Virchows Arch. A Path. Anat. and Histol. 365, 351—365 (1975)

Experimental Polymer Storage Disease in Rabbits

An Approach to the Histogenesis of Sphingolipidoses

Kichihei Miyasaki

Department of Pathology, Kobe University, School of Medicine, Kobe, Japan

Received September 2, 1974

Summary. Water-soluble polymer compound, polyvinyl alcohol (PVA), and water-insoluble polymer compounds, polyvinyl acetate (PVAc) and polystylol (PS), were administered in 297 rabbits. When high polymerized PVA, PVAc or PS were continuously injected intravenously for a long period of time, lesions resembled to those of Gaucher and Niemann-Pick diseases were developed. From these experimental results, pathological development of various sphingolipidoses found in the human body was discussed and pathological findings were analysed polymer-chemically from the chemical properties of the substances stored.

Key words: Sphingolipidoses — Lipidoses — Gaucher Disease — Niemann-Pick Disease — Fabry Disease.

Although many remains unknown on the in vivo metabolism of lipids, proteins and carbohydrates, some of diseases are known to be caused by the storage of these substances due to metabolic abnormalities. Staudinger considered that chemical studies on natural polymer compounds lead conclusion to the wrong, so that he proceeded systemic studies on synthetic polymer compounds, by regarding polyvinyl alcohol (PVA) and polyvinyl acetate (PVAc) as a model of carbohydrates and their esters, polystylol (PS) as a model of substances which dissolve in colloidal form, such as rubber, and polyacrylic acid as an indicator for proteins. According to Staudinger, our experiments were performed, taking these synthetic polymer compounds as models of in vivo polymer substances. These compounds act upon rabbits as foreign bodies and are stored abnormally. The pathological findings are similar to those found in Gaucher or Niemann-Pick disease (Miyasaki, 1959; Miyasaki and Okada, 1960; Hatano, Miyasaki, and Wakamori, 1965). Recently, we have found one of glycosphingolipidoses in necropsy (Miyasaki and Saito, 1973). Therefore, this paper discusses the pathogenesis and histogenesis of sphingolipidoses by contrasting the findings of administration of synthetic polymer compounds in rabbits with human sphingolipidoses.

Materials and Methods

Using 287 normal matured and 10 pregnant rabbits, PVA aqueous solution, PVAc aqueous emulsion and PS aqueous emulsion were administered via the vein, the mouth, the respiratory tract and the skin. The distribution of PVA and PVAc was demonstrated by the histochemical methods we originated (Miyasaki, 1959; Hatano, Miyasaki, and Wakamori, 1965). Synthetic polymer compounds used in this study are all products of the Kobunshi Kagaku Kogyo Ltd., Japan, and their brandnames are "Polysizer" for PVA, "Polysol S-3" for PVAc and "Polysol C-4" for PS.

The methods of experiments are outlined as follows: (A) *PVA administration* used 75 rabbits. (1) PVA 5 g/day mixed in diet, daily for 20 days, 11 rabbits. (2) Intratracheally 5%

PVA 0.7, 1.4 or 3.0 cc/kg injected every other day for one week, 9 rabbits. (3) Subcutaneously 2.5% PVA 0.3 cc/kg, injected daily for one or 30 days, 6 rabbits. (4) Intravenously 5% PVA 1 cc/kg injected daily for 2 weeks, 4 rabbits; 2 cc/kg injected daily for 12 weeks or 6 months, 15 rabbits; 3 cc/kg injected daily for 1, 2, 4 or 12 weeks, 20 rabbits; 4 cc/kg injected daily for one or 10 days, 5 rabbits; 5 cc/kg injected daily for 3 weeks, 3 rabbits; and 5 cc/kg injected once in 5 pregnant rabbits.

(B) *PVAc administration* used 147 rabbits. (1) Subcutaneously 30% PVAc 0.3 cc injected, 2 rabbits. (2) Intratracheally 3% PVAc 1 cc/kg injected every fourth day, four times, 3 rabbits. (3) Intravenously 5% PVAc 1 cc/kg injected daily for 1, 2, 4, 8, 12, 16 or 24 weeks, 41 rabbits; 2 cc/kg injected daily for 3 days, 1, 2, 3, 6, 12 or 24 weeks, 60 rabbits; 3 cc/kg injected daily for 6 months, 5 rabbits; 3 cc/kg injected daily for 6 months, and after that not treated for 3 months, 2 rabbits; 4 cc/kg injected daily for 1, 2, 4 or 6 weeks, 18 rabbits; and 5 cc/kg injected into 5 pregnant rabbits.

