This Week's Citation Classic®

Williams G M. Carcinogen-induced DNA repair in primary rat liver cell cultures: a possible screen for chemical carcinogens. *Cancer Lett.* 1:231-6, 1976; and **Williams G M.** Detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell cultures. *Cancer Res.* 37:1845-51, 1977.
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These papers describe the induction of DNA repair synthesis measured autoradiographically in cultured rat hepatocytes exposed to a variety of carcinogens, especially those requiring metabolic activation. The studies documented that a cell type with extensive biotransformation capability could be used reliably to detect DNA-reactive chemical carcinogens. [The *SCI*® indicates that these papers have been cited in over 135 and 275 publications, respectively.]

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Having been trained as a physician and a pathologist, I never foresaw that I would spend a large part of my adult life doing research in cell culture. My involvement in this field began in 1967 when I joined the laboratory of Elizabeth and John Weisburger at the National Cancer Institute (NCI) in Bethesda, Maryland. The Weisburgers were pioneering the NCI's program of testing chemicals for carcinogenicity, and, as one of my tasks, I was given the responsibility of developing liver cell cultures as a means for rapid detection of carcinogens. At that time the effort was directed toward establishing proliferating cultures in which transformation could be assessed. Reliable techniques for growing liver cells were nonexistent, but using cells from newborn rats we met with some success.[1]

After moving to the Fels Research Institute at Temple University in Philadelphia, I began using the techniques of M.N. Berry and D.S. Friend[2] and P.O. Seglen[3] for isolation of adult rat-liver cells in order to initiate proliferating cultures in which to extend the earlier work done with newborn-rat cells.

I was joined in related work by Brian Laishes, a postdoctoral fellow. During our collaboration, the idea (I am not sure whose it was) arose to measure, by autoradiography, carcinogen-induced unscheduled DNA synthesis (DNA repair) in the primary cultures of hepatocytes, as had been done in fibroblasts by Laishes's mentor, Hans Stich.[4] To our initial dismay, we observed no unscheduled DNA synthesis with clearly DNA-damaging agents under conditions used by other investigators. We guessed correctly that since hepatocytes are non-S-phase cells, they would have a low level of thymidine kinase and, consequently, poor utilization of the ^{3}H-thymidine. By increasing the concentration of thymidine, we observed repair.

Laishes subsequently chose to pursue other objectives at the University of Toronto. Before the work was finished, I joined the American Health Foundation in New York, where the first paper was completed. Meanwhile, I received a contract from the NCI to validate the transformation studies. I first met the project officer, Virginia Dunkel (now at the Food and Drug Administration), at a conference in Aspen, Colorado, and told her of the DNA repair studies. She agreed that these should be given priority, and the second, more extensive study was completed within a year. In this study the innovation was introduced of allowing prolonged exposure to the test chemical and ^{3}H-thymidine to permit accumulation of repair.

The main reason for the citation of these papers is that the system they describe has gradually been recognized as a useful test for carcinogens. The combination of the extensive biotransformation capability of hepatocytes with the facile measurement of DNA repair in the absence of replicative DNA synthesis is very attractive and straightforward. The test is an important component of *in vitro* screening approaches because it provides whole-cell metabolism in contrast to subcellular preparations used in most other *in vitro* systems. Hepatocytes from a variety of species, including humans, can be used, thereby providing important information for hazard assessment.

One of the unforeseen observations made while using this approach was that a number of carcinogens did not elicit repair. Based upon the structure of such chemicals and the nature of their carcinogenic effects, I postulated in 1977 a category of "epigenetic" carcinogens that did not react chemically with DNA in the manner characteristic of electrophilic carcinogens. Ten years later, this remains a controversial concept but one for which more evidence is accumulating.[5]

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 145 times.)
2. **Berry M N & Friend D S.** High-yield preparation of isolated rat liver parenchymal cells: a biochemical and fine structural study. *J. Cell Biol.* 43:506-20, 1969. (Cited 2,230 times.) [See also: **Berry M N.** Citation Classic. (Barrett J T, ed.) *Contemporary classics in the life sciences. Volume 1: cell biology.* Philadelphia: ISI Press, 1986. p. 219.]
3. **Seglen P O.** Preparation of rat liver cells. I. Effect of Ca^{2+} on enzymatic dispersion of isolated, perfused liver. *Exp. Cell Res.* 74:450-4, 1972. (Cited 235 times.)
4. **Stich H F, San R H C & Kawazoe Y.** DNA repair synthesis in mammalian cells exposed to a series of oncogenic and nononcogenic derivatives of 4-nitroquinoline 1-oxide. *Nature* 229:416-9, 1971. (Cited 100 times.)
5. **Williams G M & Weisburger J H.** Chemical carcinogens. (Klaassen C, Amdur M & Doull J, eds.) *Toxicology: the basic sciences of poison.* New York: Macmillan, 1986. p. 99-173.