JB Reflections and Perspectives

J. Biochem. 2009;146(2)149–156
doi:10.1093/jb/mvp103

Tamio Yamakawa: Dawn of Glycobiology

Akemi Suzuki*

Institute of Glycoscience, Tokai University, 1117 Kitakaname, Hiratsuka, Kanagawa, 259-1292, Japan

Tamio Yamakawa isolated a glycosphingolipid from horse erythrocyte membrane, named it hematoside, and reported the results in *Journal of Biochemistry*. This was the first paper to report that glycosphingolipids are located in the cell membrane. He also isolated a glycosphingolipid, globoside, from human erythrocytes and demonstrated for the first time that ABO blood group antigens are glycosphingolipids in the erythrocytes. He reported the correct chemical structure of sulfatide, and found seminolipid, which is unexpectedly a glyceroglycolipid, as the major glycolipid of mammalian testis and spermatozoa. He started and developed the research of glycolipid biochemistry and established the basis for the further development of glycobiology. He published most of his original work in *Journal of Biochemistry* and made great efforts to improve review process of the journal as an editor-in-chief.

Key words: ABO blood group activity, globoside, glycolipids, hematoside, neuraminic acid, sialic acid.

Abbreviations: NeuAc, *N*-acetylneuraminic acid; NeuGc, *N*-glycolyneuraminic acid; Cer, ceramide; Gb4Cer, globotetraosylceramide.

Tamio Yamakawa retired as professor at the Department of Biochemistry, Faculty of Medicine, the University of Tokyo, when he wrote a book titled *A Story of Glycolipid Research*, which covered the results of his work and events during his research life (*1*). This book was written in Japanese and unfortunately is no longer available. The description of events in this article that occurred before the author joined his laboratory in 1971 is based on that book and other review articles (*2*, *3*).

In 1943, when he was a third-year medical student, Tamio Yamakawa took an elective summer training course that involved chemical experiments in a laboratory at the Institute for Infectious Diseases, the University of Tokyo, which led him to choose a chemical approach to research. After graduating in 1944, he first joined Prof. Asano's laboratory and then Prof. Akiya's laboratory at the Institute for Infectious Diseases. As Prof. Asano was interested in the antibacterial activity of branched-chain fatty acids, he gave Yamakawa a project on the chemical synthesis of branched fatty acids. This proved to be challenging and Yamakawa also found the antibacterial effects of these molecules to be another intriguing subject. He synthesized, purified, and analysed these fatty acids using the classical methods of organic chemistry. At that time, he was attracted to R. Kuhn's work on Hildebrandt acid (*4*). Consequently, he injected the synthesized branched fatty acids into rabbits and analysed the ω-oxidized dicarboxyl acids excreted in urine. On reflection, he believed that his education in these fundamental chemical techniques and the knowledge so acquired formed the backbone of his future research. He was awarded a D. Med. Sci., which is equivalent to a PhD, from the University of Tokyo in 1948 for his dissertation 'The fate of branched-chain fatty acids in the animal body.' The papers describing these results were published in the *Journal of Biochemistry* just after World War II (*5*–*8*) (Fig. 1).

HEMATOSIDE AND HEMATAMINIC ACID

The Institute for Infectious Diseases was originally founded by Shibasaburo Kitasato, who pioneered studies on anti-sera against bacterial toxins and succeeded in treating tetanus infection with an anti-tetanus toxin in R. Koch's laboratory in 1890. During World War II, the institute was the centre for the production of anti-sera in Japan, and many horses were immunized with diphtheria and tetanus toxins. Anti-sera were valuable products, while no one was interested in the clotted blood removed from serum. Yamakawa wanted to isolate species-specific antigens from horse blood cells. Although one of his seniors belittled him, saying that the idea was a joke, Yamakawa did not abandon it. He decided to use methods he was acquainted with, that is, the purification of lipids and chemical characterization of lipid-related molecules using a large amount of discarded horse erythrocyte clot as the source material. Probably, he paid dearly in dealing with contemptuous comments from colleagues. He gently disrupted the clot to release the erythrocytes by hand. After hemolysing the erythrocytes in an excess of 0.5% acetic acid in water, the erythrocyte membranes were packed by centrifugation. He obtained about 100 g of dried membranes from 10 l of packed erythrocytes (*3*). In 1971, when the author joined his laboratory, many glass barrels measuring 60 cm in diameter and 70 cm high that he used for this preparation were still stored in a cellar at the Department of Biochemistry, Faculty of Medicine, the University of Tokyo, where he moved from the Institute for Infectious Diseases in 1966. The dried membranes were extracted with diethyl ether-methanol (1 : 1, v/v) and