(C) *PS administration* used 75 rabbits. Intravenously 5% PS 1 cc/kg injected daily for 1, 2, 4, 8, 12, 16 or 20 weeks, 26 rabbits; and 2 cc/kg injected daily for 3 days, 1, 2, 3, 4, 8 or 20 weeks, 49 rabbits.

Results

(A) PVA Administration. By oral administration, PVA is not absorbed and stored in the body of the rabbits, while by tracheal administration, it is mainly stored in the alveolar phagocytes in the lungs and is sometimes excreted in the urine, if the dosage is excessive (Table 1). By subcutaneous injection, PVA is demonstrated only in the subcutaneous histiocytes. Even after the continuous subcutaneous injection for a month, only a very small amount of PVA is found in the cells of the reticulo-endothelial system. On the contrary, PVA injected into the ear vein of rabbits begins to appear in the urine in the relatively early stage after the injection, although some of them remain in the serum for a long time (Table 2). Furthermore, continuous injection for a long period of time develops splenomegaly macroscopically and histological enlargement of the cells

Table 1. PVA storage by tracheal administration

Group	No.	Lungs					Liver	Spleen	Kidneys	Urine
		right upper lobe	right middle lobe	right lower lobe	left upper lobe	left lower lobe				
I	1	−	−	++	−	−	−	−	−	−
	2	−	−	++	−	+	−	−	−	−
	3	−	−	++	−	++	−	−	−	−
II	4	−	−	++	+	−	−	−	−	−
	5	+	−	−	++	++	−	−	−	−
	6	−	−	+	+	++	−	−	−	−
III	7	+++	+	+++	+++	+++	−	+	+	+
	8	+++	++	+++	+++	+++	−	++	−	+
	9	++	+	+++	++	+++	−	++	+	+

Group I: 5% PVA 0.7 cc/kg, intratracheal every other day for one week.
Group II: 5% PVA 1.4 cc/kg, intratracheal every other day for one week.
Group III: 5% PVA 3.0 cc/kg, intratracheal every other day for one week.

Table 2. PVA in urine and blood serum by intravenous administration

No.	After injection															
	10 min		1 hr		17 hrs		24 hrs		2 days		4 days		5 days		10 days	
	U	S	U	S	U	S	U	S	U	S	U	S	U	S	U	S
1	+		+++		+	+++										
2	+		+++		++		+	++								
3	+		+++		++		+		−	+						
4	+		+++		++		+		+	+	+	+	−	+		
5	+		+++		++		+		+	+	−	+	−	+	−	+

U = PVA in urine. S = PVA in blood serum.
5% PVA 4 cc/kg intravenously injected.

Table 3. PVA transference into fetus

	After injection									
	Mother rabbits					Fetus				
	30 min	3 hrs	8 hrs	24 hrs	72 hrs	30 min	3 hrs	8 hrs	24 hrs	72 hrs
Liver	+	++	+++	++	++	−	++	++	++	+++
Lungs	+	+	+	+	+	−	+	+	+	+
Kidneys	+	+	+	+	+	−	−	−	±	+
Blood serum	±	+	+	+	+				+	+
Amniotic fluid	−	−	+	+	−					
Placenta	+	+	++	+++	+++					

5% PVA 5 cc/kg intravenously injected into pregnant rabbits.

in the reticulo-endothelial system, such as the spleen, the liver, the bone marrow and the lymph nodes, forming a number of foam cells (Figs. 2, 4 and 6). PVA injected intravenously is also demonstrated in the epithelium of the renal tubules, the hepatic cells and the histiocytes in the interstitial tissue at the same time. The administration of low polymerized PVA results in extremely slight storage. PVA given intravenously transferres relatively easily to the fetus, but the amount is too small to form foam cells (Table 3).

