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Fishman P H & Brady R O. Biosynthesis and function of gangliosides. *Science* 194:906-15, 1976.
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This paper reviewed the biosynthesis and function of gangliosides, the sialic acid-containing glycosphingolipids that are synthesized by the sequential transfer of sugars from sugar nucleotides to the elongating glycolipid acceptor. As gangliosides are localized on the cell surface, they may participate in the transmission of membrane-mediated information. [The *SCI*® indicates that this paper has been cited in over 505 publications.]

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After receiving my PhD in biochemistry from George Washington University, I joined Roscoe O. Brady's laboratory at the National Institutes of Health as a staff fellow in July of 1970. I was to work on the altered ganglioside biosynthesis observed in DNA tumor virus-transformed cells. These cells lack gangliosides more complex than G_{M3} and the glycosyltransferase activity that converts G_{M3} to G_{M2}. My knowledge of these esoteric compounds was very limited; my biochemistry textbook contained only a few lines describing gangliosides.

For my first project I proposed assaying the other transferases in the pathway to determine whether the effect of transformation was specific to one enzyme. I began using young rat brain preparations to work out the assays. I was intrigued by Saul Roseman's review in which he proposed that gangliosides are synthesized by multiglycosyltransferases.[1] I decided to test this possibility by incubating the rat brain preparation with G_{M3} and both UDP-[^{14}C]GalNAc and -[^{3}H]Gal. I discovered that both isotopes were incorporated into one of the reaction products that I identified as G_{M1}. When I used G_{D3} for G_{M3}, I obtained the sequential synthesis of G_{D2} and G_{D1b}. Although such a pathway had been proposed, it had never been demonstrated. These exciting results became the basis for my first paper as a postdoctoral fellow.[2]

As we were in the midst of the "war on cancer," there was much interest in malignant cell surfaces, and numerous meetings were organized. Brady attended the international meetings and I attended the domestic gatherings. Such are the perks of being the laboratory chief! I often felt that it was a traveling circus—same acts, different cities. The best part was meeting other scientists in the field. While presenting my work at the 1973 Cold Spring Harbor Symposium on Cell Proliferation, I met Pedro Cuatrecasas and Morley D. Hollenberg. I knew Cuatrecasas, as he was on the examining committee when I orally defended my thesis. He was very excited about his recent studies on cholera toxin and the possibility that G_{M1} was the membrane receptor for the toxin. We decided to test some of the mouse cell lines defective in G_{M1} content and biosynthesis for their ability to bind and respond to cholera toxin. We found that the cells with detectable levels of G_{M1} had more toxin receptors and were more sensitive to the toxin than cells devoid of any detectable G_{M1}.[3] As cholera toxin still bound to the latter cells and activated adenylate cyclase, it appeared that the toxin might be a more sensitive indicator of G_{M1} than more conventional techniques. We also speculated that serum that contains gangliosides may be a source for cells unable to synthesize them.

The opportunity to pursue the latter problem arose in 1974 when I began a long and fruitful collaboration with Joel Moss of the National Heart, Lung, and Blood Institute. Moss searched the American Type Culture Collection catalogue and found a transformed mouse cell line, NCTC 2071, that had been adapted to grow in serum-free medium. We found these cells unresponsive to cholera toxin and lacking any detectable G_{M1} as well as two of the transferases required for its synthesis. We prepared [^{3}H]G_{M1} and added it to the culture medium. The cells readily took up the G_{M1} and became responsive to the toxin even when less than 20,000 molecules of G_{M1} were incorporated per cell.[4] We thus confirmed our suspicions that only trace amounts of G_{M1} were required to make a cell sensitive to the toxin. Our results were published at a crucial time, as there was growing skepticism as to whether G_{M1} was the natural receptor for cholera toxin. We later showed that NCTC 2071 cells grown in medium containing serum or gangliosides extracted from serum were very responsive to cholera toxin. These and other experiments firmly established that G_{M1} is the specific, ubiquitous cholera toxin receptor.

By 1976 a review on the biosynthesis and function of gangliosides seemed appropriate. Gangliosides had become "fashionable," especially since we had some insight into their potential function(s). I enjoyed working on the review and included all of our latest findings, many in press and in preparation.

I believe that the popularity of the article was due to it being timely, current, concise, and clear, and to its publication in *Science*. I received numerous requests to reproduce its figures, especially the cartoon I used to depict the mechanism of action of cholera toxin.

The work on cholera toxin resulted in a fundamental shift in my research as I became more interested in ganglioside function, in particular the mediation of transmembrane signaling. Exciting progress in this area is currently being made in my section by my associate Sarah Spiegel.[5]

1. **Roseman S.** The synthesis of complex carbohydrates by multiglycosyltransferase systems and their potential function in intercellular adhesion. *Chem. Phys. Lipids* 5:270-97, 1970. (Cited 950 times.)
2. **Cumar F A, Fishman P H & Brady R O.** Analogous reactions for the biosynthesis of monosialo- and disialo-gangliosides. *J. Biol. Chem.* 246:5075-84, 1971. (Cited 80 times.)
3. **Hollenberg M D, Fishman P H, Bennett V & Cuatrecasas P.** Cholera toxin and cell growth: role of membrane gangliosides. *Proc. Nat. Acad. Sci. USA* 71:4224-8, 1974. (Cited 100 times.)
4. **Moss J, Fishman P H, Manganiello V C, Vaughan M & Brady R O.** Functional incorporation of ganglioside into intact cells: induction of choleragen responsiveness. *Proc. Nat. Acad. Sci. USA* 73:1034-7, 1976. (Cited 200 times.)
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