** Isolation of plasma membranes from rat and mouse livers and hepatomas. *Methods Enzymol.* **31**:75-90, 1974.