(B) PVAc Administration. By the subcutaneous injection of PVAc presenting water-insoluble globular particles, it is localized subcutaneously and is hardly absorbed. By intratracheal administration, PVAc is phagocytosed only by the alveolar phagocytes in the lungs (Table 4). On the other hand, in the intravenous injection of PVAc, only a small amount is excreted in the urine and most of it stored in the body (Table 5). When PVAc is injected continuously into the ear vein for a long period of time, grossly marked splenomegaly along with slight enlargement of the liver and the lymph nodes is observed (Fig. 11). The cells in the reticulo-endothelial system, such as the spleen, the liver, the bone marrow, the lymph nodes, the adrenal glands and the lungs, phagocytose the injected

Table 4. PVAc storage in the lungs by tracheal administration

No.	Lungs					Liver	Spleen
	right upper lobe	right middle lobe	right lower lobe	left upper lobe	left lower lobe		
1	+	−	+	+	+++	−	−
2	−	−	++	−	+++	−	−
3	+++	++	+++	−	+	−	−

3% PVAc 1 cc/kg, every fourth day, four times.

Table 5. PVAc in urine by intravenous administration

No.	After injection														
	5 min	10 min	15 min	20 min	30 min	1 hr	1.5 hrs	2 hrs	2.5 hrs	3 hrs	3.5 hrs	4 hrs	4.5 hrs	5 hrs	5.5 hrs
1	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−
2	−	−	−	−	−	++	+++	+++	+	±	−	−	−	−	−
3	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

5% PVAc 4 cc/kg intravenously injected.

Table 6. PVAc storage after interruption of its administration

Group	No.	Liver (percent by weight)	Spleen (percent by weight)	Foam cells in lymph nodes							
				submandibular	parotid	superficial cervical	deep cervical	mediastinal	axillar	mesenterium	popliteal
I	1	4.08	1.91	++	+++	++	+	−	+	+	±
	2	5.31	1.60	+++	+++	+	++	+	+	+	++
	3	4.68	1.74	++	++++	+++	++	++	+	++	++
	4	4.98	1.49	+	++++	+	+	−	+	+	+
	5	3.82	1.90	+++	++++	+++	++	+	+	+	++
II	6	5.06	2.13	+++	++	+++	++	++++	+	+	++
	7	4.09	1.59	++	++++	++++	+++	+++	+	+	+

Group I: 5% PVAc 3 cc/kg, daily for six months.
Group II: 5% PVAc 3 cc/kg, daily for six months, and after that no treatment for three months.

PVAc and are markedly enlarged histologically, forming foam cells. Such foam cells are rarely found in the epithelium of the renal tubules, the hepatic cells and the interstitial histiocytes (Figs. 1, 3, 7–10). In this case, the storage remains unchanged three months after the interruption of PVAc, and rather a greater

Table 7. PVAc transference into fetus

	After injection									
	Mother rabbits					Fetus				
	30 min	3 hrs	8 hrs	24 hrs	72 hrs	30 min	3 hrs	8 hrs	24 hrs	72 hrs
Liver	+	+	+	+	++	−	−	±	±	±
Lungs	±	+	+	+	+	−	−	−	−	−
Kidneys	−	±	±	±	+	−	−	−	−	−
Placenta	−	±	±	+	++					
Amniotic fluid	−	−	−	−	−					

5% PVAc 5 cc/kg intravenously injected into pregnant rabbits.

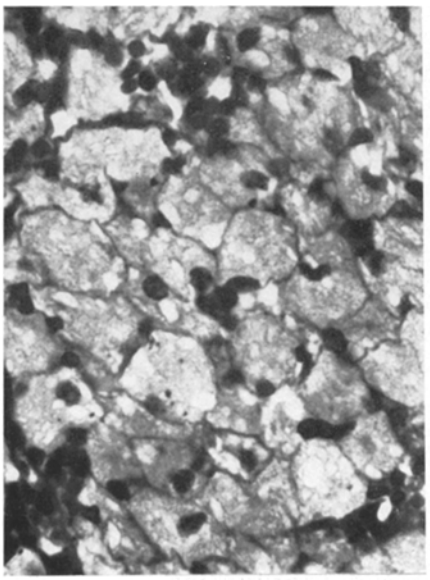
amount of PVAc is stored in the organs (Table 6). PVAc given intravenously rarely transferres to the fetus (Table 7).

