In reflecting about incidents, accomplishments, and disappointments that occurred during my career, many of them seem almost apocryphal. I acquired an interest in lipids in my third year at Harvard Medical School when I was caring for a young woman with a severe heart problem at the then-Boston City Hospital. World War II was in full swing, and medical students were intimately involved in the care and treatment of patients. She had two young children and a devoted husband, who was truly grateful for any help I could provide. One evening, while I was still on the ward, she died. You may well imagine the sadness at rounds the next morning. The next year, I was involved in the care of a 51-year-old man with a myocardial infarction. He was one of the first patients to undergo angioplasty to try to treat the condition. He died on the operating table. These distressing events made me think about trying to do something that might help such people.

At that time, virtually nothing was known about the metabolism of fatty acids or cholesterol. After an internship at the hospital of the University of Pennsylvania, I decided to try to obtain training in the biochemistry of lipids. I was awarded a National Research Council fellowship in the Department of Biochemistry at the University of Pennsylvania School of Medicine, where I worked with a superb biochemist named Samuel Gurin. He believed that the enzymatic synthesis of cholesterol proceeded from acetate through acetoacetate, which was shown to be correct using 14C-labeled acetoacetate (1). Gurin thought that acetoacetate had to be converted to a metabolically active form and that it might be phosphoenol-acetoacetic acid. I began a project to synthesize this compound to test its potential as a precursor of cholesterol, and Gurin went to England for a six-month sabbatical. Needless to say, I did not succeed in making it, nor was it ever shown to be an intermediate in cholesterol formation. When Gurin returned, we began to examine the conversion of acetate and other short-chain fatty acids and acetone to long-chain fatty acids and cholesterol in slices of rat liver tissue. We learned that the conversion of 14C-labeled acetate to long-chain fatty acids had an absolute requirement for bicarbonate in the incubation medium (2). Moreover, our findings indicated that the conversion of acetone to long-chain fatty acids occurred by its conversion to a metabolically active 2-carbon fragment that was not acetate but might be secondarily converted to acetate (1). Additional studies at that time included investigations on the impairment of fatty acid synthesis in diabetes (3) and the demonstration that insulin stimulated fatty acid synthesis, whereas growth hormone and cortisone inhibited this process (4).

Gurin and I realized that to gain insight into the biochemical reactions involved in the biosynthesis of long-chain fatty acids, we needed to find an enzyme system that catalyzed this process. Pigeon liver was known to be metabolically quite active. We decided to prime fatty acid synthesis in that organ by feeding pigeons glucose over a four-hour period before preparing homogenates of liver tissue. Using a buffer supplemented with potassium bicarbonate, we discovered that such preparations could convert 14C-labeled acetate to long-chain fatty acids (5). Eventually, a completely water-soluble enzyme system was developed (6). A marked stimulation of fatty acid for-
malonyl-CoA in the biosynthesis of long-chain fatty acids (see Fig. 2 in Ref. 11). This was the first demonstration of the involvement of malonyl-CoA in the biosynthesis of long-chain fatty acids. I erroneously deduced that the condensation between acetyl-CoA and malonyl-CoA was a Knoevenagel type of reaction. I later demonstrated that the reaction was actually a Claisen-type condensation (12).

Because of my appointment in the National Institute of Neurological Disorders and Stroke, I gradually turned my attention to lipids that were prominent in the central and peripheral nervous systems. It had been known for nearly three-quarters of a century that the major lipid on a weight basis in the brain and in myelinated nerves was galactocerebroside, which consisted of the long-chain amino alcohol sphingosine (Fig. 1A), a long-chain fatty acid, and one molecule of galactose (13). I initiated my studies in this area with an investigation of the enzymatic synthesis of sphingosine (14). The reaction is catalyzed by an enzyme complex in microsomes. Palmitoyl-CoA condenses with carbon 2 of serine in the presence of pyridoxal phosphate and Mg$^{2+}$ or Mn$^{2+}$ ions, forming a Schiff base-metal complex. A reducing material, such as TPNH, is required. Carbons 1 and 2 of sphingosine arise from carbons 3 and 2 of serine. The carboxyl carbon of serine is lost in this condensation. It must be present initially because ethanolamine does not participate in the formation of sphingosine.

