Roscoe O. Brady: Physician whose pioneering discoveries in lipid biochemistry revolutionized treatment and understanding of lysosomal diseases

Johannes M. Aerts, Department of Medical Biochemistry, Leiden University
Timothy M. Cox, Department of Medicine, University of Cambridge

Over the last half-century, understanding the manifestations and molecular pathogenesis of Gaucher disease has captured the attention of investigators and physicians. Persistent investment in this research has led, among other discoveries, to a portfolio of effective treatments for several lysosomal disorders. As an exemplary physician-investigator, Dr. Roscoe Owen Brady (1923-2016) was for a long period, the driving force behind many of these accomplishments. He set high standards for discovery based on the use of innovative research tools (including the synthesis of pure, radiolabeled natural lipid substrates) in the investigation and treatment of glycosphingolipidoses. Brady's fiercely rational approach and sheer stamina in tackling phenotypically and biochemically complex disorders is an inspiration for present-day researchers and clinicians. Indeed, as an example of scientific courage, his prodigious achievements and interdisciplinary research conducted over more than sixty years, warrant special commemoration since they are instructive for those who choose roads that are far away from the beaten track. Once established, Roscoe Brady spent his entire career as Chief of the Developmental and Metabolic neurology branch of the National Institute of Neurological Disorders and Stroke at the National Institutes of Health, Bethesda. At the Institute, Brady made seminal contributions to the understanding of glycosphingolipidoses, including the Niemann-Pick diseases, Fabry disease and Tay-Sachs disease. Here we focus principally on his prodigious and perhaps best-known accomplishments in Gaucher disease.

After obtaining his M.D. at Harvard Medical School in 1947 and completing his internship at the University of Pennsylvania, while remaining attached to the department of Medicine, Roscoe Brady took up a research fellowship award to study the biochemistry of lipids. In the group of Dr Samuel Gurin at the University, he collaborated with others to elucidate the synthesis of long chain fatty acids and in this environment of 'basic' science, became acquainted with advanced techniques in lipid research [1]. The laborious but productive training in lipid biochemistry left its mark and prepared Brady for his own programme of research into lipid chemistry in the Neurochemistry Laboratory at the Neurology Branch of the National Institutes of Health in Bethesda, Maryland (then quaintly known as the National Institute of Neurological Diseases and Blindness), which he joined in the late 1950s after two years of active clinical service in the United States Navy. After appointment as Assistant Chief in Neurochemistry, Brady investigated the enzymatic synthesis of sphingosine and developed an interest in sphingolipids [2, 3]. Almost inevitably, Gaucher's disease was among his main research interests. In January 1882, the young physician P.C.E. (Ernest) Gaucher described in his doctorate at the University of Paris, entitled: '*De l'epithelioma primitif de la rate, hypertrophie idiopathique de la rate sans leucemie',* the findings at a postmortem personally conducted at the Hôpital Cochin, on a cachectic (31 Kg) woman of 34 years with a bleeding tendency and ill-health

since the age of seven, who had died under his care. The spleen and liver were grossly enlarged (3.88 Kg and 4.77 Kg, respectively); but examination using the best technology of the day (optical microscopy), revealed spectacular findings in the sinusoids of the spleen which contained abnormal cellular infiltrates (tissue macrophages) [4]. These cells are beautifully depicted in camera lucida drawings of splenic tissue sections by Gaucher himself. Within a few decades it was realized that this patient had suffered from a distinct inheritable disease entity that was subsequently named Gaucher's disease (GD). In 1924, Emil Epstein isolated a pathological substance from the spleen of a similar patient; this material was soluble in alcohol but insoluble in acetone [5]. In the same year, analysis by the Austrian chemist, Hans Lieb, led to the discovery of gross accumulation of a glycosphingolipid - a cerebroside, as originally designated by Johannes Thudichum [6, 7]. In his paper *Cerebroside Speicherung bei Splenomegalie Typus Gaucher*, Lieb erroneously assumed that the storage material was galactosylceramide, a glycosphingolipid whose metabolism was much later shown to be disturbed in the inherited disorder Krabbe disease (caused by deficiency of the acid β-galactosidase named galactocerebrosidase) [5, 7].

