Review

Reflections on my career in analytical chemistry and biochemistry

By Charles C. SWEELEY*1,†

(Communicated by Kunihico SUZUKI, M.J.A.)

Abstract: My career has been focused in two major areas, analytical chemistry and biochemistry of complex lipids and glycoconjugates. Included here are the pioneering work on the gas chromatography of long-chain sphingolipid bases, carbohydrates, steroids and urinary organic acids. Mass spectrometry was utilized extensively in structural studies of sphingolipids, fatty acids, carbohydrates, steroids, urinary organic acids, polyisoprenoid alcohols, and juvenile hormone. Computer systems were developed for the acquisition and analysis of mass spectra, and were used for development of automated metabolic profiling of complex mixtures of metabolites. Fabry's disease was discovered to be a glycosphingolipidosis. Enzymes of lysosomal metabolism of glycosphingolipids were purified, characterized, and used in one of the first demonstrations of the feasibility of enzyme replacement therapy in a lysosomal storage disorder (Fabry's disease). Extracellular sialidases were studied to evaluate the hypothesis that they might be involved in the regulation of membrane growth factor receptors. The enzyme for hematoside synthesis was purified and characterized.

Keywords: gas chromatography, mass spectrometry, glycosphingolipids, lysosomal enzymes, sialyltransferase, Fabry's disease

It might seem odd to some that I would devote much of my professional career studying the biochemistry of sphingolipids. I say this because my early training was in chemistry; my doctoral thesis was entitled "Studies on Streptolidine, a Degradation Product of Streptothricin". I deliberately chose the University of Illinois and Professor Herbert E. Carter as a mentor for doctoral studies because I wanted to work in the field of natural product chemistry, specifically antibiotics. Carter had two laboratories, one for students working on antibiotics, and one for students working on sphingosine and sphingolipids. Little did I know that the research being done in the "sphingolipids lab" would stick to me and that I would turn to that field soon after I began postdoctoral work. My thesis research, as well as that of others in Carter's lab who worked on this antibiotic, was eventually published.[1)]

1. Beginnings

I began postdoctoral work in the Laboratory of Chemistry of Natural Products at the National Heart Institute in 1955. It was there, working in Dr. Evan Horning's laboratory, that I was to find and define what would become my major interests. My first project was on the elucidation of the chemical structure of andromedotoxin, a toxic constituent of mountain laurel (rhododendron). After several months, during which time I had made only modest progress, a group from Nagoya University[2)] announced the structure of grayanotoxin, which turned out to be the same substance.

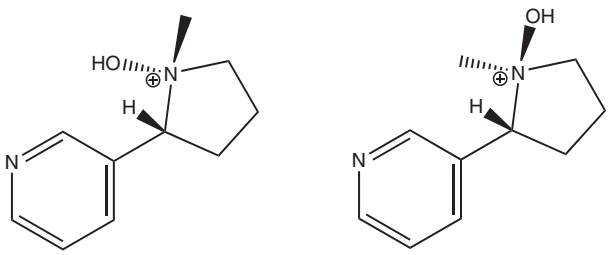
The next project was to study the mechanism of a rearrangement reaction occurring when tertiary amine N-oxides were treated with a complex of ferric ion and tartaric acid.[3)] We found that N,N-dimethylglycine-N-oxide was effectively converted to sarcosine (N-methylglycine) and formaldehyde at

This research (Figs. 2, 3 and 4) was originally published in Journal of Biological Chemistry. Polito, A.J., and Sweeley, C.C. Stereochemistry of the hydroxylation in 4-hydroxysphinganine formation and the steric course of hydrogen elimination in sphing-4-enine biosynthesis. *J. Biol. Chem.* 1971; 246, 4178–4187 © American Society for Biochemistry and Molecular Biology.

*1 Department of Biochemistry, Michigan State University, East Lansing, Michigan, U.S.A.

† Correspondence should be addressed: C. C. Sweeley, Department of Biochemistry, Michigan State University, East Lansing, Michigan 48823, U.S.A. (chucksweeley@comcast.net).

doi: 10.2183/pjab.86.822



1'S,2'S-nicotine-1'-N-oxide 1'R,2'S-nicotine-1'-N-oxide

Fig. 1. Diastereoisomers of nicotine-1′-N-oxide.

room temperature by a transfer reaction of the oxygen atom to one of the methyl groups; transfer of the oxygen to the methylene atom of the glycine moiety gave dimethylamine and glyoxylic acid. A third, pH-dependent reaction yielded carbon dioxide, formaldehyde and dimethylamine.[4] I wanted to see if the same rearrangement reactions would occur with nicotine-1′-N-oxide but discovered a complex of products which probably resulted from oxygen attack on the adjacent ring carbons as well as the N-methyl group. In preparing nicotine-1′-N-oxide, I was able to separate the two diastereoisomers resulting from the asymmetric quaternary pyrrolidine nitrogen atom (Fig. 1). Later studies by Phillipson and Handa[5] provide an excellent overview of the chemistry and enzymology of the nicotine-N-oxides.

Horning had undertaken an experiment with Charles Dalgliesh (University of London) on the metabolism of skatole (3-methylindole) in rats and I was asked to separate and identify the iron-ascorbic acid catalyzed oxidation products of skatole. This turned out to be interesting in the sense that the oxidation products, 4-, 5-, 6-, and 7-hydroxyskatoles, could be completely separated by sequential elution from silicic acid columns with 6% benzene-chloroform solvent, each giving a different color with Van den Bergh's reagent, diazotized sulfanilic acid. It was thus determined that the enzymatic oxidation product of skatole was 6-hydroxyskatole.[6]

An unexpected, career-altering opportunity came to me when Horning ordered the first gas chromatograph at the National Institutes of Health and I was given the task of setting up this machine (Barber-Colman Model 10). Our first work with this new technique was for studies of lipid metabolism, largely following studies in England and U.S. (Lipsky, Callen and others) on the use of polyester liquid phases to separate fatty acid methyl esters.[7] Later, I set out independently to apply gas-liquid chromatography (GC) to other lipids. We reported a new method to analyze sphingolipid bases in sphingomyelin and glycosphingolipids by conversion of these long-chain bases to aldehydes with periodate and separation by GC on a siliconized Celite coated with polyethylene glycol-glutarate.[8),9)] Human plasma sphingomyelin was found to contain sphingosine, dihydrosphingosine, and two unknown bases which were later shown to be sphinga-4,14-dienine and hexadecasphing-4-enine.[9)–11)]

A new method for the preparation of stationary liquid phases for GC had been developed in our laboratory, called the "solution-coating" method,[7] by which we could make column packings containing much lower amounts of liquid phase than had previously been thought to be possible. This in turn allowed us to separate larger molecules and at lower temperatures, decreasing thermal degradation during GC. Horning suggested that we try to resolve steroids on one of these columns and this turned out to be the key to opening a new field. We reported the GC behavior of several androstane-, pregnane-, and cholestane-based steroids on a polyethylene glycol-iso-phthalate column[12] and introduced the use of a General Electric silicone gum, called SE-30.[13),14)]

I left the National Heart Institute in 1960 to take an academic appointment in the Department of Biochemistry and Nutrition at the University of Pittsburgh, in the Graduate School of Public Health. I shall always be grateful for the opportunity to have worked with Evan Horning at NIH. His encouragement and wisdom were instrumental in the early development of my career.

2. Gas chromatography

When I arrived in Pittsburgh I was awarded a grant from NIH to study the biochemistry of lipids. Included in the budget was money to acquire the Barber-Colman Model 10 gas chromatograph with the Lovelock argon ionization detector.

2-1. Steroids. Our first paper was on the GC of steroids, detailing the response of the argon detector as a function of structure.[15] It was discovered that the observed molar response was dependent on the number and nature of functional groups, total oxygen content having the greatest effect. We pointed out that the application of GC to quantitative analyses of steroids would require calibration with each of the steroids in the mixture.

2-2. Fatty acids. We were interested in the lipids present in the adrenal gland since this is where many of the oxidative steps occur in the conversion

of cholesterol to steroid hormones. We developed a procedure for locating double bonds in polyenoic fatty acids from adrenal lipids by periodate-permanganate oxidation and GC[16] and reported the occurrence of a novel fatty acid (7,10,13,16-docosatetraenoic acid), which we called adrenic acid since it seemed to be in abundance only in (canine) adrenal gland.[17]

2-3. Carbohydrates. In those early days in Pittsburgh, Bill Wells and I used to drive to the university together to discuss biochemistry and to give our wives a vehicle for shopping. One day, we were talking about Wells' development of a gas chromatographic method for the analysis of bile acids, using trimethylsilyl (TMS) derivatives. It occurred to us that this reagent might be used to convert carbohydrates to volatile derivatives. By noon of that day, Wells in his laboratory in the medical school, and I in my laboratory in the Graduate School of Public Health, had independently shown that we could separate the anomeric forms of both glucose and galactose as their TMS derivatives at a relatively low GC column temperature. We asked Ronald Bentley, a faculty colleague in my department, to join us in a comprehensive study of the GC behavior of carbohydrates since he was the local carbohydrate expert. Over the next several months, the three of us along with two postdoctoral fellows in Wells' lab, Masami Makita and Toyoshi Katagi, determined the GC retention times of nearly100 carbohydrates (TMS derivatives) on a polar polyethylene glycol-succinate column and a nonpolar silicone gum, SE-52.[18)–20)] Extending these studies to other carbohydrate-containing compounds, we described a method for the determination of the carbohydrate composition of neutral glycosphingolipids and gangliosides by GC.[21]

3. Fabry's disease

I was fortunate at about this time to be introduced to a University of Pittsburgh colleague in the Pathology Department, Bernard Klionsky. He was giving me a tour of his laboratory and told me about a rare genetic disorder called Fabry's disease, supposedly a sphingomyelin disorder as reported by Scriba.[22] I was pleased that he was willing to give me a piece of formalin-fixed kidney from a Fabry patient. I was by then well-equipped with the techniques needed to characterize sphingolipids.

