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This Week's Citation Classic®

Williams G M, Weisburger E K & Weisburger J H. Isolation and long-term cell culture of epithelial-like cells from rat liver. *Exp. Cell Res.* 69:106-12, 1971; and, **Williams G M & Gunn J M.** Long-term cell culture of adult rat liver epithelia cells. *Exp. Cell Res.* 89:139-42, 1974.

[National Cancer Institute, National Institutes of Health, Bethesda, MD; and, Fels Research Institute, Temple University, School of Medicine, Philadelphia, PA]

These two papers describe reliable and practical methods for initiating and maintaining in long-term culture epithelial-like cell lines from either newborn or adult rat liver. [The *SCI*® indicates that these papers have been cited in more than 190 and 150 publications, respectively.]

Long-Term Rat Liver Epithelial Cell Cultures

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When I joined the laboratory of Elizabeth K. and John H. Weisburger at the National Cancer Institute in 1969, I was asked to take over some ongoing work culturing liver cells. The purpose was to develop an in vitro system for screening carcinogens. I quickly found out that fibroblasts dominated cultures initiated from newborn rat liver. At a seminar on immunology, I learned of a technique for separating lymphocytes from macrophages. I applied this to liver isolates and was able to enrich "epithelial-like" cells that did not attach as avidly as fibroblasts. Based on John's question to me, "What do you think liver cells would like to eat?," I formulated a new medium—Medium D. I designed this for functional epithelial cells and, with some additional refinements, developed a reproducible method and published it in the 1971 paper.

The cell lines, called TRL, for the 10-day-old rat liver, proved interesting for transformation studies,[1] but never became a practical screening system. I concluded that they were limited by a poor capacity to bioactivate carcinogens, and wondered if this could be a consequence of the fact that the lines were derived from newborn liver. As described in a previous *Citation Classic* paper,[2] I was introduced to perfusion of adult liver by Richard Hanson. Working with one of his associates, J. Martyn Gunn, we modified the procedures of Per O. Seglen[3] for liver dissociation to prepare adult hepatocytes for culture. Still interested in the nutritional requirements for culture of differentiated epithelial cells, I modified Medium D to yield Medium E, which remains in use today. The 1974 paper describes initiation from adult liver of primary monolayer cultures of hepatocytes, from which proliferating cell lines of "epithelial-like" cells were derived. I continued to work on the conditions suitable for primary cultures, together with Brian A. Laishes of Emanuel Farber's laboratory. The primary cultures of freshly isolated nonproliferating hepatocytes finally achieved the original goal of a screening test for carcinogens through measurement of chemical-induced DNA repair, as described in another *Citation Classic* paper.[4]

Returning to the subject of proliferating cell lines, the lines derived from adult liver appeared the same as those from newborn. In a further study at the American Health Foundation, we devised a procedure, together with Kazunori Furukawa, a visiting scientist from Sapporo Medical College, to enrich the clonogenic cells from adult liver that gave rise to the cell lines.[5] I suspect that the clonogenic cells may be a kind of facultative stem cell present in both newborn and adult liver. Even now, the definition of this cell type has implications for the pathogenesis of experimental liver cancer.

These papers are usually cited because investigators have made use of the methods for their specific research. Liver cell lines have been used for a variety of investigations, including the study of cellular physiology[6] and cell-to-cell communication.[7]

1. **Williams G M, Elliott J M & Weisburger J H.** Carcinoma after malignant conversion in vitro of epithelial-like cells from rat liver following exposure to chemical carcinogens. *Cancer Res.* 33:606-12, 1973. (Cited 155 times.)
2. **Laishes B A & Williams G M.** Conditions affecting primary cell cultures of functional adult rat hepatocytes. II. Dexamethasone enhanced longevity and maintenance of morphology. *In Vitro* 12:821-32, 1976. (Cited 140 times.) [See also: **Williams G M.** Parenchymal liver cell cultures. Citation Classic. *Current Contents/Clinical Medicine* 17(9):14, 27 February 1989; and *Current Contents/Life Sciences* 32(9):15, 27 February 1989.]
3. **Seglen P O.** Preparation of rat liver cells. III. Enzymatic requirements for tissue dispersion. *Exp. Cell Res.* 82:391-8, 1973. (Cited 635 times.)
4. **Williams G M.** Detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell cultures. *Cancer Res.* 37:1845-51, 1977. (Cited 350 times.) [See also: **Williams G M.** Citation Classic. *Current Contents/Life Sciences* 30(36):19, 7 September 1987.]
5. **Furukawa K, Shimada T, England P, Mochizuki Y & Williams G M.** Enrichment and characterization of clonogenic epithelia cells from adult rat liver and initiation of epithelial cell strains. *In Vitro Cell. Dev. Biol.* 23:339-48, 1987.
6. **Weinstein S P, Watts J, Graves P N & Haber R S.** Stimulation of glucose transport by thyroid hormone in ARL 15 cells: increased abundance of glucose transporter protein and messenger ribonucleic acids. *Endocrinology* 126:1421-9, 1990.
7. **Ljubimov A V, Martel N & Yamasaki H.** Response of cultured rat liver epithelial cell lines to tumor-promoting phorbol esters. *Exp. Cell Res.* 156:311-26, 1985.

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