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This Week's Citation Classic ™

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. [Div. Clinical Pathology and Dept. Pathology. Univ. California Sch. Med., San Francisco, CA]

A method is described for the high-yield preparation of suspensions of intact isolated rat hepatocytes, by perfusion of the liver with a Ca^{2+}-free medium containing collagenase. [The *SCI*® indicates that this paper has been cited in over 1,570 publications since 1969]

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"My attempts to prepare suspensions of intact isolated parenchymal liver cells (hepatocytes) began in New Zealand in 1958, with the encouragement of N.L. Edson, who recognized their potential value for metabolic studies. Employing Anderson's technique,[1] I obtained high yields of cells which, unfortunately, were virtually devoid of normal metabolic activity. This led to a three-year study in collaboration with a skilled electron microscopist, F.O. Simpson, which demonstrated that cells prepared by high-pressure perfusion of the liver suffered gross damage to cellular membranes and loss of cytoplasmic contents.[2,3] As a consequence of these studies, I developed a lasting interest in the relationship between cellular structure and function, as well as a determination to find a method for preparing intact cells.

"I continued my research under H.A. Krebs in Oxford. He discouraged further efforts directed toward isolated cell preparation, arguing very reasonably that if liver slices could not function as well as perfused liver, there was little likelihood that single cell suspensions would do so. At his direction, I spent a year establishing the perfused liver technique —experience that was subsequently to prove of great value for the preparation of isolated hepatocytes. I spent the next few years undertaking liver perfusion studies, now with an added incentive to find a satisfactory method for isolated liver cell preparation.

"In 1967,1 took up an appointment at the University of California at San Francisco. R.B. Howard, then at Stanford University, had just published a key paper [4] showing for the first time that collagenase treatment of liver slices gave preparations of isolated intact hepatocytes, albeit in low yield. Recognizing the importance of Howard's observations, I set about trying to increase the yield. It seemed logical to attempt to perfuse livers with a medium containing collagenase, and it was highly gratifying to find that this approach produced large quantities of intact isolated cells. This was one of the very few occasions in my research career when logic has been rewarded! The outstanding electron micrographs of D.S. Friend made a major contribution to the paper and many of the citations refer to morphological aspects of the work.

"The bulk of the citations, however, reflect the usefulness of the preparation as a tool for the study of hepatic metabolism. It turned out, contrary to expectations, that isolated hepatocytes perform metabolically in most instances as well as the perfused liver, while being far easier to manipulate and permitting a single liver to be used for multiple studies. In fact, when Krebs came to appreciate this, he became a strong advocate for the method and this had much to do with its rapid rise in popularity. Since then, numerous modifications of the collagenase-perfusion technique have been published (not all of them, in my view, desirable), and in consequence the number of citations for the paper underestimates perhaps fivefold the usage of the method. Nevertheless, according to *Science Citation Index*®, the paper continues to be cited with increasing frequency, presumably reflecting the entry of new workers into the field. For a review, see the proceedings of a recent conference."[5]

1. **Anderson N** G. The mass isolation of whole cells from rat liver. *Science* **117**:627-8, 1953. (Cited 165 times since 1955.)
2. **Berry M N.** Metabolic properties of cells isolated from adult mouse liver. *J. Cell Biol.* **15**: 1-8, 1962.
3. **Berry M N & Simpson F O**. Fine structure of cells isolated from adult mouse liver. *J. Cell Biol.* **15**:9-17, 1962.
4. **Howard R B, Christensen A K, Gibbs F A & Pesch L A.** The enzymatic preparation of isolated intact parenchymal cells from rat liver. *J. Cell Biol.* **35**:675-84, 1967. (Cited 125 times.)
5. **Harris R A & Cornell N W,** eds. *Isolation, characterization, and use of hepatocytes: proceedings of the International Symposium on Isolation. Characterization, and Use of Hepalocyles held at Indiana University School of Medicine. Indianapolis. October* **22-***24. I982.* New York: Elsevier Biomedical. 1983. 660 p.