*To whom correspondence should be addressed. Tel: +81-463-58-1211 (ext. 4643); Fax: +81-463-50-2432;
E-mail: akmszk@tokai-u.jp

Fig. 1. **Tamio Yamakawa.**

then with chloroform-methanol (1 : 1, v/v). The resulting dark grey extract was dissolved in a minimal amount of chloroform and then precipitated with a large excess of acetone. The precipitate reacted with orcinol, indicating the presence of neuraminic acid (*9*).

Yamakawa wrote that he learned about ganglioside and neuraminic acid in 1950 from an article by S.J. Thannhauser in the *Annual Review of Biochemistry* published in 1943 (*10*) at a public library, which was the only place scientists could read recent publications after the long unavailability of such information due to World War II. He thought that his material from horse erythrocytes might be ganglioside because his fraction and Klenk's ganglioside both reacted with orcinol. Yamakawa also prepared Klenk's ganglioside from bovine brain and compared it with his material. He named the horse erythrocyte glycolipid 'hematoside' to differentiate his glycolipid from Klenk's ganglioside. Analytical results indicated that the hematoside was composed of 1 mol each of fatty acid, sphingosine and an acidic sugar with an unknown structure that he named hemataminic acid, and 2 mol of galactose (*9*).

Sphingosine is a long chain base and the name was coined by J.L.W. Thudichum, a German clinician with training in chemistry, who isolated lipids from the human brain and published his life work in a book titled *A Treatise on the Chemical Constitution of the Brain* in 1884 (*11*). He also coined the names 'cerebroside', 'ceramide', 'sphingomyelin' and 'cephalin', and is considered to be the father of sphingolipid biochemistry and biology. Yamakawa wrote, 'In May, 1965, the 16th Colloquium of The German Society for Physiological Chemistry was held in Mosbach, a small city by the Neckar River, and I was invited to this meeting by E. Klenk. After the meeting, a ceremony took place in Büdingen, near Frankfurt, to dedicate a memorial plate indicating the house where Thudichum was born on the 27th of August, 1829, and I had the pleasure of attending the ceremony. His research was centered on the chemical analysis of the brain, to search for changes of brain components in diseases, such as cholera and typhoid fever, and to understand the pathogenesis of these diseases (*2*).'

When Yamakawa isolated hematoside, he found that it contained an acidic sugar with an unknown structure, hemataminic acid, which resulted in a purple colour on reacting with orcinol, similar to neuraminic acid, as Klenk had discovered (*9*). In 1935, Klenk isolated a white phosphorus-free substance from the brain of a patient with Niemann–Pick disease that gave a purple colour on reacting with orcinol, and subsequently purified this substance in much better yield from the brains of patients with Tay–Sachs' disease (*12*, *13*). In 1941, Klenk named this substance ganglioside (*14*) because its concentration was very high in ganglions (a mass of nerve cells). He treated the ganglioside with methanolic hydrochloric acid, isolated a crystallized substance exhibiting the colour reaction with orcinol, and named this neuraminic acid based on its acidic nature and the source of the substance (*15*). Earlier, in 1936, Blix had purified a polyhydroxyamino acid-like substance from bovine submaxillary mucin using a hot water treatment and in 1952 named this substance sialic acid (*16*). In addition, R. Kuhn isolated an acidic sugar from cow colostrum and named it lactaminic acid (*17*). The determination of the structures of hemataminic acid, neuraminic acid, sialic acid and lactaminic acid proved to be an exciting topic and prominent scientists such as E. Klenk, G. Blix and R. Kuhm, as well as newcomers such as Hiyama in Hirosaki and Yamakawa, proposed their own structures. Hiyama's proposal regarding a degradation product of sialic acid on alkaline treatment became the basis of the correct structure proposed by A. Gottschalk in Australia (*18*). All of these substances turned out to be the same molecule. Blix, Gottschalk and Klenk agreed to the proposal that the name sialic acid be used as the acyl derivatives of neuraminic acid (*19*).