3-1. Novel glycosphingolipids and classification as a sphingolipid storage disease. It did not take long to find that this kidney contained abnormal amounts of two novel glycosphingolipids, which were partially characterized as a galactosyl-1,4-galactosyl-1,4-glucosylceramide (GL-3) and a galactosyl-1,4-galactosylceramide.[23] There were no other abnormal neutral lipids or phospholipids in the sample. Thus, it was concluded that Fabry's disease, an X-linked metabolic disease, should be classified as a sphingolipidosis. The structural comparison of the GL-3 in Fabry's disease with those of globoside, a tetrahexosyl neutral glycosphingolipid discovered by Yamakawa and Suzuki in the stroma of erythrocytes[24] and lactosylceramide, which had been found in the neutral glycosphingolipid fraction of human plasma,[25] suggested the possibility that GL-3 is an intermediate in the metabolism of globoside. My thoughts turned to methods which might be used to prove this supposition and to determine whether the abnormal amounts of GL-3 in Fabry's disease resulted from an abnormality in the biosynthetic pathway or from a deficiency of a galactosidase involved in the metabolism of globoside.

3-2. Stereochemistry of GL-3 (globo-triaosylceramide). In 1971, we reported the anomeric configurations of the glycosidic linkages in GL-3 from Fabry kidney.[26] We were mistaken in assigning an nmr peak for the terminal galactose as a β-configuration. This galactosidic linkage was shown to be in the α-configuration in several laboratories.[27)–30)] An accurate historic account on the chemistry of GL-3 can be found in Sweeley *et al.*[31] The complete chemical structure of GL-3, consistent with the other glycolipids in the globo family of neutral glycosphingolipids, is galactopyranosyl-α1-4-galactopyranosyl-β1-4-glucopyranosyl-β1-1′-ceramide.

3-3. Structural studies of other glycosphingolipids. Structural studies of glycolipids include reports on a glycolipid from *Aspergillus niger*,[32] the structure of a ceramide tetrahexoside hapten from rat lymphosarcoma,[33] a pentaglycosylceramide from canine intestine and kidney,[34] the sphingolipid composition of human platelets,[35] and structures of the glycosphingolipids of membrane fractions of normal and transplanted canine kidney.[36]

3-4. Stable isotope-labeled glucose for metabolic studies. We wanted to do *in vivo* metabolic studies of the globo-type glycosphingolipids in Fabry patients but concluded that use of radioactively labeled tracers was not feasible. Stable isotope-labeled sugars were just becoming available and we obtained a sample of perdeuteroglucose ($^2H_7$-glucose) from Merck, Sharpe and Dohme in Montreal. Analysis of mixtures of the TMS derivatives of the protium

and deuterium forms gave GC peaks that were slightly wider than those of either sugar alone, suggesting the possibility of a chromatographic isotope effect. We needed GC columns with greater resolving power (theoretical plates) to test this possibility. Capillary columns were just then becoming known, mainly in Europe, and were not commercially available. Bentley and I therefore decided to make a long packed column from 1/8 inch (i.d.) copper tubing. We packed 8 foot sections of this tubing with 3% SE-30 stationary phase and coupled 6 sections together with Swagelok fittings. The result was a GC column with approximately 40,000 theoretical plates. Mixtures of TMS glucose and glucose-d_7 were completely resolved by this column in an F & M Model 400 gas chromatograph, with retention times of about 153 minutes for the protium form.[37] Curiously, the peak for the deuterium labeled glucose was somewhat broader than that of the protium form (31,000 plates for the deuterium form versus 40,000 plates for the protium form). It was interesting that the deuterium-labeled form of glucose (β-anomer) eluted from the GC column **before** the protium form even though it had the higher molecular weight (547 vs 540 for the TMS derivatives). We demonstrated that one could use this GC column to determine the relative amounts of labeled and unlabeled glucose from metabolic studies down to as low as 0.5% of the labeled species. Other groups were reporting chromatographic isotope effects at about this same time; a detailed study of the chromatographic isotope effect was reported by Peter Klein.[38] We concluded from studies of band broadening factors and gas and liquid phase diffusion coefficients that separation of the protium and deuterium forms could be attributed to differences in vapor pressure and differential solubilities in the liquid phase.[39] We were unable to account for the band broadening differences.

3-5. Levels of globo-type glycosphingolipids. Returning to the problem of neutral glycosphingolipid metabolism in patients with Fabry's disease, there were several avenues that needed to be pursued in addition to the use of stable isotopes. Dennis Vance, a graduate student in my laboratory, was interested in this project as his dissertation research. He analyzed the levels of neutral glycosphingolipids in normal[40] and Fabry plasma and red cells.[41] The circulating level of the GL-3 was elevated about three-fold in the plasma of all Fabry patients (hemizygotes) studied and also was elevated in two female heterozygotes. The red cell levels of globoside were reduced in the Fabry patients while the amount of GL-3 was normal. There was little if any digalactosylceramide in either plasma or red cells of the Fabry patients. This substance had been shown by Martensson to be a constituent of the glycolipid fraction in normal kidney.[42]

3-6. Chromatographic isotope effect in GC of TMS derivatives of deuterium-labeled glucose. Having concluded that we would have to use a stable isotope to study glycosphingolipid metabolism in Fabry's disease, Vance set out to select a suitable isotope-labeled glucose as a substrate for these studies while I was leaving for Stockholm (1965) to determine how to utilize the newly developed LKB combined GC-mass spectrometer (LKB-9000) for our work. I had submitted a supplement to my NIH grant requesting funds to purchase this instrument before I began my sabbatical in Sweden. In Ragnar Ryhage's laboratory at the Karolinska Institute I was introduced to the LKB-9000 prototype and given permission to use this instrument for my research. I had the good fortune to have the assistance of a young medical student from Columbia University, Ian Fries, and a visiting professor from St. Louis University, William Elliott, to work with me on this project. The first thing we did was to take mass spectra as frequently as we could during the elution of a 50/50 mixture of TMS protium and d_7 glucoses from a 2-meter 3% SE-30 column (approximately 15 second intervals). The band width was about 2 minutes so that we were able to obtain about 10 mass spectra. Collecting the mass spectra with an oscillographic recorder, I measured the intensities of several ions for the protium and d_7 forms, and plotted ion intensity versus time. As suspected from our earlier studies by GC, the d_7 form eluted before the protium form, with about 30 seconds difference in retention time. Armed with this result, I approached Ryhage and told him it would not be possible to use GC-MS to determine isotope ratios because they were changing throughout the elution of the mixture. After substantial deliberation among Ryhage's engineers, they came up with an answer, which was to switch the accelerating voltage back and forth between two values chosen to focus two ions, one for the protium form and one for the d_7 form. Fries built the device, we demonstrated that it worked, and thus was born one of the earliest reports on selected ion monitoring even though we called it the accelerating voltage alternator and did not give a name to the method,[43] which has since become widely used and called various names including mass fragmentography.

Table 1. Micromolar levels of neutral glycosphingolipids in plasma and incorporation of 6,6-$^2H_2$-glucose in normal and Fabry's disease plasmas

Glycolipid[a]	Plasma Pool Size[b]		% 6,6-$^2H_2$-Glucose[c]	
	Normal	Fabry	Normal	Fabry
Glucosylceramide	9.8	7.8	0.8	0.8
Lactosylceramide	5.5	4.7	0.8	1.6
Globotriaosylceramide	2.1	7.6	0.4	0.1
Globoside	2.8	3.1	0.7	0.4

[a]Globotriaosylceramide is the name given in the nomenclature for glycolipids[109] for the Fabry GL-3.
[b]Micromolar concentrations in plasma.
[c]Percent incorporation of labeled glucose into the glucose moiety of the plasma glycosphingolipid.