I began to investigate the formation of glycosphingolipids and found an enzyme in microsomes of young rat brain tissue that catalyzed the incorporation of radioactive glucose and galactose into cerebrosides using uridine diphosphate galactose as the hexose donor (15). The formation of cerebrosides was of particular interest because it had been
At that time, no information was available concerning the catabolism of cerebrosides. Experiments were therefore undertaken to examine the enzymatic breakdown of glucocerebroside. The first investigation was an attempt to detect the release of free glucose from unlabeled glucocerebroside that had been isolated from the spleens of patients with Gaucher disease. No increment of free glucose that might have originated from glucocerebroside could be detected in surviving animal or human spleen tissue preparations. The reason for this inability to detect cerebrosidase catabolism in this manner was that glucose is extensively metabolized and largely converted to CO₂ under those conditions. A second attempt to examine glucocerebroside metabolism was carried out by radiolabeling glucocerebroside throughout the molecule with tritium. That approach was accomplished by exposing unlabeled glucocerebroside to ³H in a sealed vessel for several days: the Wilzbach technique. Under those conditions, covalently bound hydrogen atoms were replaced with ³H. However, the background radioactivity from ³H-glucocerebroside prepared by that procedure was too great to permit metabolic investigations in vitro.

To overcome these obstacles to investigate glucocerebroside catabolism, I decided that the chemical synthesis of glucocerebroside should be undertaken. I read an article by David Shapiro and H. M. Flowers at the Weizmann Institute of Science in Rehovot, Israel, describing the chemical synthesis of sphingomyelin (20). I wrote to Shapiro telling him that I would like to go to Israel and work with him on the preparation of radioactive sphingomyelin so that we might use it to identify the metabolic defect in Niemann-Pick disease, in which excessive quantities of that lipid accumulate. He wrote back saying that he did not have access to the use of radioactive materials, but, if I could find support, he would come to the United States and help me prepare radioactively labeled glucocerebroside to look at its metabolism in Gaucher disease. I approached Richard Masland, my institute’s director, and he provided Shapiro with modest support for the project. Shapiro came to the NIH and, working with Julian Kanfer, synthesized two preparations of [¹⁴C]glucocerebroside. Radioactivity was introduced into the fatty acid moiety in one preparation and into the glucose portion in the other. Experiments with these labeled lipids revealed that all mammalian tissues contain an enzyme called glucocerebrosidase (glucosylceramide β-glucosidase) that catalyzes the hydrolytic cleavage of glucose from glucocerebroside (21).

Experiments performed in 1964 revealed that the metabolic defect in Gaucher disease is a deficiency of glucocerase.

### Reflections: Unearthing a Biochemical Rosetta Stone

**Known since 1924 that Gaucher disease, the most prevalent hereditary metabolic storage disorder of humans, was characterized by an accumulation of cerebrosides.** The German physician H. Lieb thought that it was galactocerebroside (16). However, the optical rotation of the aqueous cleavage product was incompatible with that deduction. In 1934, the French chemist A. Aghion correctly identified the accumulating glycolipid as glucocerebroside (Fig. 1B) (17).

Patients with Gaucher disease experience a number of problems that can lead to death. Among them are massive enlargement of the spleen and liver; severe anemia; low blood platelets, leading to easy bruising and frequent hemorrhages; reduced white blood cell count; skeletal damage, including Erlenmeyer flask deformity of the distal femur; multiple fractures of the hips, spine, and elsewhere; and a predisposition to multiple myeloma. From 1934 until the early 1960s, there was considerable speculation about the metabolic basis of Gaucher disease (18). I undertook studies to try to resolve this dilemma. Because of the possibility of an error of carbohydrate metabolism that resulted in substitution of glucose for galactose in the cerebrosides of patients with Gaucher disease, the first investigation was an examination of galactose tolerance in a patient with Type 1 Gaucher disease. It was anticipated that, if patients could not carry out the intermediary steps required for galactose metabolism, including the formation of UDP-galactose, their cerebrosides would contain only glucose. This appeared not be the case because galactose tolerance was found to be normal in a patient with Gaucher disease.

The next experiments to identify the metabolic basis of Gaucher disease were performed with surviving slices of spleen tissue obtained from patients with the disorder who underwent splenectomy because of severe hematological difficulties. Investigations with [¹⁴C]glucose and [¹⁴C]galactose revealed that both of the hexoses were efficiently utilized as precursors of both glucocerebroside and galactocerebroside, thereby eliminating an abnormality in the pathway of cerebroside synthesis in Gaucher disease. The rate of glucocerebroside formation was then compared in spleen slices obtained from patients with Gaucher disease with the rate in similar preparations from two patients with Niemann-Pick disease and one patient with idiopathic thrombocytopenic purpura. That investigation revealed that there was no increase in the rate of glucocerebroside formation in the tissues derived from the patients with Gaucher disease. Those observations led to the prediction that a defect in glucocerebroside catabolism was the basis of Gaucher disease (19).
rebrosidase (22). Those initial observations were substan-
tiated in a more extensive study carried out the next year
(23). Patients with Type 1 (non-neuronopathic) Gaucher
disease exhibited glucocerebrosidase activity that aver-
aged ~11% of the normal activity. Patients with Type 2
(acute neuronopathic) Gaucher disease had extremely low
glucocerebrosidase activity (1–3% of the normal activity),
and patients with Type 3 (subacute neuronopathic) Gau-
cher disease had glucocerebrosidase activity that was gen-
erally between that in Types 1 and 2. The deficiency of
glucocerebrosidase in Gaucher disease has been univer-
sally confirmed. In time, it was shown that glucocerebro-
sidase is primarily a lysosomal enzyme (24). Because the
highest concentration of the glucocerebrosidase activity is
in lysosomes, Gaucher disease is properly classified as a
lysosomal storage disorder. Maximal catalytic activity of
this enzyme occurs at pH 5.5–5.9.