Yamakawa wrote 'I submitted a paper describing these results (about hematoside) to the *Journal of Biochemistry* published by The Japanese Biochemical Society in 1950 (*9*) and at the same time sent a letter to Klenk for his opinion on my results. Six months later, I received a letter from him indicating that he had read my paper with much interest. He also mentioned that he had studied a glycolipid isolated from human erythrocytes, and this glycolipid did not contain neuraminic acid, but galactosamine. All ganglioside preparations he had obtained so far contained galactosamine; therefore, I should check for the presence of galactosamine in hematoside. Furthermore, I needed to determine the optical rotation of hemataminic acid before I reached the conclusion that neuraminic acid and hemataminic acid were the same molecule. Subsequently, I confirmed that hematoside did not contain hexosamines; therefore, hematoside is different from brain ganglioside, and that optical rotation values are the same for hemataminic

Fig. 2. **E. Klenk visited T. Yamakawa at the Department of Biochemistry, Faculty of Medicine, the University of Tokyo, in August 1967.**

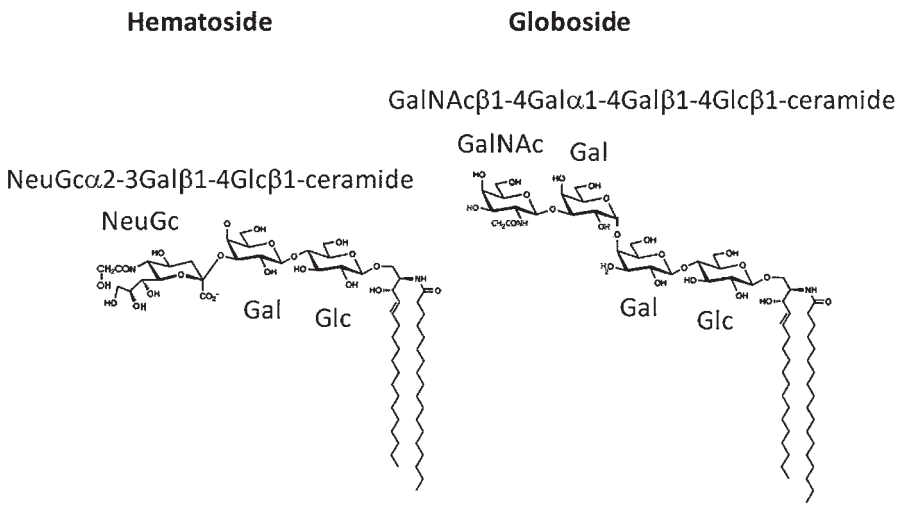

Fig. 3. **Structures of hematoside and globoside.**

acid and neuraminic acid, thus both should be the same molecule. I also decided to confirm Klenk's results for human erythrocyte glycolipid' (*2*) (Fig. 2). Finally, hematoside isolated from horse erythrocytes was determined to be *N*-glycolylneuraminic acidα2-3galactoseβ1-4-glucoseβ1-ceramide (Fig. 3). This was the first demonstration that glycolipids are located in the cell membrane and that sialic acid is exposed on the cell membrane.

REGULATION OF SIALIC ACID EXPRESSION

In 1964, Handa and Yamakawa reported on dog erythrocyte glycolipids. The major glycolipid was a hematoside containing 73% N-acetylneuraminic acid (NeuAc) and 27% *N*-glycolylneuraminic acid (NeuGc) (*20*). Interestingly, Klenk had reported that dog erythrocyte hematoside contained 100% NeuAc (*21*). Later, S. Hamanaka and S. Handa in Yamakawa's group examined the erythrocyte glycolipids of individual dogs and various breeds of dogs with the help of veterinarians. The difference turned out to be due to the geographical origin of the dogs. Dogs originating from Europe, Russia and America had a hematoside containing NeuAc exclusively, while several breeds of Japanese, Korean and Chinese dogs had NeuGc in their hematosides (*22*). Klenk used European dog erythrocytes and Handa and Yamakawa used pooled Japanese dog erythrocytes. A pedigree analysis

of one family of Shiba dogs, which are of Japanese origin, indicated that the expression of hematosides containing NeuGc was inherited dominantly over that of hematosides containing NeuAc. Historically, dogs have accompanied humans, and these results provide a line of evidence supporting one route of the original Japanese ancestors through the Korean peninsula from northern China (*3*).