The correct structure of the storage lipid in Gaucher disease as ß-glucosylceramide (glucocerebroside; GlcCer), was reported in spleen tissue obtained from a severely affected young girl, who died at the age of nine years with a neurological variant of Gaucher disease and massive visceromegaly and other features; the patient had been treated by splenectomy. This was documented by another French physician, Henriette Aghion, of l'Hôpital Rothschild: she had secured the expertise of a pharmacy intern, M. Lardé, based at a neighbouring institution (L'Hôpital Trousseau). In her doctoral dissertation in Medicine, like that of Ernest Gaucher also awarded by the University of Paris, entitled '*La Maladie de Gaucher dans l'Enfance (forme cardio-rénale*)', Aghion noted the unequivocal identification of glucose by her collaborator [9]. The pathological glucocerebroside was confirmed (by the reducing properties, melting point and ozazone) of the cognate hexose liberated by hydrolysis of the purified material after prolonged treatment with acid-methanol. The definitive confirmation of glucosylceramide as the pathological storage lipid was presented by the work of Rosenberg and Chargaff in 1958 [10].

Of crucial importance at the outset of his investigations into Gaucher disease, Roscoe Brady formulated a clear research question: '*what is the basic cause of the glucocerebroside accumulation?*' He examined this problem in a highly systematic manner – the hallmark of his scientific approach. Keenly employing state-of-the-art techniques using radioactive lipid precursors synthesized in collaboration with expert chemists, David Shapiro and Julian Kanfer, together with Eberhard Trams, examined capacity for de novo formation of glucocerebroside by splenic tissue. In contrast to what had been assumed earlier, the biosynthesis was not impaired [11]. Subsequently, in a series of investigations he provided compelling evidence for defective breakdown of GlcCer in GD. The activity of an acid β-glucosidase named glucocerebrosidase was shown to be markedly reduced in homogenates of spleen and other tissues obtained from Gaucher patients [12-14]. Independently, Dr Desmond Patrick at the Institute of Child Health (University College London) at Great Ormond Street Hospital, London using an artificial β-glucosidic substrate, the deficiency of acid β-glucosidase activity was also shown in [15]. Ernest Beutler developed soon afterwards a more sensitive and facile enzymatic assay for diagnostic purposes based on a fluorogenic artificial substrate [16]. A few years before, Christian de Duve and colleagues had identified lysosomes as sub-cellular acidic compartments involved in macromolecular degradation that harbour a portfolio of specific enzymes with acid pH optima for this purpose. The presence of a glucosylceramide-degrading acid β-

glucosidase and other acid hydrolases with activity against sphingolipid substrates in lysosomes was first reported in 1968 by Dr Neil Weinreb, then working in the Brady laboratory at the National Institutes of Health [17]. These findings demonstrated the central rôle of the lysosome in the breakdown and recycling of cellular sphingolipids implicated in several important diseases, including Niemann-Pick diseases A and B and Fabry disease.

Identification of the primary defective protein, lysosomal acid β-glucosidase, in Gaucher disease, initiated another phase of endeavour: the investigation of enzymes. Here again Brady formulated another crucial and rational question: '*what is the nature of glucocerebrosidase and how is it impaired in Gaucher disease?'* This question was particularly relevant on account of the appreciable residual activity that was obtained with assays employing natural substrates [13, 14]. Purification and characterization of glucocerebrosidase presented many difficulties: the relative low abundance of the protein; its tendency to adhere to membranes and other hydrophobic proteins as well its intrinsic heterogeneity due to variably sialylated N-linked glycans. With formidable persistence, assisted by Peter Penchev, Scott Furbish and others, the enzyme was ultimately purified to homogeneity from human placenta. Later, the entire 495 amino acid sequence was meticulously determined by sequencing based on the Edman-degradation method; in addition, the glycan composition was determined by mass spectrometry. These were truly heroic achievements at that time [18-20]. Again, this fundamental work provided a solid basis for improved diagnosis of Gaucher disease and characterization of the mutant enzyme in patients employing specific antibodies [21]. Moreover, the detailed knowledge which had been gained about the enzyme greatly facilitated subsequent applications in the direction of therapeutic administration of enzyme and gene transfer – in short, a clear pathway of translational research.