3-7. *In vivo* synthesis and turnover of plasma and erythrocyte globo-type neutral glycosphingolipids in Fabry's disease. The biosynthesis and metabolism of plasma glycosphingolipids was studied in a patient with Fabry's disease, using 6,6-$^2H_2$-glucose[44] as a tracer, and subsequently in a pig, using uniformly ^{14}C-labeled glucose.[45),46] Table 1 shows the mean micromolar levels of each of the globo-type neutral glycosphingolipids in normal and Fabry plasma.[40),41] The incorporation of glucose into these glycosphingolipids in a Fabry patient and a control was measured by GC-MS following the infusion of 35 g of 6,6,-$^2H_2$-glucose with a 5 g priming dose into each subject, using the ion intensities at m/z 200 and m/z 202 from mass spectra of the 2,3,4,6-tetra-O-acetyl methyl glucosides, representing the glucose moiety of the glycolipids. Maximum incorporation of the labeled glucose occurred in all of the glycolipids about 48 hours after glucose infusion, and the label disappeared at logarithmic rates in all of the glycosphingolipids except the Fabry GL-3 (globotriaosylceramide) (Table 1). Our results strongly suggested that the accumulating GL-3 in Fabry plasma was not derived from a site of biosynthesis (presumably liver) of the other neutral glycosphingolipids.[44)–46]

3-8. Metabolism of neutral glycosphingolipids in porcine plasma and erythrocytes. Dawson and Tao obtained the same results in porcine plasma, which contained the same mixture of neutral glycosphingolipids as were present in human plasma.[45),46] Maximum incorporation of ^{14}C-labeled glucose occurred in the plasma lipids about 24 hours after injection of 5 mCi of glucose into young Yorkshire pigs. After about 9 days the radioactivity had disappeared from all of the plasma glycolipids. Label in the erythrocyte lactosylceramide, GL-3 and globoside persisted, however, until about 60 days when the labeled erythrocytes reached senescence. At that time, label reappeared in the plasma pools of lactosylceramide, GL-3 and globoside. Glucosylceramide behaved differently, however, because of a dynamic equilibrium between the plasma and erythrocyte pools of this lipid. We concluded from these studies that erythrocyte lacotosylceramide, GL-3 and globoside were synthesized in bone marrow and incorporated into the erythrocyte membrane, where they remained until the erythrocytes reached senescence, when these glycosphingolipids were lost from the membrane and appeared in plasma pools without breakdown and re-synthesis somewhere. By comparing the amount of radioactive glucose in the plasma and erythrocyte pools at maximum incorporation at 24 hours and 60 days, it was clear that the major source of the plasma neutral glycosphingolipids was globoside from the erythrocyte.

4. Structural studies and enzymatic synthesis of sphingolipid bases

Studies on the chemistry and biosynthesis of long-chain aliphatic bases from sphingolipids continued in my laboratory at the University of Pittsburgh and at Michigan State University, where I moved in 1968. Gaver reported a new method for the methanolysis of sphingolipids and direct determination of the long-chain bases by GC of the trimethylsilylated derivatives[47] and the N-acetyl-O-trimethylsilylated derivatives.[48] Using the LKB-9000, Polito worked out a method to determine the positions of double bonds in unsaturated long-chain sphingolipid bases by mass spectrometry of the osmium tetroxide oxidation products by GC-MS of the N-acetyl-O-trimethylsilyl derivatives,[49] which gave characteristic fragmentation at the vicinal trimethylsilyloxy groups derived from double bonds. Tetradecasphing-4-enine and hexadecasphing-4-enine were found to be the principal long-chain bases in larvae and adults of *Musca domestica*,[50] and iso-branched-chain sphinganines were the long-chain bases in phospho-sphingolipids from *Bacteroides melaninogenicus*.[51] In the scorpion we discovered eicosasphinga-4,11-dienine and eicosasphing-11-enine.[52]

The biosynthesis of sphingolipid bases was a very active field in the 1950's and 1960's and the mechanism had been shown to involve a pyridoxal-phosphate (PLP) catalyzed condensation of serine with palmitoyl CoA and concomitant loss of carbon dioxide. We were interested in details of the

mechanism involved and initiated studies on the biosynthesis of sphinganine, sphing-4-enine and 4-hydroxysphinganine. Since it had been shown by Stodola that *Hansenula ciferrii* produced fully and partially acetylated extracellular sphingolipid bases in relatively large amounts, Thorpe chose this species of yeast for her doctoral studies.[53] The principal products were tetracetyl-4-hydroxysphinganine and triacetylsphinganine. It had been reported that the synthesis of 4-hydroxysphinganine in whole yeast cells was similar to the pathway for sphing-4-enine and sphinganine in animal systems.[54] We were interested in the origin of the oxygen atoms in these long-chain bases and used ^{18}O-labeled water and ^{18}O-labeled molecular oxygen for these studies. We were able to show that none of the three oxygen atoms in 4-hydroxysphinganine was derived from molecular oxygen. Curiously, the incorporation of ^{18}O from labeled water was nearly as predicted for C-3 but almost none was incorporated at C-4, leaving us to conclude that perhaps the C-4 oxygen atom was derived in yeast from another source in the medium.[53] Previous studies had not ruled out the possibility that 3-oxosphinganine might be an intermediate in the biosynthesis of long-chain bases. We synthesized the 3-oxo products of sphinganine and sphing-4-enine from their N-acetyl derivatives by selective oxidation at C-3 with chromic anhydride in a mixture of dry benzene and pyridine (1:1) and reported their mass spectral behavior.[54] As a doctoral dissertation project, Polito chose to examine the incorporation of various deuterium-labeled palmitates into sphinganine and sphing-4-enine.[55] One purpose of this work was to determine whether sphing-4-enine is an obligatory intermediate in the synthesis of 4-hydroxysphinganine in yeast. It was found that perdeuteropalmitate ($^2H_{31}$-palmitate) was incorporated into 4-hydroxysphinganine with loss of only one deuterium atom, excluding sphing-4-enine and 3-oxosphing-4-enine as intermediates since the 4-hydroxysphinganine would be labeled with 29 deuterium atoms (Fig. 2). We then synthesized 2*R* and 2*S*, and 3*R* and 3*S* monodeuteropalmitates and incubated them with a cell-free particulate fraction from yeast (*H. ciferri*). The results showed that the pro-*R*-hydrogens at C-2 and C-3 of palmitate are removed in the formation of sphing-4-enine, which is consistent with a mechanism involving an antiperiplanar elimination process, presumably from 3-oxosphinganine.[55] Finally, the yeast incorporated (*S*)-[2-$^2H_1$] palmitate into 4-hydroxysphinganine with retention of the deuterium atom whereas deuterium was

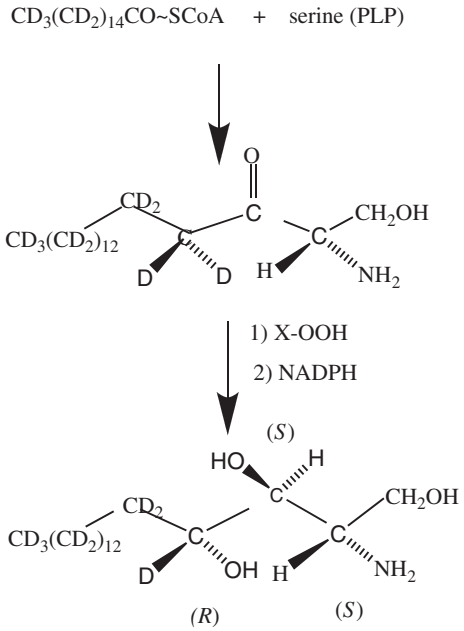


Fig. 2. Conversion of [$^2H_{31}$]-palmitic acid to 4-hydroxysphinganine in yeast with loss of one deuterium atom from C-2 of palmitate.[55]

completely lost from (*R*)-[$^2H_1$]-palmitate in the conversion. The hydroxylation step to 4-hydroxysphinganine therefore proceeds with retention of configuration at C-4 of the precursor base (Fig. 3).[55] Finally, Hammond, a postdoctoral associate, studied the biosynthesis of unsaturated sphingosines in cell-free extracts of oysters, which Hayashi and Matsubara had shown to contain sphinga-4,8-dienine.[56] The primary objective in Hammond's study was to determine at which step in the biosynthesis the double bonds are introduced, at the fatty acid stage or a long-chain base. Using GC-mass spectrometry to identify the bases, and incubations with ^{14}C- and ^3H-labeled serine and palmitate, with and without coincubation with sphinganine, sphing-4-enine or 3-oxosphinganine, we showed clearly that desaturation to the mono- and di-unsaturated long-chain bases occurred with 3-oxosphinganine, followed by reduction to sphing-4-enine and sphinga-4,8-dienine.[57] Finally, Krisnangkura and I established that the α-hydrogen atom of serine was lost in the synthesis of sphinganine in rat microsomal fractions, using [2,3,3-$^2H_3$]-serine as substrate, from which we concluded that the mechanisms of 3-oxosphinganine synthesis involves the replacement of the α-hydrogen atom and the carboxyl group of serine in the PLP complex by a proton and the palmitoyl group (Fig. 4).[58]

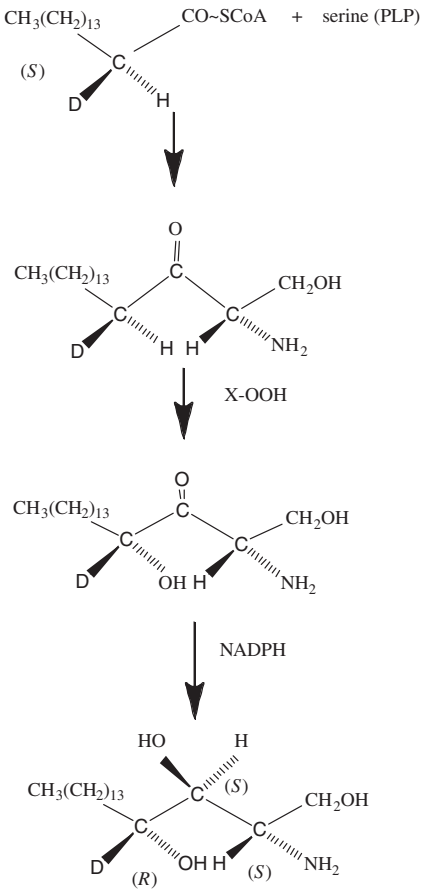


Fig. 3. Conversion of *S*-[^{2}H$_1$]-palmitate to 4-hydroxysphinganine by hydroxylation of 3-oxosphinganine and with loss of the *pro-R* hydrogen of serine and retention of configuration.[55]

5. Enzymes of glycosphingolipid metabolism

I can discuss only briefly our extensive studies of lysosomal enzymes of glycosphingolipid metabolism, post-translational processing reactions in their biosynthesis, and enzyme replacement therapy in Fabry's disease.

