

This Week's Citation Classic

Rambourg A & Leblond C P. Electron microscope observations on the carbohydrate-rich cell coat present at the surface of cells in the rat. *J. Cell Biol.* 32:27-53, 1967.
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Periodic acid-silver methenamine, a technique for glycoprotein detection on the electron microscope, stains the surface of a wide variety of cells. The absence of staining in the regions where adjacent plasma membranes fuse to form tight junctions indicates that the stained material is located at the outer surface of the plasma membrane. [The SCI® indicates that this paper has been cited in over 615 publications since 1967.]

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"In the early 1960s, the literature on the features of the cell surface was in a state of confusion. Some claimed that a carbohydrate-rich cell coat was typical of cancer cells.¹ Others described a carbohydrate-containing fuzz on intestinal cells, but only at their apical surface,² although the lateral membranes had been shown to stain with the periodic acid (PA)-Schiff technique for carbohydrates.³ Moreover, at that time, the long held view that a protein-rich 'cement' kept cells together was being abandoned by electron microscopists in favor of desmosomes and junctional complexes. The spark that made it possible to reach a unified view on the presence of carbohydrates at the cell surface arose from a fortuitous observation.

"In 1964, when, as a young MD, I joined C.P. Leblond's group at McGill University, Marian Neutra was investigating the biogenesis of glycoproteins by radioautography. Her histologic slides were stained by the PA-Schiff technique, but counterstained by hematoxylin. On a PA-Schiff stained section

which I counterstained with toluidine blue instead of hematoxylin, the delicate plasma membrane appeared as a fine purplish line against a pale blue background. Thus, the PA-Schiff staining of the plasmalemma previously masked by hematoxylin became visible. This was confirmed in some 50 different cell types of the rat. We concluded that the plasmalemma of all cells included glycoproteins.⁴ Moreover, since the staining disappeared along tight junctions, where the outer surfaces of adjacent plasma membranes were known to fuse, we further postulated that the layer staining for glycoprotein was exclusively located on the outside of the plasmalemma.⁴

"Since the work had been performed with the light microscope, the resolving power of which was thought by some authors not to allow the visualization of the plasma membrane, these conclusions were met with widespread criticism. It was thus decided to use the electron microscope and to adapt a modification of the PA-Schiff technique, i.e., the PA-silver methenamine method which had been used in the department.⁵ The first attempts at staining the plasmalemma were plagued with silver precipitates and unspecific reactions. After many months of work, reproducible results were obtained, and the observations made on the light microscope were confirmed, as described in the present article.

"The success of this article is attributed to its presenting a broad but simple concept which unifies the views of microscopists postulating the existence of fuzz-like or cement-like carbohydrates at the cell surface, as well as the conclusions of biochemists extracting carbohydrates from the plasmalemma of cancer or blood cells. Our results, which were published in the widely read *Journal of Cell Biology*, indicated that carbohydrates were a constant feature of the cell surface and, indeed, it is now accepted⁶ that the plasma membrane contains glycoproteins and glycolipids, the carbohydrate chains of which are exposed on its outer surface."

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3. Pechter H & Leblond C P. Histochemical analysis of cell membranes and associated structures as seen in the intestinal epithelium. *Amer. J. Anat.* 102:1-31, 1958.
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