Having identified and characterized the culprit enzyme in Gaucher disease, Roscoe Brady formulated another essential research question: '*can exogenous glucocerebrosidase correct the defect?'.* The target cell for correction was defined by him as the tissue macrophage, the primary pathological storage cell first identified by Ernest Gaucher. In a series of systematic investigations, the importance of glycan composition of glucocerebrosidase for its delivery to lysosomes of macrophages was identified [22]. Critically, Roscoe Brady and his colleagues exploited the newly recognized receptor-mediated endocytosis of proteins to lysosomes and the unique presence of mannose-receptors on macrophages [22]. This allowed him and by now the multidisciplinary research team he assembled, including clinicians, molecular biologists and biochemists such as John Barranger, Norman Barton, Edwards Ginns and Gary Murray, to design an appropriate enzyme with mannose-terminated glycans. The initial reports of the Brady team on clinical responses to human placental glucocerebrosidase with glycan modification to expose terminal mannose ligands manufactured in collaboration with Genzyme were seminal in the therapeutic realization of this extraordinary programme of research [23, 24]. The clinical success of this rational stratagem for treatment, often termed enzyme replacement therapy, is well documented: rightly, Roscoe Brady's achievements were acknowledged by the Lasker Foundation Clinical Medical Research Award, the National Medal of Technology and Innovation and the Kovalenko Medal from the National Academy of Sciences. The astonishing success of enzyme therapy in reversing, as well as preventing the principal visceral and haematological manifestations of Gaucher disease in children and adults, was not only life-changing for patients all over the world but also transformed the entire field of research into lysosomal disorders. From the outset, even with the placenta-derived human tissue enzyme, not only did the success of therapy revive the interest of investigators and clinicians in these disorders, but it boosted

the biopharmaceutical development of recombinant glucocerebrosidase preparations produced on different expression platforms and by several competing companies. Later, by then able to take advantage of opportunities offered by Orphan Drug legislation in many countries, the commercial success of enzyme therapy in Gaucher disease greatly incentivized the introduction of other genetically engineered recombinant lysosomal hydrolases for treating corresponding lysosomal disorders.

Enzyme therapy is effective in correcting the pathological phenotype of tissue macrophages present in the viscera and the attendant clinical complications of Gaucher disease. However, it is unable to address the manifestations of established disease associated with fibrotic scarring and inflammation in for example the skeleton; and clearly parenteral enzyme cannot prevent neurological manifestations characteristic of patients with the more severe forms of disease. Roscoe Brady also took up this latter formidable challenge and together with Dr Raphael Schiffmann (presently Baylor Research Institute, Dallas), also investigated the efficacy of administration of enzyme to the brain [25-27]. In parallel, he fostered the work on gene therapy by his fellow Stefan Karlsson who subsequently also generated now widely used mouse models of Gaucher disease [28, 29]. Gene therapy research was sustained successfully by Jeff Medin (presently University of Toronto); latterly, Stefan Karlsson (presently at Lund University) has generated third-generation advanced lentiviral vectors with the promise of human application [30]. Future success of gene therapy of neuronopathic Gaucher disease soon may also be attributable to Brady's fundamental contributions - including the joint work with Mia Horowitz, Ari Zimran and others on the characteristics of the glucocerebrosidase gene and its pathological mutations (GBA1, locus 1q21) [31]. Late in life, Roscoe Brady remained eager to stimulate definitive and well thought-out alternative investigation of treatments for Gaucher disease; and his deep knowledge of sphingolipid biochemistry, enzymology and molecular biology facilitated exploration of molecular chaperone therapy as well as potential therapeutic use of HDAC inhibitors [32-34]. Over the years, he promoted the work of innumerable acolytes and talented young research scientists worldwide – often providing invaluable guidance for the clear formulation and realization of their ideas.