5-1. α-Galactosidase A and α-galactosidase B. Dean was able to purify α-galactosidase A and α-galactosidase B to homogeneity from human liver[59)–61)] and showed that α-galactosidase B is active with α-N-acetylgalactosamine-containing substrates and probably functions in cells as an α-galactosaminidase.[61)] Sung purified α-N-acetylgalactosaminidase from porcine liver.[62)] For use in enzyme replacement therapy, we partially purified α-galactosidase A from Cohn Fraction IV-1 of human plasma on a scale sufficient to provide enough enzyme for therapy.[63),64)]

5-2. Post-translational processing of oligosaccharide chains of α-galactosidases A and B. While I was on sabbatical at MIT in Robbins' laboratory, I undertook a study of the synthesis of the carbohydrate chains of α-N-acetylgalactosaminidase in cultured human fibroblasts, using [2-^{3}H$_1$]-mannose and ^{32}P$_i$ as substrates.[65)] We concluded from these studies that post-translational reactions led to high-mannose oligosaccharide moieties on the enzyme and that a precursor contained phosphorylated residues. In contrast, the N-linked oligosaccharide chains of α-galactosidase A were found to contain both high-mannose and complex, sialylated chains in the enzyme obtained from cultured Chang liver cells.[66)]

5-3. Enzyme replacement therapy in Fabry's disease. A pilot study of enzyme replacement was studied with splenic and plasma forms of α-galactosidase A in two brothers with Fabry's disease.[67)] Six doses of the enzyme were administered by intravenous infusion over a 117-day period. The circulating half-life of the splenic enzyme was about 10 minutes whereas that of the plasma isozyme was about 70 minutes, presumably because the plasma form was more highly sialylated.[67)] Both isozymes decreased the level of plasma GL-3 to approximately 50% of pre-infusion levels, the plasma form having a more prolonged effect on the level of GL-3. There were no immunological consequences from the multiple injections of these enzymes. Desnick has since extended these studies to many more patients and developed a bioengineered α-galactosidase A that is now on the market and being used to treat patients.[68)]

5-4. Enzymatic synthesis of GM$_3$ ganglioside (hematoside). Studies by Hakomori on the regulation of membrane-bound growth factor receptors by GM$_3$ ganglioside[69)] prompted me to become interested in the enzymatic synthesis of this ganglioside and in its catabolism by extracellular sialidases, hypothesizing that they might be involved in the metabolism of GM$_3$ on the cell membrane. For substrates in the purification of the sialyltransferase (SAT-1) from rat liver Golgi apparatus,[70)] lactosylceramide was synthesized chemically by Kanemitsu[71)] and CMP-[^{14}C$_{4,5,6,7,8,9}$]-sialic acid of high specific activity was obtained from New England Nuclear. The enzyme activity was extracted from Golgi-enriched microsomal fractions with lauryldimethylamine oxide, chromatographed first on an affinity column of CMP-hexanolamine-Sepharose and then on an affinity column of lactosylceramide

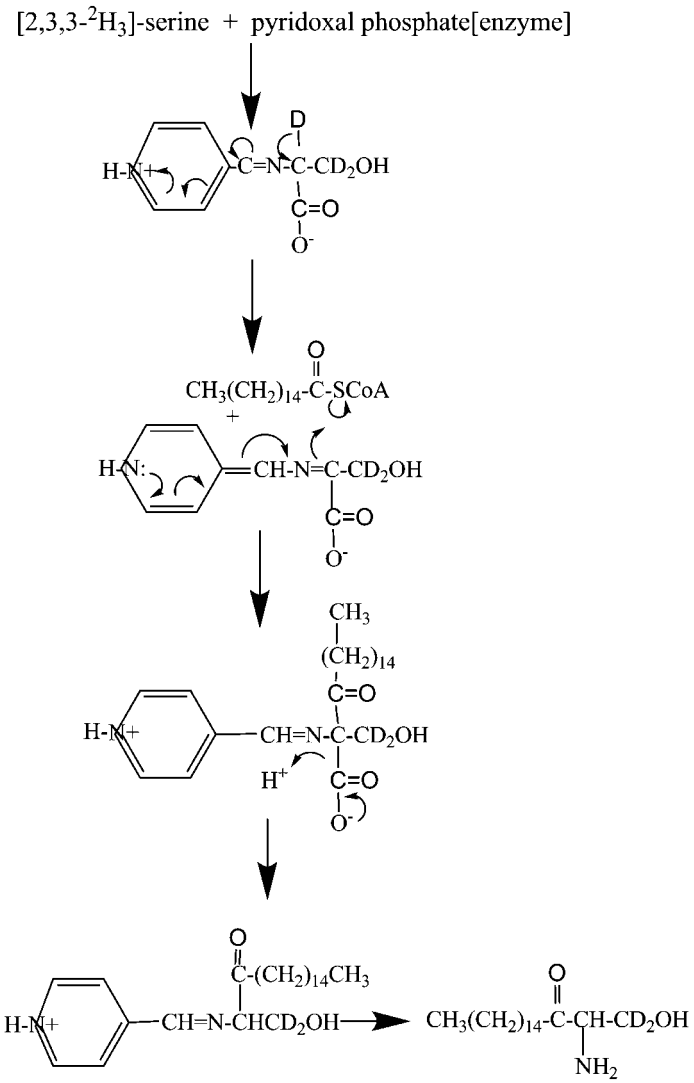


Fig. 4. Loss of deuterium from C-2 of [$^2H_3$]-serine precedes decarboxylation and addition of the palmitoyl group in its conversion to 3-oxosphinganine in whole cells of yeast (*Hansenula ciferri*).[58]

aldehyde-Sepharose. The final purification step was on an immunoaffinity column containing an anti-SAT-1 monoclonal antibody. Overall purification was about 37,000-fold yielding a single spot on gel electrophoresis. SAT-1 specificity was determined with various neutral and sialo-glycosphingolipids and sialo-glycoproteins. Lactosylceramide was the preferred sialic acid acceptor although glucosylceramide, galactosylceramide and asialo-GM_1 ganglioside served as substrates to a lesser extent.[70]

5-5. Metabolism of GM_3 ganglioside. This ganglioside was first isolated from horse erythrocytes in 1951 by Yamakawa and Suzuki[72] and was shown to consist of sphingosine, fatty acid, glucose, galactose and N-glycolylneuraminic acid (NeuGc). Substitution of the amino group of neuraminic acid was shown to be species-specific, human erythrocytes containing N-acetylneuraminic acid (NeuAc).[73] We were interested in the metabolism of GM_3 ganglioside by extracellular sialidases as a possible mechanism for the regulation of their role when bound to growth factor receptors.[74),75] Although we were able to measure the activity of a GM_3 sialidase in the cultured medium of human fibroblasts[76] we could not tell whether the sialidase in the medium or a membrane-bound sialidase was involved in the turnover of GM_3 ganglioside. The soluble sialidase had an optimum activity at pH 6.5 and increased with cell

density, reaching a maximum at confluency.[76] In support of our hypothesis about the turnover of GM_3 in these cells, we found that GM_3 labeled with ^{14}C in the sialic acid residue and ^{3}H in the long-chain base (ceramide) lost the label preferentially in the sialic acid residue with little or no loss of label in the ceramide moiety.[76),77]

These results were extended by Ogura, who showed that the addition of lactosylceramide (100 μM) to the fibroblast culture medium increased DNA synthesis three-fold within 24 hours and cell density two-fold after 48 hours.[78] We concluded that these results were compatible with a mechanism by which the proliferation of human fibroblasts is regulated by the relative levels of GM_3 and lactosylceramide in the plasma membrane.[78]

6. Mass spectrometry data systems and metabolic profiling

I would now like to summarize briefly the work that Jack Holland and I did over many years on computer systems for the acquisition and analysis of mass spectral data. Our work together began shortly after I joined the faculty at Michigan State University. Our first computers were a PDP-8i and PDP-8e from DEC. Programming was done in machine language by a group of students (many of them were honors undergraduates). Our first paper was on the development of an on-line computer system for single focusing mass spectrometry.[79] This was followed by a report on computer-controlled multiple ion detection in combined gas chromatography-mass spectrometry (GC-MS)[80] and development of a computer system for selected ion monitoring of multi-component mixtures by computer control of accelerating voltage and magnetic field strength.[81] This allowed investigators to determine several substances in mixtures at the very high sensitivity obtained by selected ion monitoring. The next step was to develop methods for the automated determination of many substances in a mixture and this led to the development of MSSMET, a computer system for metabolic profiling.[82)–87] We utilized metabolic profiling to examine the urinary organic acid fraction in natural early-onset insulin-dependent diabetic dogs[88] and in studies of the turnover of [U-^{14}C]-glucose into various metabolites in lactic acidemias.[89] This technique was utilized not only in studies of urinary organic acids but also in the analysis of urinary steroids.[90]

6-1. Sound as a sense of perception in GC analyses. Metabolic profiling was also extended to a new and novel detection system using musical sounds instead of graphs or tables to analyze normal and abnormal samples of urine.[91] Intensities at the apex of each GC peak were converted to frequencies and played on a digital keyboard, higher notes reflecting greater concentrations of metabolites. This was one of the first reports on the use of sound as a sense of perception in the field of analytical chemistry and became known whimsically in the press world-wide as "musical urines."