PERSPECTIVES

R. Schauer extensively analysed the structures, distributions, degradation and biosynthesis of sialic acids (*23*). His group proposed that the expression of NeuGc is regulated by CMP-NeuAc hydroxylase, which converts CMP-NeuAc into CMP-NeuGc (*24*). We purified the hydroxylase from mouse liver cytosol and found that the hydroxylation requires the hydroxylase, cytochrome b5, and NADH-dependent cytochrome b5 reductase (*25–27*). Yamakawa's finding was critical to the success of the purification. In the author's laboratory, Y. Kozutsumi found that fractions A and B separated from mouse liver cytosol by ion exchange chromatography were required for the hydroxylation activity. In addition, he measured the hydroxylase activity in the lysate of horse erythrocytes because he knew Yamakawa had discovered that the horse erythrocyte membrane contained a hematoside carrying NeuGc as the major component, and Kozutsumi thought that horse erythrocyte lysate might still retain hydroxylase activity. However, this was not the case. Then, he conducted a mixing experiment, using fractions A and B and horse erythrocyte lysate. It turned out that the lysate was able to replace fraction B and reconstitute the hydroxylase activity together with fraction A. Using fraction A, an active component equivalent to that in fraction B was purified from the horse erythrocyte lysate. Its amino acid sequence matched that of soluble cytochrome b5. NADH-cytochrome b5 reductase was also detected in fraction A and separated from the hydroxylase by gel filtration chromatography. Finally, the hydroxylase was purified from mouse liver cytosol and partial amino acid sequences were determined. The amino acid sequence deduced from a cloned complementary DNA (cDNA) sequence of mouse hydroxylase confirmed that it was a new protein lacking a membrane-spanning domain (*27*). CMP-NeuGc is synthesized in the cytosol from CMP-NeuAc, transported into the internal cisternae of the Golgi complex, and used by sialyltransferases as a sugar donor.

Normal human tissues do not contain glycolipids or glycoproteins carrying NeuGc, and in humans, NeuGc-containing glycolipids are detected only in colon cancers (*28*). To determine the underlying molecular mechanism, a human cDNA homologous to mouse hydroxylase was cloned and a 92-bp deletion was found in the human sequence (*29*). Unfortunately, our cDNA was not full length. Varki's group reported the complete sequence, confirmed the 92-bp deletion, and demonstrated that the deletion created a stop codon and possibly produced a short, inactive protein (*30*). His group also demonstrated that the deletion occurred in human ancestors after they had diverged from the chimpanzee (*30*) and reported the molecular mechanism of the deletion in collaboration with N. Takahata (*31*, *32*). Interestingly, the suppression of CMP-NeuAc hydroxylase mRNA expression in the brain is conserved in mice and humans, and possibly in other mammals, because other mammals have very high NeuAc contents in the brain. The suppression of NeuGc expression in the brain is conserved among mammals and may be related to maintaining brain function common to mammals. Kozutsumi's group produced CMP-NeuAc hydroxylase gene-targeted mice; using these mice, the suppression of hydroxylase transcription was found in B cells in the germinal centre of the spleen as a physiological process of B-cell activation (*33*).

ANTIGENS ON ERYTHROCYTES

The discovery of hematoside in the horse erythrocyte membrane prompted Yamakawa to examine the glycolipids of erythrocyte membranes isolated from other mammals: humans, sheep, rabbits and guinea pigs. He wrote 'I was very much interested in erythrocyte glycolipids, because they were so different among species. I analyzed erythrocyte glycolipids of various species of mammals (*34*). I believed that erythrocyte glycolipids reflect certain properties of mammalian species, such as the shape of animals. And I thought that glycolipid difference might be the basis of immunological difference among mammalian erythrocytes' (*35*). As mentioned earlier, he prepared a human erythrocyte membrane to confirm Klenk's results (*36*) and obtained a glycolipid. Human erythrocyte glycolipid contained galactosamine, but not neuraminic acid, and these results were consistent with those of Klenk. He named this glycolipid 'globoside' due to the globular structure of the obtained crystal-like substance (*37*). The term 'globo' is now used as a prefix to indicate glycolipids containing a Galα1-4Galβ1- structure in the core. However, Klenk described the first human erythrocyte glycolipid, globotetraosylceramide, whose structure was subsequently determined to be GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-Cer (Fig. 3).