This discovery was of primary importance concerning
the underlying cause of lysosomal storage disorders. In
fact, the American Chemical Society had a cover story
depicting it with me in tennis clothes on the cover (25).
Once the etiology of Gaucher disease was established, the
enzymatic defects in other hereditary metabolic storage
disorders were rapidly elucidated. These included Niemann-Pick disease in 1966 (26); Fabry disease in 1967
(27), and Tay-Sachs disease in 1969 (28, 29). Practical ben-
efit soon followed. Facile tests were developed to identify
patients based on enzyme assays in white blood cells (30)
and cultured skin fibroblasts (31), the detection of het-
erozygous carriers of these disorders (32), and the prenatal
diagnosis of these conditions (33–35).

The year after the discovery of the enzymatic defect in
Gaucher disease, I proposed that enzyme replacement
therapy should be investigated to treat patients with met-
abolic storage disorders (36). I wanted to use a human
source of the missing enzymes, and one evening I thought
of the placenta. The next day, I went into the laboratory,
homogenized some fresh placental tissue, and found that
it did contain several sphingolipid hydrolases. The quan-
tities were not great, but I began to work with this source.
Although encouraged by those observations, two major
impediments to successful enzyme replacement therapy
for patients were immediately encountered. My colleagues
and I worked for one year to obtain 9 mg of purified pla-
cental glucocerebrosidase. When the enzyme was injected
into a third patient with Gaucher disease, we did not
observe a significant reduction of glucocerebroside in the
liver, and there was no decrease of glucocerebroside in the
circulation. She had accumulated 20 times more glueco-
crebroside in the liver than the first patient and 10 times
more than the second. It was apparent that we would need
much larger quantities of the enzyme to expect consistent
benefit. A purification procedure based on hydrophobic
column chromatography was developed (42). Although
the enzyme was obtained in good yield and was perfectly
active with [14C]glucocerebroside as substrate, another
potentially formidable problem was encountered. Gluco-
cerebrosidase purified in this fashion was taken up primar-
ily by hepatocytes in the liver, in which there is no accu-
mulation of glucocerebroside. The lipid accumulates in
macrophages, such as the Kupffer cells in the liver. We
needed to devise a strategy to divert glucocerebrosidase from
hepatocytes to macrophages. Three important lessons were
learned. 1) Glucocerebrosidase and other lysosomal enzymes
are glycoproteins. Glucocerebrosidase has four oligosacca-
rside side chains. 2) Macrophages have a lectin on the plasma
cell membrane with high affinity for mannose-terminal gly-
coproteins. 3) The molecules of mannose on placental gluco-
cerebrosidase are shielded from interacting with the lectin by
death of three disaccharides: N-acetylneuraminic acid, galactose,
and N-acetylgalcosamine. To expose the mannose residues,
glucocerebrosidase was treated sequentially with three exo-
glycosidases: neuraminidase, β-galactosidase, and hex-
osaminidase (43). Glucocerebrosidase modified in that man-
ner is endocytosed by macrophages 50 times more effectively
than unmodified glucocerebrosidase. Administration of ade-
this writing, more than 5,600 patients with Gaucher disease throughout the world are benefiting from enzyme replacement therapy. Enzyme replacement therapy has been approved also for patients with Fabry disease and four additional lysosomal storage disorders.

I am certain that readers of these reflections will have acquired a clear concept of how arduous and time-consuming these investigations were. I feel very fortunate to have had the support of my co-workers, my institute directors at the NIH, and the members of many scientific review boards during the lengthy period required to bring enzyme replacement therapy to fruition.

Editor's Note—The work reported in this Reflections article is of dramatic importance in indicating how carefully performed basic biochemical studies can lead to dramatic therapeutic successes.

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