6-2. Time array detection by time-of-flight mass spectrometry. During the 1980's a group of scientists in the Chemistry and Biochemistry Departments at Michigan State University were working on the development of a detector that could be used with time-of-flight mass spectrometry for high-speed gas chromatographic analyses. The system they developed was capable of collecting up to 10,000 mass spectra per second. We were interested in the utilization of this new tool in metabolic profiling studies and demonstrated its capabilities with organic acid metabolites from urine, collecting 10 scan files per second and allowing profiling analyses to be carried out in about 15 minutes instead of more than 80 minutes required by capillary GC with magnetic sector instruments.[92]

7. Collaborations

Mass spectrometers with GC inlets were not widely available in the 1960's and 1970's and I had always made our instrumentation available for service analyses as well as collaborations when interpretations of mass spectral data were requested. Several of these collaborations were especially notable and should be discussed here.

7-1. Fast–atom bombardment mass spectrometry. I was fortunate to have the opportunity to work with Yoko Ohashi on one of the early papers on the use of fast-atom-bombardment mass spectrometry (FAB-MS) of gangliosides and neutral glycosphingolipids. She and Doug Gage at Michigan State University extended our earlier work on the analysis of trimethylsilylated glycosphingolipids using electron-impact MS, demonstrating that FAB-MS in the positive and negative modes is useful in determining structural features with prominent molecular ions and fragment ions to determine long-chain base composition and saccharide sequences up to and including GM_2 ganglioside.[93]

7-2. Insect juvenile hormone. In 1966, Roller and Dahm (University of Wisconsin Zoology Department) visited my laboratory at the University of Pittsburgh with a lipid called juvenile

hormone, which they had isolated from the abdomens of adult male *Hyalophora cecropia*, the giant silkworm moth.[94] Using GC-MS for analyses of the hormone, we found a molecular ion at m/z 294 and fragment ions at M-18 and M-32 suggesting an empirical formula of $C_{18}H_{30}O_3$. Catalytic hydrogenation yielded a product with a molecular ion at m/z 284, suggesting an empirical formula of $C_{18}H_{36}O_2$. These results could be interpreted to mean that juvenile hormone contained three double bonds or rings including an oxygen atom that was eliminated by hydrogenation (either an epoxide or a tertiary alcohol group). Fragment ions of the hydrogenation product also indicated that the hormone was a methyl ester (m/z 74) with branching at C-7. The position of the labile oxygen and its nature were determined by GC-MS of the products obtained by catalytic hydrogenation with a poisoned catalyst (palladium on barium sulfate with triethylamine). Several days after Roller and Dahm had returned to Wisconsin, I concluded that the mass spectral data were consistent with the structure shown in Fig. 5. This structure was unusual and unexpected and Roller delayed publication until it was confirmed by nmr spectroscopy (Barry Trost), which also allowed assignment of the trans configurations to the two double bonds.[95] This structure was later confirmed by chemical synthesis in several laboratories.

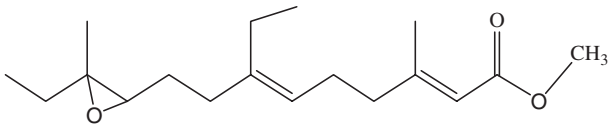


Fig. 5. Chemical structure of juvenile hormone from *Hyalophora cecropia*.[95]

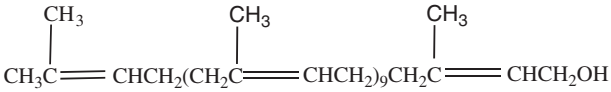


Fig. 6. Chemical structure of bacterial polyisoprenoid alcohol involved in peptidoglycan synthesis.[97]

7-3. Polyisoprenoid alcohols. The peptidoglycan of bacterial cell walls is synthesized by a sequence of reactions involving a membrane-bound phospholipid which is bound to the oligosaccharide chain during extension of the carbohydrate moiety.[96] Strominger called me from the University of Wisconsin (Pharmacology Department) to ask if I would help him identify this lipid. Not having analyzed molecules of the size of phospholipids I asked him to carry out acid-catalyzed methanolysis and I would see if I could identify the products (presumed to be fatty acids) by GC-MS. When the sample arrived, I was unable to find any fatty acid methyl esters by GC-MS and so I tried to analyze the sample in the direct probe of the LKB9000. A mass spectrum was obtained for a substance with an unusually high molecular weight, showing a very weak molecular ion at m/z 766 and a fragment ion at M-18 (m/z 748). There were clusters of fragment ions separated by 68 mass units and a very intense ion at m/z 68, which were taken to be derived from a polyisoprenoid alcohol composed of 11 isoprene and a terminal primary alcoholic functional group.[97] The chemical structure of this undecaprenol is shown in Fig. 6. A similar alcohol, called bactoprenol, had been isolated from lactobacilli.[98] This same polyisoprenoid alcohol was simultaneously reported to be involved in the biosynthesis of the lipopolysaccharides in the bacterial cell wall of Salmonella.[99] I identified this polyprenol as well in hydrolysates sent to me by Lennarz, who found it to be a structural intermediate in mannan synthesis in *Micrococcus lysodeikticus*.[100] A much larger polyisoprenol (dolichol), containing -20 isoprene units, was discovered by Behrens and Leloir as a carrier in the synthesis of the oligosaccharide chains of glycoproteins in rat liver.[101]

7-4. Kallikreins. At a meeting in Japan in the 1980's, I met with the late Professor Hiroshi Moriya of the Science University of Tokyo. He asked if I would be interested in a project on kallikrein, a glycoprotein in human plasma. I agreed and was pleased to have visits to my laboratory by several of his young scientists over the next several years.[102),103]

7-5. An inhibitor of sphing-4-enine biosynthesis and mass spectrometry of complex oligosaccharides. Finally, Professor Fumito Matsuura (Fukuyama University) and I have enjoyed a long and fruitful association, beginning with his postdoctoral work in my laboratory in 1979. He and Soltysiak worked on the synthesis of D,L-α-fluoropalmitic acid[104] and showed that this fatty acid is a strong inhibitor of sphing-4-enine synthesis.[105] He was involved in our studies of permethylated oligosaccharide chromatography[106] and was the leader in structural studies of urinary oligosaccharides from patients with mannosidosis.[107] He also developed a method for the analysis of asparagine-linked neutral oligosaccharides from α1-acid glycoprotein, bovine fetuin, human chorionic gonadotro-

pin, porcine thyroglobulin, and human IgG and reported the elution profiles of 39 oligosaccharide derivatives on TSKgel Amide 80 and Wakosil 5C18-200 columns.[108)]

Conclusions

By now the work I have described is ancient history. Such progress has been made over the past 15 years that my studies are but faint memories in the dusty archives of science. But I lived in exciting times, times that marked the beginnings in most of the areas of my research. It was the beginning of gas chromatography, nearly the beginning of mass spectrometry in the biomedical sciences, the beginning of chemistry and metabolism of sphingolipids, and certainly the beginning of what we now know about intermediary metabolism in man. Our generation provided a foundation upon which modern investigation in these fields has grown and prospered. It was indeed exciting, and I am fortunate to have known and considered as my friends some of the giants in chromatography and sphingolipid research.

Acknowledgements

I am indebted to the students, postdoctoral associates and visiting scientists that made these studies possible. Not all of their work has been cited in this review and I apologize for those who were not included, not because of the significance of their work but rather by size restraints. Much of this research was supported by research grants and a mass spectrometry regional resource grant from the National Institutes of Health. I am grateful to Professor Tamio Yamakawa for his encouragement in the preparation of this review. For my many visiting scientists and friends in Japan I would like to say that I am now retired, enjoying my grandchildren, an occasional round of golf, and participating as a musician and composer in a local community concert band where I play trombone. My only musical composition to date is a concert band march called New Horizons, which can be found and heard at a web site, www.sweeleymusicpublishing.com.

