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This Week's Citation Classic®

Bierman E L, Stein O & Stein Y. Lipoprotein uptake and metabolism by rat aortic smooth muscle cells in tissue culture. *Circ. Res.* 35:136-50, 1974.
[Lipid Research Lab., Dept. Medicine B, Hadassah Univ. Hospital; Dept. Experimental Medicine and Cancer Research, Hebrew Univ.-Hadassah Medical Sch., Jerusalem, Israel; and Div. Metabolism and Gerontology, Univ. Washington, Seattle, WA]

This paper describes for the first time the separate steps of binding, internalization, and degradation of lipoproteins by cultured mesenchymal cells and details the methods used to study the metabolic fate of various classes of lipoproteins after their interaction with cells. [The *SCI*® indicates that this paper has been cited in over 550 publications.]

Lipoprotein Interaction with Cultured Cells

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The experiments described in this paper were performed during my sabbatical year as a Guggenheim Fellow in the laboratory of Olga and Yechezkiel Stein at Hadassah University Hospital in Jerusalem. I learned the then newly developed technique of culturing primate aortic smooth muscle cells at the University of Washington prior to my departure and set up the method in the Steins' laboratory. We decided on testing the uptake and metabolism of lipoproteins by aortic cells using a rat model system, because of the long experience in that lab working with radiolabeled rat lipoproteins. The initial idea was to measure iodinated lipoprotein uptake and metabolism after their incubation with a cultured cell layer, washing and then trypsinizing the cells to release them from the dish. I can recall the initial excitement of seeing a surge of released radioactivity upon trypsinization indicating that lipoproteins were being released from the cell surface, separate from those being internalized. We now had a method for assessing lipoprotein cell surface binding, a method that subsequently was further developed using dextran or heparin in J.L. Goldstein and M.S. Brown's laboratory[1] and formed part of the methodology used in their Nobel Prize-winning discovery of the low density lipoprotein (LDL) receptor.

Another aspect of assessment of lipoprotein interaction with cells was the development of a technique to measure lipoprotein protein degradation to trichloracetic acid (TCA) soluble fragments free of iodide, which might have been produced by simple deiodination of the apoprotein without degradation. This involved a technique based on a two-phase partitioning of iodine into a chloroform layer after iodide oxidation with H_2O_2, suggested by previous work done in the Steins' laboratory. This method has become widely used as a standard technique by many investigators studying lipoprotein degradation by cells[2] and may be partly responsible for the frequent citation of this paper.

These early studies also were the first to describe the process of "reverse endocytosis" of lipoproteins by cells and formed the basis of subsequent studies of retroendocytosis of LDLs.[3,4] They also formed the basis of many of the studies in our two laboratories during the past 15 years on the many pathobiological influences affecting the metabolism of a variety of lipoproteins by cultured arterial cells.[5,6] It is interesting that the work in my first *Citation Classic*[7] was performed during my first year of research training, and the work in this, my second *Citation Classic*, was performed during a year of "retraining." It must be time for another sabbatical.

1. **Goldstein J L, Basu S K, Brunschede G Y & Brown M S.** Release of low-density lipoprotein from its cell surface receptor by sulfated glycosaminoglycans. *Cell* 7:85-95, 1976. (Cited 440 times.)
2. **Goldstein J L & Brown M S.** Binding and degradation of low density lipoproteins by cultured human fibroblasts. *J. Biol. Chem.* 249:5153-62, 1974. (Cited 800 times.)
3. **Aulinskas T H, Van Der Westhuyzen D R, Bierman E L, Gevers W & Coetzee G.** Retroendocytosis of low density lipoprotein by cultured bovine aortic smooth muscle cells. *Biochim. Biophys. Acta* 664:255-65, 1981. (Cited 35 times.)
4. **Greenspan P & St. Clair R W.** Retroendocytosis of low density lipoprotein. Effect of lysosomal inhibitors on the release of undegraded ^{125}I-low density lipoprotein of altered composition from skin fibroblasts in culture. *J. Biol. Chem.* 259:1703-13, 1984. (Cited 30 times.)
5. **Oram J F, Chait A & Bierman E L.** Lipoprotein and cholesterol metabolism in cultured arterial smooth muscle cells. (Campbell J & Campbell G, eds.) *Vascular smooth muscle in culture.* Boca Raton, FL: CRC Press, 1986. p. 61-80.
6. **Stein Y & Stein O.** Interaction between serum lipoproteins and cellular components of the arterial wall. (Scanu A M, Wissler R W & Getz G S, eds.) *The biochemistry of atherosclerosis.* New York: Dekker, 1979. p. 313-44. (Cited 5 times.)
7. **Bierman E L, Dole V P & Roberts T N.** An abnormality of nonesterified fatty acid metabolism in diabetes mellitus. *Diabetes* 6:475-9, 1957. (Cited 315 times.) [See also: **Bierman E L.** Citation Classic. (Barrett J T, ed.) *Contemporary classics in clinical medicine.* Philadelphia: ISI Press, 1986. p. 230.]

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