In a brief autobiographical article, Roscoe Brady revealed that as a third-year clinical medical student at Harvard during the dark days of World War II, two encounters with young patients who suffered fatal complications of heart disease moved him to seek a life-long scientific path in lipid research [35]. Rather than pursue a specialist career in what would have been 'patch-up' medicine or cardiac surgery, and at a time when practically nothing was known about the metabolism of fatty acids or cholesterol, he planned his unconventional career departure. Immediately after completing his internship at the University hospital of Pennsylvania, Brady applied for and was awarded a National Research Council fellowship in the Department of Biochemistry at the University of Pennsylvania School of Medicine.

During his entire career, Brady was intrigued by the potential toxicity of lipid metabolites – this fascination was later extended to the glycosphingolipidoses as the portfolio of opportunities opened up at the Neurochemistry laboratory in the National Institutes of Health and as his biochemical skills and confidence expanded. In general, research interests in metabolites, a popular research topic up to the nineteen sixties, waned: the characterization of proteins, logically followed by genetics, as the science of molecular biology became accessible to experiment, soon claimed centre stage in biomedical research. During a long and highly productive working life, Roscoe Brady witnessed these

transitions - and it is notable that he seemed easily to negotiate the conceptual and the technical inventiveness that innovation demanded. Indeed, he had a striking capacity to integrate methodological advances and bring new platforms of discovery to bear on the research being undertaken in his laboratory.

When first in the laboratory, Brady carried out demanding studies on the biochemistry of fatty acid biosynthesis: he used his empirical knowledge gained from the study of enzyme-catalyzed acetyl transthiolation reactions, to discover the critical involvement of malonyl-CoA in the biosynthesis of long-chain fatty acids - ultimately identifying the mechanism of the condensation reaction responsible [36-38]. This work, initiated again during hard times (of the Korean war, 1952-4), and during secondment as head of the clinical chemistry laboratory at the National Naval Medical Center in Bethesda, was completed after his precocious appointment to the combined basic research laboratories of the then National Institute of Neurological Diseases and Blindness and the National Institute of Mental Health. From this time, in an environment enriched by neuroscience and enthusiastic chemists, Roscoe Brady's research interests naturally moved to the metabolism of lipid molecules of importance in nervous tissue. In work of prescient significance, he embarked on investigations into the biosynthesis of the principal lipid of the brain, the sphingolipid, galactocerebroside (galactosylceramide). Sphingolipids are defined by a unique component, sphingosine - a long-chain amino alcohol with enigmatic properties so named by its discoverer, JLW Thudichum. Cerebrosides, of which glucosylceramide that is found in excess in Gaucher disease is a class member, contain long-chain fatty acids and sugar moieties linked to the sphingosine moiety. After defining the requirements for formation of sphingosine *de novo* from palmitoyl CoA and L-serine [39], Brady and colleagues set out systematically to explore biosynthesis of cerebrosides: in brain microsomes, incorporation of glucose and galactose was found to depend on the presence of energy-rich nucleotide sugars, which had been identified in the contemporaneous (and ultimately Nobel prize-winning) discoveries of Luis Leloir [40]. These investigations, involving UDP-glucose and UDP-galactose as hexose donors - combined with Brady's deep knowledge of the demanding methodology - immediately made possible exploration of the key biosynthetic reactions leading to formation of all three component moieties of the cerebrosides. In a synergistic collaboration with David Shapiro from The Weizmann Institute of Science, Brady was able to develop sophisticated radio-labelled substrates suitable for investigating the fundamental defect in Gaucher disease. Later he studied the metabolism of more complex sphingolipids, including the globosides and gangliosides implicated in related disorders of iconic pathological obscurity; with this, the stage was set for discoveries in what grew exponentially generate an important and competitive field of enzymology. While Okada and O'Brien were the first to discover the enzyme defect in hexosaminidase A responsible for Tay-Sachs disease, Brady's laboratory rose rapidly to a dominant position. This was further evident from studies of sphingomyelin metabolism implicated in classical Niemann-Pick disease and the identification of alpha-galactosidase deficiency responsible for Anderson-Fabry disease.