References

1) Carter, H.E., Sweeley, C.C., Daniels, E.E., McNary, J.E., Schaffner, C.P., West, C.A. *et al.* (1961) Streptothricin and streptolin: the structure of streptolidine (roseonine). J. Am. Chem. Soc. **83**, 4296–4297.
2) Kakisawa, H., Kurono, M., Takahashi, S. and Hirata, Y. (1961) Structure of grayanotoxin-I and -III. Tetrahedron Lett. **2**, 59–67.
3) Fish, M.S., Sweeley, C.C., Johnson, N.M., Lawrence, E.P. and Horning, E.C. (1956) Chemical and enzymatic rearrangements of N,N-dimethylamino acid oxides. Biochim. Biophys. Acta **21**, 196–197.
4) Sweeley, C.C. and Horning, E.C. (1957) Rearrangement and decarboxylation reactions of N,N-dimethylglycine oxide. J. Am. Chem. Soc. **79**, 2620–2625.
5) Phillipson, J.D. and Handa, S.S. (1975) Nicotine-N-oxides. Phytochemistry **14**, 2683–2690.
6) Horning, E.C., Sweeley, C.C., Dalgliesh, C.E. and Kelly, W. (1959) Mammalian hydroxylation in 6 position of the indole ring. Biochim. Biophys. Acta **32**, 566–567.
7) Horning, E.C., Moscatelli, E.A. and Sweeley, C.C. (1959) Polyester liquid phases in gas-liquid chromatography. Chem. Ind. 751–752.
8) Sweeley, C.C. (1959) A gas chromatographic method for sphingosine assay. Biochim. Biophys. Acta **36**, 268–271.
9) Sweeley, C.C. and Moscatelli, E.A. (1959) Qualitative microanalysis and estimation of sphingolipid bases. J. Lipid Res. **1**, 40–47.
10) Sweeley, C.C. (1963) Purification and partial characterization of sphingomyelin from human plasma. J. Lipid Res. **4**, 402–406.
11) Polito, A.J., Akita, T. and Sweeley, C.C. (1968) Gas chromatography and mass spectrometry of sphingolipid bases. Characterization of sphinga-4,14-dienine from human plasma sphingomyelin. Biochemistry **7**, 2609–2614.
12) Sweeley, C.C. and Horning, E.C. (1960) Microanalytical separation of steroids by gas chromatography. Nature **187**, 144–145.
13) Vanden Heuvel, W.J.A., Sweeley, C.C. and Horning, E.C. (1960) Separation of steroids by gas chromatography. J. Am. Chem. Soc. **82**, 3481–3482.
14) Vanden Heuvel, W.J.A., Sweeley, C.C. and Horning, E.C. (1960) Microanalytical separation by gas chromatography in the sex hormone and bile acid series. Biochem. Biophys. Res. Commun. **3**, 33–36.
15) Sweeley, C.C. and Chang, T.-C.L. (1961) Gas chromatography of steroids. relation of structure to molar response in an argon ionization detector. Anal. Chem. **33**, 1860–1863.
16) Chang, T.-C.L. and Sweeley, C.C. (1962) Studies of canine adrenal polyenoic acids: Locating double bonds by periodate-permanganate oxidation and gas-liquid chromatography. J. Lipid Res. **3**, 170–176.
17) Chang, T.-C.L. and Sweeley, C.C. (1963) Characterization of lipids from canine adrenal glands. Biochemistry **2**, 592–604.
18) Bentley, R., Sweeley, C.C., Makita, M. and Wells, W.W. (1963) Gas chromatography of sugars and other polyhydroxy compounds. Biochem. Biophys. Res. Commun. **11**, 14–18.

19) Sweeley, C.C., Bentley, R., Makita, M. and Wells, W.W. (1963) Gas-liquid chromatography of trimethylsilyl derivatives of sugars and related substances. J. Am. Chem. Soc. **85**, 2497–2507.
20) Wells, W.W., Katagi, T., Bentley, R. and Sweeley, C.C. (1964) Gas chromatography of sugar phosphates. Biochim. Biophys. Acta **82**, 408–411.
21) Sweeley, C.C. and Walker, B. (1964) Determination of carbohydrates in glycolipids and gangliosides by gas chromatography. Anal. Chem. **36**, 1461–1466.
22) Scriba, K. (1950) Zur pathogenese des angiokeratoma corporis diffusum Fabry mit cardio-vasorenalem symptomenkomplex. Verh. Deutsch. Ges. Path. **34**, 221–226.
23) Sweeley, C.C. and Klionsky, B. (1963) Fabry's disease: classification as a sphingolipidosis and partial characterization of a novel glycolipid. J. Biol. Chem. **238**, 3148–3150.
24) Yamakawa, T. and Suzuki, S. (1952) The chemistry of the lipids of posthemolytic residue or stroma of erythrocytes. III. globoside, the sugar-containing lipid of human blood stroma. J. Biochem. **39**, 393–402.
25) Svennerholm, E. and Svennerholm, L. (1962) Isolation of blood serum glycolipids. Acta Chem. Scand. **16**, 1282–1284.
26) Sweeley, C.C., Snyder, P.D. and Griffen, C.E. (1971) Chemistry of glycosphingolipids in Fabry's disease. Chem. Phys. Lipids **4**, 393–408.
27) Kint, J.A. (1970) Fabry's disease, α-galactosidase deficiency. Science **167**, 1268–1269.
28) Romeo, G. and Migeon, B.R.M. (1970) Genetic inactivation of the α-galactosidase locus in carriers of Fabry's disease. Science **170**, 180–181.
29) Handa, T., Ariga, T., Miyatake, T. and Yamakawa, T. (1971) Presence of α-anomeric glycosidic configuration in the glycolipids accumulating in kidney with Fabry disease. J. Biochem. **69**, 625–627.
30) Hakomori, S., Siddiqui, B., Li, Y.-T., Li, S.-C. and Hellerquist, C.G. (1971) Anomeric structures of globoside and ceramide trihexoside of human erythrocytes and hamster fibroblasts. J. Biol. Chem. **246**, 2271–2277.
31) Sweeley, C.C., Klionsky, B., Krivit, W. and Desnick, R.J. (1972) Fabry's disease: glycosphingolipid lipidosis. *In* The Metabolic Basis of Inherited Disease (eds. Stanbury, J.B., Wyngaarden, J.B. and Fredrickson, D.S.). McGraw-Hill, New York, 3rd ed., pp. 663–687.
32) Laine, R.A., Griffin, P.F., Sweeley, C.C. and Brennan, P.J. (1972) Monoglucosyloxyoctadecenoic acid, a glycolipid from *Asperigillus niger*. Biochemistry **6**, 2267–2271.
33) Laine, R., Sweeley, C.C., Li, Y.T., Kisic, A. and Rapport, M.M. (1972) On the structure of cytolipin R, a ceramide tetrahexoside hapten from rat lymphosarcoma. J. Lipid Res. **13**, 519–524.
34) Sung, S.S., Esselman, W.J. and Sweeley, C.C. (1973) Structure of a pentahexosylceramide (Forssman hapten) from canine intestine and kidney. J. Biol. Chem. **248**, 6528–6533.
35) Tao, R.V., Sweeley, C.C. and Jamieson, G.A. (1973) Sphingolipid composition of human platelets. J. Lipid Res. **14**, 16–24.
36) Esselman, W.J., Ackerman, J.R. and Sweeley, C.C. (1973) Glycosphingolipids of membrane fractions from normal and transplanted canine kidney. J. Biol. Chem. **248**, 7310–7317.
37) Bentley, R., Saha, N.C. and Sweeley, C.C. (1965) Separation of protium and deuterium forms of carbohydrates by gas chromatography. Anal. Chem. **37**, 1118–1122.
38) Hatchey, D.L., Wong, W.W., Boutton, T.W. and Klein, P.D. (1987) Isotope ratio measurements in nutrition and biomedical research. Mass Spectrom. Rev. **6**, 289–328.
39) Saha, N.C. and Sweeley, C.C. (1968) Gas chromatographic characteristics in the separation of protium and deuterium forms of trimethylsilyl sugars. Anal. Chem. **40**, 1628–1633.
40) Vance, D.E. and Sweeley, C.C. (1967) Quantitative determination of the neutral glycosylceramides in human blood. J. Lipid Res. **8**, 621–630.
41) Vance, D.E., Krivit, W. and Sweeley, C.C. (1969) Concentration of glycosylceramides in plasma and red cells in Fabry's disease, a glycolipid lipidosis. J. Lipid Res. **10**, 188–192.
42) Martensson, E. (1966) Neutral glycolipids of human kidney. Isolation, identification and fatty acid composition. Biochim. Biophys. Acta **116**, 296–308.
43) Sweeley, C.C., Elliott, W.H., Fries, I. and Ryhage, R. (1966) Mass spectrometric determination of unresolved components in gas chromatographic effluents. Anal. Chem. **38**, 1549–1553.
44) Vance, D.E., Krivit, W. and Sweeley, C.C. (1975) Metabolism of neutral glycosphingolipids in plasma of a normal human and a patient with Fabry's disease. J. Biol. Chem. **250**, 8119–8125.
45) Dawson, G. and Sweeley, C.C. (1970) *In vivo* studies on glycosphingolipid metabolism in porcine blood. J. Biol. Chem. **245**, 410–416.
46) Tao, R. (1973) Biochemistry and metabolism of mammalian blood glycosphingolipids. Ph.D. dissertation, Michigan State University.
47) Gaver, R.C. and Sweeley, C.C. (1965) Methods for methanolysis of sphingolipids and direct determination of long-chain bases by gas chromatography. J. Am. Oil Chem. Soc. **42**, 294–298.
48) Gaver, R.C. and Sweeley, C.C. (1966) Chemistry and metabolism of sphingolipids. 3-oxo derivatives of N-acetylsphingosine and N-acetyldihydrosphingosine. J. Am. Chem. Soc. **88**, 3643–3647.
49) Polito, A.J., Naworal, J. and Sweeley, C.C. (1969) Determination of the structures of sphingolipid bases by combined gas chromatography-mass spectrometry. Biochemistry **8**, 1811–1815.
50) Bieber, L.L., O'Connor, J.D. and Sweeley, C.C. (1969) The occurrence of tetradecasphing-4-enine and hexadecasphing-4-enine as the principal sphingosines of *Musca domestica* larvae and