(C) PS Administration. Although PS is a water-insoluble globular particle similar to PVAc, histochemical methods for demonstration have not yet been established. Therefore, the details on its in vivo distribution cannot be discussed. However, same as PVAc, continuous injection of PS into the ear vein for a long period of time causes obvious splenomegaly (Fig. 11) and the cells in the reticulo-endothelial system, such as the liver, the spleen, the bone marrow and the lymph nodes, form marked foam cells (Fig. 5).

(D) Laboratory Findings on Experiments by Intravenous Injection. Polymer used in this study is markedly stored in the cells in the reticulo-endothelial system only when injected into the ear vein. Therefore, this paper describes only the laboratory findings on experiments by intravenous injection. Congo red index increases in each case and is histologically correlated with the degree of storage of each substance, so that the function of the reticulo-endothelial system is considered to decrease (Fig. 12). Also in the examination of self-respiration in the liver and the spleen by using Warburg pressure gauge and in the enzymatic examination using succinic acid oxidation enzyme, the function seems to decrease as the polymer is stored greater (Fig. 13). No abnormalities are seen in the liver function tests. Total protein content in the serum and amino-N, K, Na and Cl in the serum are not markedly changed. No variation is also found in the values of plasma lipoids, serum cholesterol, serum bilirubin, and blood sugar. Although the values of red blood cells and hemoglobin tend to decrease, the iron content in the serum and color index is not markedly changed (Fig. 14). No variation is found in white blood cells, blood coagulation time and bleeding time. In addition, the erythrocyte sedimentation rate is markedly increased only in the case to which PVA is given intravenously, estimating the direct effect of PVA upon the erythrocyte sedimentation (Miyasaki, 1958).

Discussion

Although the definite mechanisms of synthesis and catabolism of sphingolipids are poorly understood, most cases of the sphingolipidoses are known to



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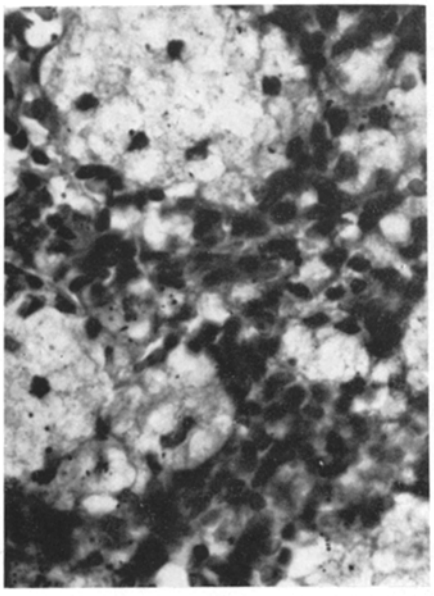

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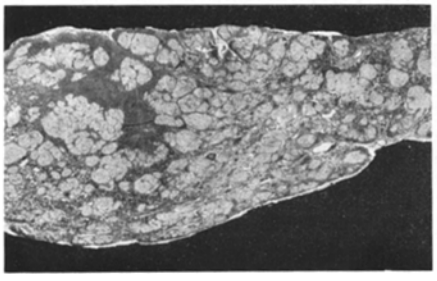

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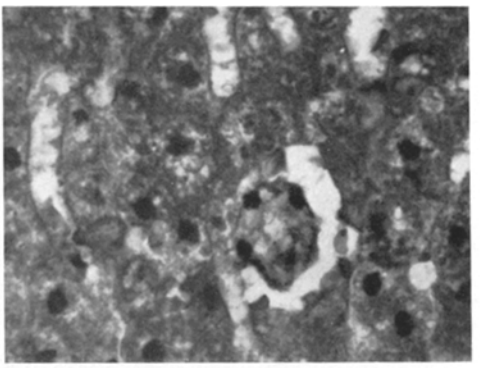