Yamakawa spoke about an event that occurred when he presented data on human erythrocyte glycolipids to the Annual Meeting of the Japanese Biochemical Society. After his talk, Prof. H. Masamune of Tohoku University asked him whether he had tested the ABO blood group antigen activity of isolated glycolipid fractions. Incidentally, S. Hakomori, who identified the molecular mechanism involved in the expression of ABO blood group antigens in 1990 (*38*), was Masamune's student and seemed to be very interested in the presentation. This question triggered Yamakawa's subsequent work on the isolation and structural characterization of ABO blood group antigens, and he together with T. Iida found that his glycolipid fractions contained A and B antigen activities (*39*, *40*). The active fractions were separated from globoside *via* silica gel column chromatography and named globoside II and III (*41*) (Fig. 4). This work was the first demonstration that erythrocyte glycolipids exhibit biological activities. The structures of A, B and O antigens isolated from ovarian cysts were

studied extensively by W. Morgan and W. Watkins in UK, E. Kabat in USA and many other groups, and were finally determined to be GalNAcα1-3(Fucα1-2) Galβ1-, Galα1-3(Fucα1-2)Galβ1- and Fucα1-2Galβ1- in 1971. E. Kabat cited Yamakawa's work in his book (*42*) as follows, 'The final product gave a "clear colloidal solution" in water: reducing sugar (as galactose) 53–56%, hexosamine (as glucosamine HCl) 15.6%, N 2.3%; about 2 μg inhibited hemagglutinating doses of anti-A or anti-B. Yamakawa and Iida (*39*) have reported that globoside contains fatty acids and sphingosine, acetylchondrosamine, D-galactose, and D-glucose, but no amino acid, L-fucose, or D-glucosamine. These findings suggest that globoside may be contaminated with a trace of blood group substance. Further studies are indicated.' Kabat considered the A and B antigens on erythrocytes to be glycoproteins in nature and the antigenic activities of Yamakawa's glycolipid fractions were attributed to a trace amount of contaminating active glycoproteins. However, this was not the case and we now know that the active fractions contain A and B glycolipids together with globoside, which is the major glycolipid of human erythrocyte membranes. Yamakawa wrote that 'In 1953, we found that glycolipid fractions of human erythrocytes inhibited hemagglutination of erythrocytes by ABO blood group antiserum (*39*). In the 1960s, W.T.J. Morgan and W. Watkins, and independently, E. Kabat and his group isolated and determined the structures of ABO blood group antigens from various body fluids and the mucin of ovarian cysts. These antigens were glycoproteins in nature. However, I believed that major ABO antigens on the surface of erythrocytes were glycolipids and not glycoproteins. Hakomori completed the structural determination of ABO blood group active glycolipids and his group recently revealed the molecular mechanism responsible for the production of ABO antigens by the determination of the glycosyltransferase gene structures (*38*).' In Yamakawa's laboratory, S. Ando continued the purification of glycolipids from human erythrocytes and reported the glycolipid composition of blood group A and B erythrocytes shown in Table 1 (*43*).

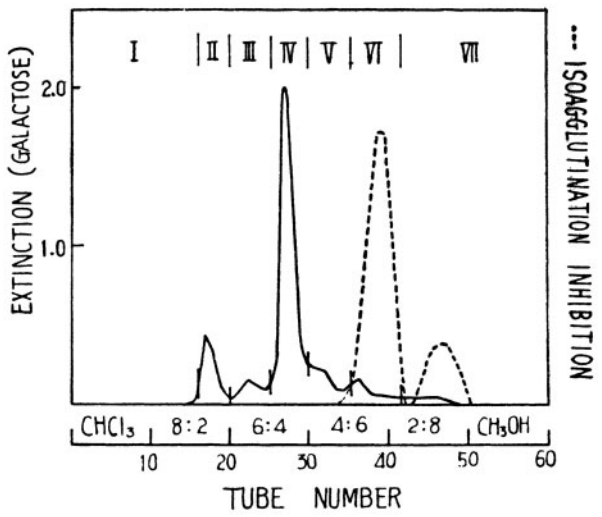

Fig. 4. **Demonstration of ABO blood group antigen activities in glycolipid fractions of human erythrocytes.** Glycolipids were separated by silica gel column chromatography and fractions were tested by a haemagglutination inhibition test. Solid line indicates the content of galactose and dotted line haemagglutination inhibition activity with anti-A antibody. Two peaks with A antigen activity were detected and separated from globoside, the major glycolipid of human erythrocytes (*41*).