adults. Biochim. Biophys. Acta **187**, 157–159.
51) White, D.C., Tucker, A.N. and Sweeley, C.C. (1969) Characterization of the iso-branched sphinganines from the ceramide phospholipids of *Bacteroides melaninogenicus*. Biochim. Biophys. Acta **187**, 527–532.
52) O'Connor, J.D., Polito, A.J., Monroe, R.E., Sweeley, C.C. and Bieber, L.L. (1970) Characterization of invertebrate sphingolipid bases: occurrence of eicosasphinga-4,11-dienine and eicosasphing-11-enine in scorpion. Biochim. Biophys. Acta **202**, 195–197.
53) Thorpe, S.R. and Sweeley, C.C. (1967) Chemistry and metabolism of sphingolipids. On the biosynthesis of phytosphingosine by yeast. Biochemistry **6**, 887–897.
54) Greene, M.L., Kaneshiro, T. and Law, J.H. (1965) Studies on the production of sphingolipid bases by the yeast *Hansenula ciferri*. Biochim. Biophys. Acta **98**, 582–588.
55) Polito, A.J. and Sweeley, C.C. (1971) Stereochemistry of the hydroxylation in 4-hydroxysphinganine formation and the steric course of hydrogen elimination in sphing-4-enine biosynthesis. J. Biol. Chem. **246**, 4178–4187.
56) Hayashi, A. and Matsubara, T. (1970) On the occurrence of sphinga-4,8-dienine in oyster glycolipid. Biochim. Biophys. Acta **202**, 228–230.
57) Hammond, R.K. and Sweeley, C.C. (1973) Biosynthesis of unsaturated sphingolipid bases by microsomal preparations from oysters. J. Biol. Chem. **248**, 632–640.
58) Krisnangkura, K. and Sweeley, C.C. (1976) Studies on the mechanism of 3-ketosphinganine synthesis. J. Biol. Chem. **251**, 1597–1602.
59) Dean, K.J. and Sweeley, C.C. (1979) Studies on human liver α-galactosidases. I. Purification of α-galactosidase A and its enzymatic properties with glycolipid and oligosaccharide substrates. J. Biol. Chem. **254**, 9994–10000.
60) Dean, K.J. and Sweeley, C.C. (1979) Studies on human liver α-galactosidases. II. Purification and enzymatic properties of α-galactosidase B (α-N-acetylgalactosaminidase). J. Biol. Chem. **254**, 10001–10005.
61) Dean, K.J. and Sweeley, C.C. (1979) Studies on human liver α-galactosidases. III. Partial characterization of carbohydrate-binding specificities. J. Biol. Chem. **254**, 10006–10010.
62) Sung, S.S. and Sweeley, C.C. (1980) Purification and partial characterization of porcine liver α-N-acetylgalactosaminidase. J. Biochem. **255**, 6589–6594.
63) Bishop, D.F. and Sweeley, C.C. (1978) Plasma α-galactosidase A: properties and comparisons with tissue α-galactosidases. Biochim. Biophys. Acta **525**, 399–409.
64) Bishop, D.F., Wampler, D.E., Sgouris, J.T., Bonefield, R.J., Anderson, D.K., Hawley, M.C. *et al.* (1978) Pilot scale purification of α-galactosidase A from Cohn fraction IV-1 of human plasma. Biochim. Biophys. Acta **524**, 109–120.
65) Sweeley, C.C., LeDonne, N.C. and Robbins, P.W. (1983) Post-translational processing reactions involved in the biosynthesis of lysosomal α-N-acetylgalactosaminidase in cultured human fibroblasts. Arch. Biochem. Biophys. **223**, 158–165.
66) LeDonne, N.C., Fairley, J.L. and Sweeley, C.C. (1983) Biosynthesis of α-galactosidase A in cultured Chang liver cells. Arch. Biochem. Biophys. **224**, 186–195.
67) Desnick, R.J., Dean, K.J., Grabowski, G., Bishop, D.F. and Sweeley, C.C. (1979) Enzyme therapy in Fabry disease: differential *in vivo* plasma clearance and metabolic effectiveness of plasma and splenic α-galactosidase A isozymes. Proc. Natl. Acad. Sci. USA **76**, 5326–5330.
68) Desnick, R.J. (2004) Enzyme replacement and enhancement therapies for lysosomal diseases. J. Inherit. Metab. Dis. **27**, 385–410.
69) Bremer, E.G., Schlessinger, J. and Hakomori, S. (1986) Ganglioside mediated modulation of cell growth. Specific effects of GM_3 on tyrosine phosphorylation of the epidermal growth factor receptor. J. Biol. Chem. **261**, 2434–2440.
70) Melkerson-Watson, L.J. and Sweeley, C.C. (1991) Purification to apparent homogeneity by immunoaffinity chromatography and partial characterization of the GM_3 ganglioside-forming enzyme, CMP-sialic acid:lactosylceramide α-2,3-sialyltransferase (SAT-1) from rat liver golgi. J. Biol. Chem. **266**, 4448–4457.
71) Kanemitsu, K. and Sweeley, C.C. (1986) Synthesis of double labeled lactosylceramide. Glycoconj. J. **3**, 143–151.
72) Yamakawa, T. and Suzuki, S. (1951) The chemistry of the lipids of posthemolytic residue or stroma of erythrocytes. I. Concerning the ether-insoluble lipids of lyophilized horse blood stroma. J. Biochem. **38**, 199–212.
73) Yamakawa, T. (2005) Studies on erythrocyte glycolipids. Proc. Jpn. Acad., Ser. B **81**, 52–63.
74) Usuki, S. and Sweeley, C.C. (1988) Consideration of a functional role of an extracellular sialidase secreted by cultured fibroblasts. Indian J. Biochem. Biophys. **25**, 102–105.
75) Sweeley, C.C. (1993) Extracellular sialidases. *In* Advances in Lipid Research, Vol. 26 (eds. Bell, R.M., Merrill, A.H. and Hannun, Y.A.). Academic Press, New York, pp. 235–252.
76) Usuki, S., Lyu, S.C. and Sweeley, C.C. (1988) Sialidase activities of cultured human fibroblasts and the metabolism of GM_3 ganglioside. J. Biol. Chem. **263**, 6847–6853.
77) Ogura, K., Ogura, M., Anderson, R.L. and Sweeley, C.C. (1992) Peroxidase-amplified assay of sialidase activity toward gangliosides. Anal. Biochem. **200**, 52–57.
78) Ogura, K. and Sweeley, C.C. (1992) Mitogenic effects of bacterial neuraminidase and lactosylceramide on human cultured fibroblasts. Exp. Cell Res. **199**, 169–173.
79) Sweeley, C.C., Ray, B.D., Wood, W.I., Holland, J.F. and Krichevsky, M.I. (1970) On-line digital