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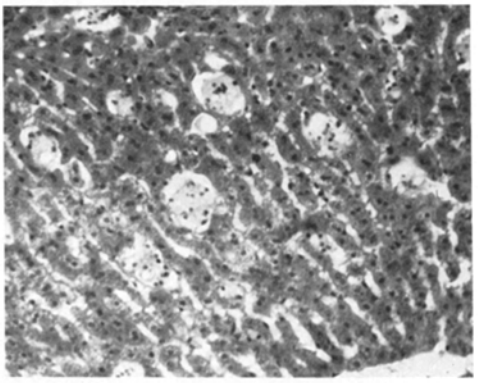

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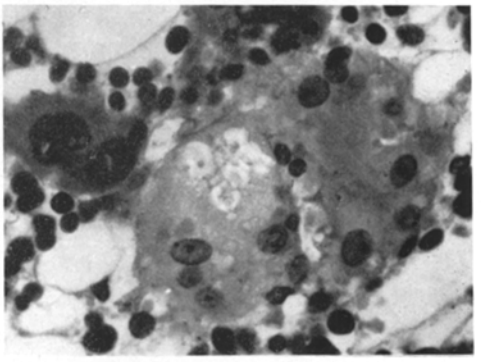

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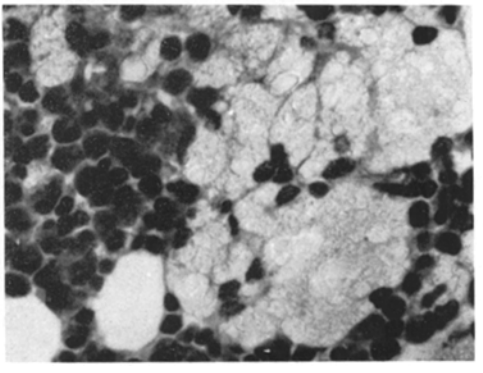

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be caused by the disturbances of lipolytic enzymes (Fig. 17). Solubility of each sphingolipid in water is chemically thought to augment according to the increase of the number of sugar chain. Ceramide, glucocerebroside, galactocerebroside and sphingomyelin similarly to PVAc and PS tend to act upon the living body as *foreign bodies*. Therefore, in Gaucher disease with β-glucosidase defect (Brady *et al.*, 1965a, b, 1966a; Beutler and Kuhl, 1970), Niemann-Pick disease with sphingomyelinase defect (Brady *et al.*, 1966b; Kanfer *et al.*, 1966; Sloan *et al.*, 1969), and Krabbe disease with β-galactosidase defect (Brady *et al.*, 1965c; Hajra *et al.*, 1966; Suzuki and Suzuki, 1970), each increased substance is estimated to tend to be easily stored mainly in phagocytes as a foreign body.

Autopsy cases with Gaucher and Niemann-Pick diseases in Japan revealed splenomegaly, slight enlargement of the liver and the lymph nodes and anemia, and foam cells are histologically observed in the spleen, the liver, the bone marrow and the lymph nodes (Figs. 15 and 16). These autopsy findings are closely resembled to the findings of intravenous injection of PVAc or PS in our experiment, although the stored substances are different. It is assumed that the difference between Gaucher and Niemann-Pick diseases is resulted from the delicate difference in the amount of increasingly stored substance and the chemical structure of the substances in each disease. Both Gaucher and Niemann-Pick diseases may be caused by the enzyme defects in some of the cells except the brain. Therefore, both diseases are considered as the condition in which the lipids released in the blood due to the enzyme defects are mainly stored in the reticulo-endothelial system. Both Gaucher and Niemann-Pick diseases show no increase of lipids in the blood, and likewise our experiment revealed that PVAc and PS are not increased in the blood, in spite of the administration of these substances into the blood. So-called globoid cells found in the brain in Krabbe disease are not seen in Gaucher or Niemann-Pick disease, and foam cells are only found around the small blood vessels in the brain of Gaucher or Niemann-Pick disease. Similar findings are seen by the injection of PVAc and PS, suggesting the presence of blood-brain barrier. It is considered that Krabbe disease is a metabolic disorder of galactocerebroside developed in the brain and shows marked phagocytosis only in phagocytes of the brain. This finding is supported from the experiment on intracranial injection of galactocerebroside (Suzuki, 1970).