PERSPECTIVES

The purification and structural characterization of glycolipids from erythrocytes, cancer tissues and cultured cancer cells were carried out extensively by Hakomori's group (*44*). His contributions to cancer biology and cell biology are very well known and the structures of purified glycolipids and glycans and monoclonal antibodies raised against these molecules became excellent tools for glycobiology. S. Naiki and D. Marcus determined that globoside was P antigen, which a very small part of the human population lacks (*45*).

Table 1. **Composition of glycolipids in human erythrocytes with A or B blood group (*3*, *43*).**

Structures	μg/100 ml packed erythrocytes	Comments
Glc-Cer	279	
Gal-Glc-Cer	2,211	LcCer
Gal-Gal-Glc-Cer	1,021	Gb3Cer
GlcNAc-Gal-Glc-Cer	47	Lc3Cer
GalNAc-Gal-Gal-Glc-Cer	9,600	globoside, Gb4Cer
GalNAc-GalNAc-Gal-Gal-Glc-Cer	7	IV^3GalNAcβ-Gb4Cer
Gal-GlcNAc-Gal-Glc-Cer	553	nLc4Cer
Gal-Gal-GlcNAc-Gal-Glc-Cer	5–10	P1 antigen IV^4Galα-nLcCer
GalNAc-(Fuc-)Gal-GlcNAc-Gal-Glc-Cer	124	in erythrocytes with A blood group IV^3GalNAcα, IV^2Fucα-nLcCer
Gal-(Fuc-)Gal-GlcNAc-Gal-Glc-Cer	120	in erythrocytes with B blood group IV^3Galα, IV^2Fucα-nLcCer
NeuAc-Gal-Glc-Cer	355	hematoside, GM3
NeuAc-Gal-GlcNAc-Gal-Glc-Cer	426	IV^3NeuAcα-nLc4Cer

STRUCTURE ANALYSIS OF GLYCOLIPIDS

Gas-liquid chromatography (GLC) was a Yamakawa's favourite tool. He introduced GLC for the analysis of sugar components for the first time in Japan, which was very early on in terms of worldwide use (*46*). GLC is still a powerful method for determining the substitution position of carbohydrates after the methylation of free hydroxyl groups. Hakomori's permethylation method using carbanion in dimethyl sulphoxide was published in the *Journal of Biochemistry* and became very popular (*47*). GLC analysis of partially methylated methylglycosides prepared from permethylated glycolipids by acidic methanolysis was applied to purified glycolipids. In 1962, Yamakawa needed C6-subsituted galactose as a reference for GLC analysis and prepared it from sulfatide because Blix in 1933 had first reported that the sulfate of sulfatide was attached at the C6 position of galactose (*48*), which was again described in 1955 by Thannhauser and Schmidt (*49*). Surprisingly, GLC analysis of permethylated sulfatide gave the peaks of 2,4,6-trimethyl-methylgalctoside, but not 2,3,4-trimethy-methygalactoside, indicating that the sulfate is attached at the C3 position of galactose, not C6. Yamakawa wrote that he became feverish the next day due to this unexpected finding, which based on a 2-day experiment led to the revision of biochemistry textbooks. He sent a communication describing this finding to the *Journal of Biochemistry* (*50*) and mailed its reprints to glycolipid biochemists. Several months later, P. Stoffyn reported in *Biochimica et Biophysica Acta* that sulfate is attached at the C3 position of the galactose of sulfatide (*51*). When Yamakawa visited Folch in McLean Hospital in Boston in 1964, he met Stoffyn, who said, 'I had got the result of the C3 substitution of sulfate and discussed it with Folch in much excitement. When I was back to my lab, within less than 10 min, Folch rushed to me, showing the reprint of Yamakawa's paper and he said that it had been already done by Yamakawa.'

In addition, A. Makita reported the correct structure of ganglioside, GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ1-ceramide, purified from the brain of a patient with Tay–Sachs' disease, revising previously reported structures (*52*). Y. Seyama purified GalNAcβ1-4Galβ1-4Glcβ1-ceramide from guinea pig erythrocytes (*53*), and Y. Eto reported that the major glycolipid of rabbit erythrocytes was Galα1-3Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ1-ceramide (*54*). Forssman antigen was purified from sheep erythrocytes in Yamakawa's laboratory, and the final structure was reported by Suddiqui and Hakomori (*55*). Hamanaka reported that mouse erythrocytes showed strain differences (*56*) and these polymorphic variations were further subjected to genetic analysis (*3*).