computer system for high speed single focusing mass spectrometry. Anal. Chem. **42**, 1505–1516.
80) Holland, J.F., Sweeley, C.C., Thrush, R.E., Teets, R.E. and Bieber, M.A. (1973) On-line computer-controlled multiple ion detection in combined gas chromatography-mass spectrometry. Anal. Chem. **45**, 308–314.
81) Young, N.D., Holland, J.F., Gerber, J.N. and Sweeley, C.C. (1975) Selected ion monitoring for multi-component analysis by computer control of the accelerating voltage and the magnetic field. Anal. Chem. **47**, 2373–2376.
82) Sweeley, C.C., Young, N.D., Holland, J.F. and Gates, S.C. (1974) Rapid computerized identification of compounds in complex biological mixtures by gas chromatography-mass spectrometry. J. Chromatogr. A **99**, 507–517.
83) Gates, S.C., Smisko, M., Ashendal, C., Young, N., Holland, J.F. and Sweeley, C.C. (1978) Automated simultaneous qualitative and quantitative analysis of complex organic mixtures with a gas chromatography-mass spectrometry-computer system. Anal. Chem. **50**, 433–441.
84) Gates, S.C., Dendramis, N. and Sweeley, C.C. (1978) Automated metabolic profiling of organic acids in human urine. I. description of methods. Clin. Chem. **24**, 1674–1679.
85) Gates, S.C., Sweeley, C.C., Krivit, W., DeWitt, D. and Blaisdell, B.E. (1978) Automated metabolic profiling of organic acids in human urine. II. Analysis of urine samples from healthy adults, sick children, and children with neuroblastoma. Clin. Chem. **24**, 1680–1689.
86) Gates, S.C. and Sweeley, C.C. (1978) Quantitative metabolic profiling based on gas chromatography. Clin. Chem. **24**, 1663–1673.
87) Holland, J.F., Leary, J.J. and Sweeley, C.C. (1986) Advanced instrumentation and strategies for metabolic profiling. J. Chromatogr. A **20**, 3–26.
88) Shigematsu, Y., Sweeley, C.C., Schall, W.D. and Gossain, V. (1988) Urinary organic acids in natural early-onset insulin-dependent diabetic dogs. Acta Paediatr. Jpn. **30**, 285–293.
89) Kassel, D.B., Glerum, M., Robinson, B.H. and Sweeley, C.C. (1988) Determination of [U-^{14}C]-glucose turnover into various metabolite pools for the differential diagnosis of lactic acidemias. Anal. Biochem. **176**, 382–389.
90) Vrbanac, J.J., Braselton, W.E. Jr., Holland, J.F. and Sweeley, C.C. (1982) Automated qualitative and quantitative metabolic profiling analysis of urinary steroids by a gas chromatography-mass spectrometry data system. J. Chromatogr. A **239**, 265–276.
91) Sweeley, C.C., Holland, J.F., Towson, D.S. and Chamberlin, B.A. (1987) Interactive and multi-sensory analysis of complex mixtures by an automated gas chromatography-mass spectrometry system. J. Chromatogr. A **24**, 173–181.
92) Schulte, G.A., Chamberlin, B.A., Sweeley, C.C., Watson, J.T. and Allison, J. (1992) Complex mixture analysis based on gas chromatography-mass spectrometry with time array detection using a beam deflection time-of-flight mass spectrometer. J. Chromatogr. A **590**, 329–339.
93) Ohashi, Y., Gage, D.A. and Sweeley, C.C. (1991) Gangliosides and neutral glycosphingolipids. *In* Techniques in Diagnostic Human Biochemical Genetics: A Laboratory Manual (ed. Hommes, F.). Wiley-Liss, New York, pp. 239–265.
94) Roller, H. and Bjerke, J.S. (1965) Purification and isolation of juvenile hormone and its action in *Lepidopteran* larvae. Life Sci. **4**, 1617–1624.
95) Roller, H., Dahm, K.H., Sweeley, C.C. and Trost, B.M. (1967) The structure of the juvenile hormone. Angew. Chem. Int. Ed. Engl. **6**, 179–180.
96) Anderson, J.S., Matsuhashi, M., Haskin, M.A. and Strominger, J.L. (1967) Biosynthesis of the peptidoglycan of bacterial cell wall. II. Phospholipid carriers in the reaction sequence. J. Biol. Chem. **242**, 3180–3190.
97) Higashi, Y., Strominger, J.L. and Sweeley, C.C. (1967) Structure of a lipid intermediate in cell wall peptidoglycan synthesis: A derivative of a C_{55} isoprenoid alcohol. Proc. Natl. Acad. Sci. USA **57**, 1878–1884.
98) Thorne, K.J.I. and Kodicek, E. (1966) The structure of bactoprenol, a lipid formed by lactobacilli from mevalonic acid. Biochem. J. **99**, 123–127.
99) Wright, A., Dankert, M., Fennesey, P. and Robbins, P.W. (1967) Characterization of a polyisoprenoid compound functional in O-antigen biosynthesis. Proc. Natl. Acad. Sci. USA **57**, 1798–1803.
100) Scher, M., Lennarz, W.J. and Sweeley, C.C. (1968) The biosynthesis of mannosyl-1-phosphoryl polyisoprenol in *Micrococcus lysodeikticus* and its role in mannan synthesis. Proc. Natl. Acad. Sci. USA **59**, 1313–1320.
101) Behrens, N.H. and Leloir, L.F. (1970) Dolichol monophosphate glucose: an intermediate in glucose transfer in liver. Proc. Natl. Acad. Sci. USA **66**, 153–159.
102) Sweeley, C.C., Hollingsworth, R., Gage, D., Ikekita, M. and Moriya, H. (1989) Fast atom bombardment mass spectrometry (FAB-MS): analysis of complex carbohydrate chains of tissue kallikreins. Adv. Exp. Med. Biol. **247A**, 533–538.
103) Kamada, M., Ikekita, M., Kurahashi, T., Aoki, K., Kizuki, K., Moriya, H. *et al.* (1990) Generation of a different type of β-kallikrein from porcine pancreatic α-kallokrein by the action of chymotrypsin—observation of proteolytic processing occurring around the "kallikrein autolysis loop" region. Chem. Pharm. Bull. (Tokyo) **38**, 1053–1057.
104) Matsuura, F., Soltysiak, R.M., Fairley, J.L. and Sweeley, C.C. (1984) Facile synthesis of ^{3}H-labeled α-fluoropalmitic acid and stereoisomers of α-fluoropalmitic acid. Bull. Fac. Genl. Ed., Fukuyama Univ. **8**, 77–83.
105) Soltysiak, R.M., Matsuura, F., Bloomer, D. and Sweeley, C.C. (1984) (*D*,*L*)-α-Fluoropalmitic acid inhibits sphingosine base formation and accumulates in membrane lipids of cultured mammalian

cells. Biochim. Biophys. Acta **792**, 214–226.
106) Wang, W.T., Matsuura, F. and Sweeley, C.C. (1983) Chromatography of permethylated oligosaccharide alditols. Anal. Biochem. **134**, 398–405.
107) Jardine, I., Matsuura, F. and Sweeley, C.C. (1984) Electron impact mass spectra of reduced and permethylated urinary oligosaccharides from patients with mannosidosis. Biomed. Mass Spectrom. **11**, 562–568.
108) Matsuura, F., Ohta, M., Murakami, K., Hirano, K. and Sweeley, C.C. (1992) The combination of normal phase with reversed phase high performance liquid chromatography for the analysis of asparagine-linked neutral oligosaccharides labeled with p-aminobenzoic ethyl ester. Biomed. Chromatogr. **6**, 77–83.
109) Chester, M.A. (1997) Nomenclature of glycolipids. Pure Appl. Chem. **69**, 2475–2487.

(Received June 22, 2010; accepted July 12, 2010)

Profile

Charles Sweeley was born in Williamsport, Pennsylvania in 1930. He is a graduate of the University of Pennsylvania (B.S., Chemistry, 1952) and the University of Illinois (Ph.D., Biochemistry, 1955). He began his research career at the National Institutes of Health in Dr. Evan Horning's laboratory, where together they established the first gas chromatography laboratory at NIH. They pioneered the development of gas chromatography for the separation of steroids and he was the first to report the gas chromatography of long-chain sphingolipid bases. In 1960, he joined the faculty in biochemistry at the University of Pittsburgh, Graduate School of Public Health. Continuing his work on biochemical applications of gas chromatography, he reported the chemistry of several new sphingolipid bases, and pioneered with colleagues the gas chromatography of carbohydrates in a paper in 1963 that drew international attention and was one of the 500 most cited papers in the 1960's. He published one of the first papers on selected ion monitoring in combined gas chromatography-mass spectrometry. Combining his knowledge about the separation of fatty acids, carbohydrates and sphingolipid bases, he discovered the biochemical basis of the X-linked genetic disorder, Fabry's disease, reporting the partial structures of two novel glycosphingolipids isolated from the Fabry kidney. He determined the levels of the globo type glycosphingolipids in normal and Fabry plasma and erythrocytes and studied their biosynthesis and metabolism using stable isotope-labeled glucose as a tracer. He proposed the correct chemical structure of the insect juvenile hormone in 1967 and established the chemical structure of the polyisoprenoid alcohol involved in peptidoglycan biosynthesis using combined gas chromatography-mass spectrometry. In 1968 he accepted an academic position as Professor of Biochemistry at Michigan State University where he continued his studies on the biochemistry of sphingolipids. His interests during that time included studies of the lysosomal enzymes of glycosphingolipid metabolism, enzyme replacement therapy in Fabry's disease, post-translational processing reactions in lysosomal enzyme synthesis, effects of tumor promoters on glycolipid metabolism, regulation of glycolipid metabolism in normal and transformed cells in culture, and isolation and characterization of glycolipid antigens. He collaborated extensively with Holland on biomedical applications of gas chromatography and mass spectrometry, leading to the first fully automated computer system for mass spectrometry and the development of metabolic profiling of urinary organic acids for disease diagnosis. He was Chairperson of the Department of Biochemistry at Michigan State University from 1979 to 1985, Guggenheim Fellow in Stockholm in 1970, Michigan Scientist of the Year in 1988, and University Distinguished Professor in 1990. He was awarded an honorary degree from Ghent University in Belgium in 1982 and the Anachem Award from the American Chemical Society in 2001.