As shown above, profound methodological understanding and a systematic but interdisciplinary approach to biological research proved critical for a succession of discoveries in the whole field of sphingolipid biochemistry by the Brady Laboratory at NIH. These were translated into studies of molecular pathogenesis, diagnostic enzymology and genetics - as well as applied therapeutic science. A striking outcome of the work programme reflects two contrasting but clinically validated means of treating glycosphingolipid diseases: targeted molecular therapy involving replacement of the missing

factor (usually a matrix enzyme) or the use of specific inhibitory molecules to rebalance the rate of glycosphingolipid biosynthesis with its rate of breakdown. This latter stratagem, so-called substrate-reduction therapy, later taken up by Norman Radin, Frances Platt and James Shayman and their associates. It is notable that Brady's early excursions into the sphingolipid field involved studies of Gaucher disease: eventually it was the prodigious efforts of his laboratory in this disease that yielded his most productive and worthwhile discoveries as applied to medicine. These achievements were not only recognized by his early election as a Member of the National Academy of Sciences and award of a Lasker prize and other prestigious awards, but they gave Roscoe Brady a deep personal satisfaction. This depth of feeling is no more evident than in images in which he is shown in the company of patients whose lives were dramatically changed by his enduring commitment to clinical research [35].

Although Roscoe Brady remained faithful to his first love of lipid biochemistry, his lifetime of scientific work retained a strong translational perspective; and throughout, his underlying motivation to improve clinical practice and develop credible therapies was undimmed. At times he could appear brusque with those who had a superficial scientific understanding, but his adherence to invariant biological principles was unerring and his behaviour in the presence of distressed patients was dignified and caring. From the scientific aspect, Roscoe Brady's advice carefully to re-examine metabolite abnormalities in glycosphingolipidoses using advanced mass spectrometric techniques should be taken to heart. Indeed, in Gaucher disease it is not only glucosylceramide that accumulates, but also the unacylated sphingolipid, glucosylsphingosine – concentrations of which are elevated several hundred-fold in body fluids and tissues. That the same phenomenon also holds true for Fabry disease where, in addition to the excessive globotriaosylceramide, the water-soluble base, globotriaosylsphingosine is prominently increased, was a consequential finding that delighted Dr Brady [41]. There is mounting evidence that that the minor glycosphingoid base abnormalities in several of the sphingolipidoses disease contribute to tissue injury and other manifestations: the pathological overproduction of glucosylsphingosine has been linked to B-cell lymphoma [42, 43] and it has been recently reported that Gaucher-related glycolipids act as auto-antigens driving B-cell proliferation - thus directly promoting development of multiple myeloma, a blood cell cancer occurring with a greatly increased frequency in patients with Gaucher disease [44]. Excessive galactosylsphingosine is widely regarded as toxic to oligodendroglia and their precursors in Krabbe disease [45] and excessive globotriasylsphingosine has been reported to be toxic for nociceptive peripheral neurons and podocytes in Fabry disease patients [46, 47]. Dr Brady's admonitions to explore fundamental aspects of disease may also be justified in terms of physiological understanding, since deacylation of complex sphingolipids to water-soluble derivatives may represent a natural means for their transport and ultimate disposal.

