

This Week's Citation Classic®

Laishe B A & Williams G M. Conditions affecting primary cell cultures of functional adult rat hepatocytes. I. The effect of insulin. *In Vitro* 12:521-32, 1976.
[Fels Research Institute and Department of Pathology, Temple University School of Medicine, Philadelphia, PA]

These papers (including references 1, 2, and 3) describe techniques for dissociating rat liver and isolating a high yield of viable hepatocytes for initiation of monolayer cultures representative of the hepatic parenchyma. The properties of hepatocyte cultures were studied and factors affecting their quality were examined. [The *SCI*® indicates that this paper and the three others in the series have been cited (individually or in some combination) a total of 507 times (162, 130, 157, and 58 cites, respectively) in 380 publications.]

Parenchymal Liver Cell Cultures

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December 9, 1988

In developing liver culture systems for the detection of chemical carcinogens, I found that measurement of DNA repair synthesis in freshly isolated hepatocytes was a useful method, as described in a previous *Citation Classic*.⁴ This approach requires a fresh preparation of hepatocytes for each test series, and consequently it was important to have a technique for isolation and culture of hepatocytes that was highly efficient and reproducible. In earlier work, I was introduced to liver perfusion by a colleague at the Fels Research Institute, Richard Hanson. Together with one of his associates, Martyn Gunn, I adapted the procedures of P.O. Seglen⁵ for liver dissociation to isolation of hepatocytes for establishment of monolayer cultures to yield proliferating epithelial cell lines.⁶ Once the utility of the primary cultures was demonstrated, various aspects of liver perfusion and hepatocyte isolation, mainly derived from the procedures of M.N. Berry and D.S.

Friend⁷ and Seglen,⁵ and of culture of hepatocytes were examined in detail. The conduct of a large number of experiments first at Fels and then at the American Health Foundation over a period of about four years, in collaboration with a number of other co-workers who appear as coauthors on the papers, led to a method that was refined for primary culture of hepatocytes as monolayers.

Two of the most significant technical innovations that we introduced were the use of collagenase for liver perfusion at a concentration in activity units/ml rather than mg/ml and a short attachment interval of 1-2 hr for initiation of cultures. The latter step allowed the attachment of virtually only viable hepatocytes and thereby resulted in cultures of high quality.

In the course of these studies, we examined the effects of hormones, identifying an enhancement of attachment efficiency mediated by insulin and a prolongation of the longevity of cultures by corticosteroids. We studied various media and showed that, after attachment, hepatocytes could be well-maintained in serum-free medium E, which had been developed earlier for proliferating liver cell cultures.⁶ We documented the production of plasminogen activator and a neutral protease by cultured hepatocytes and showed that this was suppressed by corticosteroids. This period was perhaps the most exciting in my research career. My coinvestigators were enthusiastic and extremely capable (two, who were research assistants at the time, M.F. Laspia and D. Scaramuzzino, have subsequently obtained PhDs), and we seemed to be learning something new every day, including a number of interesting observations that were never published.

The papers that are the subject of this commentary usually have been cited individually for their description of any one of the aforementioned findings. The basic method for isolation and culture of hepatocytes that emerged from these studies is still one of the better techniques available. Many workers use it or the main elements of it, especially the medium E, which is now provided by several commercial sources. In general, advances have been made in techniques for culturing parenchymal liver cells, and their use in research has burgeoned.⁸

1. Laishe 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.
2. Williams G M, Bermudez E & Scaramuzzino D. Rat hepatocyte primary cell cultures. III. Improved dissociation and attachment techniques and the enhancement of survival by culture medium. *In Vitro* 13:809-17, 1977.
3. Williams G M, Bermudez E, San R H C, Goldblatt P J & Laspia M F. Rat hepatocyte primary cultures. IV. Maintenance in defined medium and the role of production of plasminogen activator and other proteases. *In Vitro* 14:824-37, 1978.
4. 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. (Cited 150 times.); and Williams G M. Detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell cultures. *Cancer Res.* 37:1845-51, 1977. (Cited 310 times.) [See also: Williams G M. *Citation Classic. Current Contents/Life Sciences* 30(36):19, 7 September 1987.]
5. Seglen P O. Preparation of rat liver cells. III. Enzymatic requirements for tissue dispersion. *Exp. Cell Res.* 82:391-8, 1973. (Cited 540 times.)
6. Williams G M & Gunn J M. Long-term cell culture of adult rat liver epithelial cells. *Exp. Cell Res.* 89:139-42, 1974. (Cited 130 times.)
7. Berry M N & Friend D S. High-yield preparation of isolated rat liver parenchymal cells. *J. Cell Biol.* 43:506-20, 1969. (Cited 2,490 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.]
8. Rauckman E J & Padilla G, eds. *The isolated hepatocyte: use in toxicology and xenobiotic biotransformations.* New York: Academic Press, 1987. 292 p.