SEMINOLIPID AND THE *JOURNAL OF BIOCHEMISTRY*

When the author joined Yamakawa's laboratory in 1971, the author's research subject was the purification and structure characterization of an unknown glycolipid in pig testis. Epididymis was used to purify hexosaminidase and the remaining testis was subjected to lipid extraction. The purified glycolipid had an absorption peak due to an ester linkage in its infrared spectra, and a gas chromatography component analysis showed that palmitic acid was the fatty acid and galactose the carbohydrate. Yamakawa and the rest of us felt that these components were too simple and the presence of the ester linkage was due to contaminating glycerolipids. I. Ishizuka joined Yamakawa's laboratory and took charge of this project. Within a few months, he reached the conclusion that the glycolipid was a glyceroglycolipid: 1-*O*-alkyl-2-*O*-acyl-3-(β3′-sulfogalactosyl)glycerol. This success was due to his experience with glyceroglycolipids that he had isolated from the bacteria (*57*). Yamakawa named the novel glycolipid seminolipid. It was a new unexpected finding that testis contained a glyceroglycolipid as the major glycolipid because we had believed that plants and bacteria contained glyceroglycolipids, but not glycosphingolipids, as the major glycolipids, and that mammals contained glycosphingolipids, but not glyceroglycolipids. Testis is a unique tissue in terms of the type of glycolipid. Ishizuka reported the results at the annual meeting of the Japanese Lipid Biochemistry Society in June 1972, and we had to submit a six-page abstract since the society asked for a presentation of most the recent and additional preliminary results to enhance discussion. Yamakawa was invited to the 6th International Carbohydrate Symposium held at Wisconsin University in August 1972, where he gave a presentation on seminolipid. We had submitted the first manuscript about seminolipid to the *Journal of Biochemistry* in May 1972, and the paper was published in January 1973, 8 months later (*58*). Meanwhile, we were surprised to find a paper submitted in July 1972 and published in the September issue of *Biochemical and Biophysical Research Commununications* that reported exactly the same structure for a glycolipid purified from rat testis (*59*). This prompted Yamakawa to improve the review process of the *Journal of Biochemistry* to shorten the time required for publication when he was editor-in-chief from 1977 to 1982. Succeeding editors-in-chief have continued this effort and now the first response to authors is sent within 3 weeks at most and manuscripts are made available on a website soon after acceptance.

PERSPECTIVES

Testis and spermatozoa contained seminolipid and a precursor glycolipid, 1-*O*-alkyl-2-*O*-acyl-3-βgalactosyl-glycerol, and the enzymes involved in the biosynthesis were determined to be sulfotransferase and β-galactosyltransferase, respectively. Both enzymes use glycerolipids (1-*O*-alkyl-2-*O*-acyl-3-βgalactosyl-glycerol and 1-*O*-alkyl-2-*O*-acyl-glycerol) and sphingolipids (Gal-ceramide and ceramide) as precursors. The products 3′sulfo-Gal-ceramide (sulfatide) and Gal-ceramide are the major components of myelin. Sulfotransferase and β-galactosyltransferase knockout mice have been established and pathological phenotypes in the testis and myelin were reported (*60–62*). In both knockout mice, spermatocyte meiosis was blocked (*63*). The physiological functions of these two glycolipids still remain unknown but can be unexpected ones.

After the discovery of a seminolipid in boar testis, Ishizuka devoted his research life to sulfated glycolipids and contributed to structural and functional studies of kidney sulfated glycolipids (*64*). He proposed new nomenclature for sulfated glycolipids, SM4 for 3′ sulfo-Galβ1-ceramide, SM3 for 3′ sulfo-Galβ1-4Glcβ1-ceramide, SM2 for GalNAcβ1-4(3′sulfo-)Galβ1-4Glcβ1-ceramide, and so on. Regrettably, he could not continue his research because he contracted pancreatic cancer and died in 2005.

EPILOGUE

Yamakawa published almost all of his work in the *Journal of Biochemistry*. His pioneering work contributed greatly to enhancing the international reputation of this journal. Biology tells us that the monopoly or domination of one particular material, species or anything will cause serious problems in the future. Glycobiology is a science that deals with biological diversity, which is the product of evolution and critical to the fate of species. It is sincerely hoped that glycobiology and our *Journal of Biochemistry* can both survive under heavy competition pressure.

ACKNOWLEDGEMENTS

The author thanks Drs N. Taniguchi, K. Suzuki and T. Yamakawa for the suggestions in writing this article.

CONFLICT OF INTEREST

None declared.

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