Envoi

In a long and creative life of discovery and one populated by gifted colleagues and high achievement, Dr Brady's reputation should move honorably to that offered by posterity. Here it places on us a duty to crystallize his legacy in context. In this great man, we have a person whose skill and staying power marks him out on account of his revolutionary introduction of an effective molecular therapy for a diverse and challenging lysosomal disease – thus far beyond expectations realizing Christian de Duve's original prediction. The therapeutic achievement alone, with all the attendant economy of orphan medicinal drugs, is a legacy of immense human and tangible value. We also have in Dr Roscoe

Brady a gifted physician-scientist educated in the teutonic tradition of biochemistry, who sublimated his sympathetic feelings and engaged prodigious innate abilities to attain the highest level of scientific attainment, the better to discover and unravel the complexities of human disease. There was much that was private and discreet in Dr Brady and he rarely gave away his true feelings; but large numbers of colleagues and associates as well as patients and industrial collaborators held him in thrall. For his contributions to medicine and to science, brought about by his intellectual leadership and capacity to share; for his imagination as an investigator, as well as sheer doggedness, he commands our deepest and enduring respect.

References

1. Brady RO, Gurin S (1952). The biosynthesis of fatty acids by cell-free or water-soluble enzyme systems. J. Biol. Chem. 199: 421-431
2. Brady RO, Koval GJ (1957) Biosynthesis of sphingosine *in vitro*. J. Am. Chem. Soc. 79, 2648-9
3. Brady RO (2010). Benefits from unearthing "a biochemical Rosetta Stone". J. Biol. Chem. 285:41216-21.
4. Gaucher PCE (1882). De l'epithelioma primitif de la rate, hypertrophie idiopathique de la rate sans leucemie. Faculte de Medecine, These de Paris.
5. Epstein E (1924). Beitrag zur Chemie der Gaucherschen Krankheit. Biochem Z. 145, 398.
6. Lieb H (1924). Cerebroside Speicherung bei Splenomegalie Typus Gaucher. Ztschr. Physiol. Chem. 140, 305–313.
7. Thudichum JL (1884). A treatise on the chemical constitution of the brain. Bailliere, Tindall and Cox, London.
8. Suzuki K, Suzuki Y (1970). Globoid cell leukodystrophy (Krabbe's disease): deficiency of galactocerebroside β-galactosidase. Proc. Natl. Acad. Sci. U. S. A. 66, 302–9.
9. Aghion H (1934). La Maladie de Gaucher dans l'enfance. Faculte de Medecine, These de Paris.
10. Rosenberg A, Chargaff E (1958). A reinvestigation of the cerebroside deposited in Gaucher's disease. J Biol Chem. 1958 Dec;233(6):1323-6.
11. Trams EG, Brady RO (1960). Cerebroside synthesis in Gaucher's disease. J. Clin. Invest. 39, 1546-50.
12. Brady RO, Kanfer J, Shapiro D (1965). The metabolism of glucocerebrosides. I. Purification and properties of a glucocerebroside-cleaving enzyme from spleen tissue. J. Biol. Chem. 240, 39–42.
13. Brady RO, Kanfer JN, Shapiro D (1965). Metabolism of glucocerebrosides. II. Evidence of an enzymatic deficiency in Gaucher's disease. Biochem Biophys Res Commun. 18,221-5.
14. Brady RO, Kanfer JN, Bradley RM, Shapiro D (1966). Demonstration of a deficiency of glucocerebroside-cleaving enzyme in Gaucher's disease. J Clin Invest. 45, 1112–1115.
15. Patrick AD (1965). A deficiency of glucocerebrosidase in Gaucher's disease. Biochem J. 97, 17C–18C.
16. Beutler E, Kuhl W (1970). Detection of the defect of Gaucher's disease and its carrier state in peripheral-blood leucocytes. Lancet. 1(7647):612-3.
17. Weinreb NJ, Brady RO, Tappel AL (1968). The lysosomal localization of sphingolipid hydrolases. Biochim. Biophys. Acta 159, 141–6.
18. Pentchev PG, Brady RO, Hibbert SR, Gal AE, Shapiro D (1973). Isolation and characterization of glucocerebrosidase from human placental tissue. J. Biol. Chem. 248, 5256–5261
19. Furbish F.S, Blair HE, Shiloach J, Pentchev PG, Brady RO (1977). Enzyme replacement therapy in Gaucher's disease: large-scale purification of glucocerebrosidase suitable for human administration. Proc. Natl. Acad. Sci. U.S.A. 74, 3560–3.
20. Takasaki S, Murray GJ, Furbish FS, Brady RO, Barranger JA, Kobata A (1984). Structure of the N-asparagine-linked oligosaccharide units of human placental beta-glucocerebrosidase. J Biol Chem. 259(16):10112-7.
21. Ohashi T, Hong CM, Weiler S, Tomich JM, Aerts JM, Tager JM, Barranger JA (1991). Characterization of human glucocerebrosidase from different mutant alleles. J Biol Chem. 266:3661-7.

22. Brady RO (2003). Enzyme replacement therapy: conception, chaos and culmination. Philos Trans R Soc Lond B Biol Sci. 358:915-9.
23. Barton NW, Furbish FS, Murray GJ, Garfield M, Brady RO (1990) Therapeutic response to intravenous infusions of glucocerebrosidase in a patient with Gaucher's disease. Proc. Natl. Acad. Sci. U.S.A. 87, 1913–6.
24. Barton NW, Brady RO, Dambrosia JM, DiBisceglie AM, Doppelt SH, Hill SC, Mankin HJ, Murray GJ, Parker RI, Argoff CE, Grewal RP, Yu K-T (1991) Replacement therapy for inherited enzyme deficiency: macrophage-targeted glucocerebrosidase for Gaucher's disease. N. Engl. J. Med. 324, 1464–70.
25. Schiffmann R, Heyes MP, Aerts JM, Dambrosia JM, Patterson MC, DeGraba T, Parker CC, Zirzow GC, Oliver K, Tedeschi G, Brady RO, Barton NW (1997). Prospective study of neurological responses to treatment with macrophage-targeted glucocerebrosidase in patients with type 3 Gaucher's disease. Ann Neurol. 42:613-21.
26. Lonser RR, Walbridge S, Murray GJ, Aizenberg MR, Vortmeyer AO, Aerts JM, Brady RO, Oldfield EH (2005). Convection perfusion of glucocerebrosidase for neuronopathic Gaucher's disease. Ann Neurol. 57:542-8.
27. Lonser RR, Schiffman R, Robison RA, Butman JA, Quezado Z, Walker ML, Morrison PF, Walbridge S, Murray GJ, Park DM, Brady RO, Oldfield EH (2007). Image-guided, direct convective delivery of glucocerebrosidase for neuronopathic Gaucher disease. Neurology 68:254-61.
28. Dunbar CE, Kohn DB, Schiffmann R, Barton NW, Nolta JA, Esplin JA, Pensiero M, Long Z, Lockey C, Emmons RV, Csik S, Leitman S, Krebs CB, Carter C, Brady RO, Karlsson S (1998). Retroviral transfer of the glucocerebrosidase gene into CD34+ cells from patients with Gaucher disease: in vivo detection of transduced cells without myeloablation. Hum Gene Ther. 9:2629-40.
29. Enquist IB, Lo Bianco C, Ooka A, Nilsson E, Månsson JE, Ehinger M, Richter J, Brady RO, Kirik D, Karlsson S (2007). Murine models of acute neuronopathic Gaucher disease. Proc Natl Acad Sci U S A. 104:17483-8.
30. Dahl M, Doyle A, Olsson K, Månsson JE, Marques AR, Mirzaian M, Aerts JM, Ehinger M, Rothe M, Modlich U, Schambach A, Karlsson S (2015). Lentiviral gene therapy using cellular promoters cures type 1 Gaucher disease in mice. Mol. Ther. 23:835-44.
31. Horowitz M, Tzuri G, Eyal N, Berebi A, Kolodny EH, Brady RO, Barton NW, Abrahamov A, Zimran A (1993). Prevalence of nine mutations among Jewish and non-Jewish Gaucher disease patients. Am J Hum Genet. 53:921-30.
32. Abe A, Gregory S, Lee L, Killen PD, Brady RO, Kulkarni A, Shayman JA (2000). Reduction of globotriaosylceramide in Fabry disease mice by substrate deprivation. J Clin Invest. 105:1563-71.
33. Brady RO, Yang C, Zhuang Z (2013). An innovative approach to the treatment of Gaucher disease and possibly other metabolic disorders of the brain. J Inherit Metab Dis. 36:451-4.
34. Yang C, Swallows CL, Zhang C, Lu J, Xiao H, Brady RO, Zhuang Z (2014). Celastrol increases glucocerebrosidase activity in Gaucher disease by modulating molecular chaperones. Proc Natl Acad Sci U S A. 111:249-54.
35. Brady RO (2010). Benefits from Unearthing “a Biochemical Rosetta Stone.” J Biol Chem. 285: 41216-21.
36. Brady RO, Stadtman E R. (1954) Enzymatic thiol transacetylation. J. Biol. Chem. 211: 621-29;
37. Brady RO (1958). The enzymatic synthesis of fatty acids by aldol condensation. Proc. Natl. Acad. Sci. U.S.A. 44, 993-98;

38. Brady, R. O. (1960) Biosynthesis of fatty acids. II. Studies with enzymes obtained from brain. J. Biol. Chem. 235, 3099-103.
39. Brady R O, Koval G J (1957). Biosynthesis of sphingosine in vitro. J. Am. Chem. Soc. 79: 2648-49.
40. Buton R M, Sodd MA, Brady R O (1958). The incorporation of galactose into galactolipids. J. Biol. Chem. 233: 1053-60.
41. Aerts JM, Groener JE, Kuiper S, Donker-Koopman WE, Strijland A, Ottenhoff R, van Roomen C, Mirzaian M, Wijburg FA, Linthorst GE, Vedder AC, Rombach SM, Cox-Brinkman J, Somerharju P, Boot RG, Hollak CE, Brady RO, Poorthuis BJ (2008). Elevated globotriaosylsphingosine is a hallmark of Fabry disease. Proc Natl Acad Sci USA. 105, 2812–2817.
42. Pavlova E, Wang S, Archer J, Dekker N, Aerts J, Karlsson S, Cox T (2013). B cell lymphoma and myeloma in murine Gaucher's disease. J. Pathol. 231, 88–97.
43. Pavlova E V, Archer J, Wang S, Dekker N, Aerts JM, Karlsson S, Cox TM (2015). Inhibition of UDP-glucosylceramide synthase in mice prevents Gaucher disease-associated B-cell malignancy. J. Pathol. 235, 113–124.
44. Nair S, Branagan AR, Liu J, Boddupalli CS, Mistry PK, Dhodapkar MV (2016). Clonal Immunoglobulin against Lysolipids in the Origin of Myeloma. N Engl J Med. 374, 555-561.
45. Suzuki K (1998). Twenty-five years of the "Psychosine Hypothesis": a personal perspective of its history and present status. Neurochem Res. 23, 251–259.
46. Choi L, Vernon J, Kopach O, Minett MS, Mills K, Clayton PT, Meert T, Wood JN (2015). The Fabry disease-associated lipid Lyso-Gb3 enhances voltage-gated calcium currents in sensory neurons and causes pain. Neurosci Lett. 594, 163-168.
47. Sanchez-Niño MD, Carpio D, Sanz AB, Ruiz-Ortega M, Mezzano S, Ortiz A (2015). Lyso-Gb3 activates Notch1 in human podocytes. Hum Mol Genet. 24, 5720-5732.