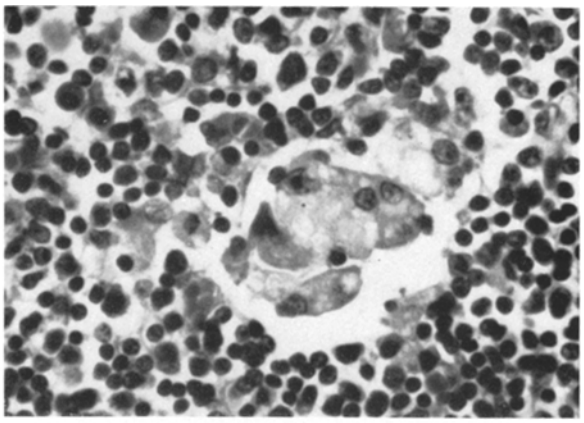
Figs. 1 and 2. Foamy swollen reticulum cells in the *spleen*. H-E stain. Original magnification $\times 200$. Fig. 1: 5% PVAc 1 cc/kg intravenously daily for 4 months. Fig. 2: 5% PVA 2 cc/kg intravenously daily for 6 months

Fig. 3. Foamy swollen reticulum cells in the *superficial cervical lymph node*. 5% PVAc 3 cc/kg intravenously daily for 6 months, and no treatment for next 3 months. H-E stain. Original magnification $\times 5$

Figs. 4 and 5. Foamy swollen Kupffer cells in the *liver*. H-E stain. Original magnification $\times 200$ in Fig. 4, and $\times 50$ in Fig. 5

Figs. 6 and 7. Foamy swollen reticulum cells in the *bone marrow*. H-E stain. Original magnification $\times 200$.

Figs. 4 and 6: 5% PVA 2 cc/kg intravenously daily for 6 months. Fig. 5: 5% PS 1 cc/kg intravenously daily for 4 months. Fig. 7: 5% PVAc 1 cc/kg intravenously daily for 4 months



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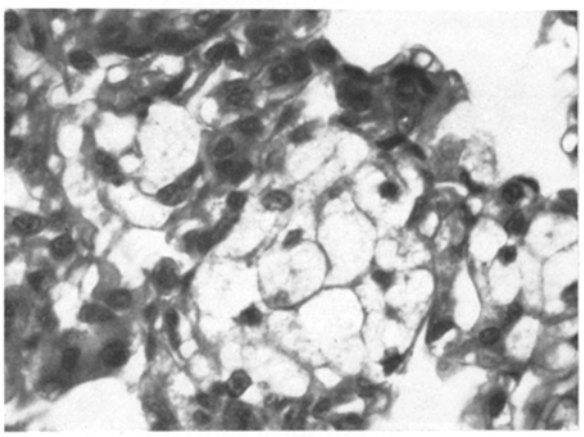

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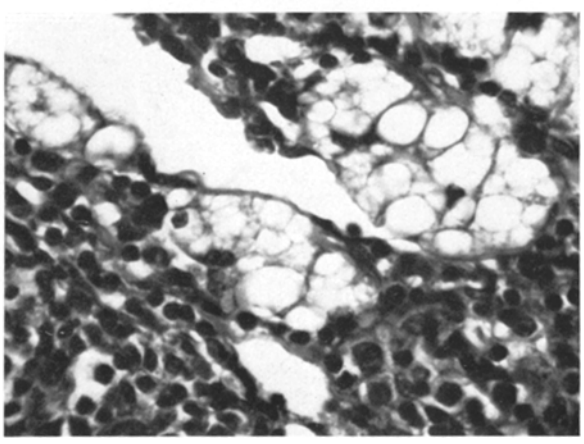

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Fig. 8. Foamy swollen reticulum cells in the *thymus*. Intravenous injection of 5% PVAc 3 cc/kg daily for 6 months. H-E stain. Original magnification ×200

Fig. 9. Foamy swollen alveolar phagocytes in the *lungs*. Intravenous injection of 5% PVAc 3 cc/kg daily for 6 months. H-E stain. Original magnification ×200

Fig. 10. Foamy swollen reticulum cells in the *paratine tonsil*. Intravenous injection of 5% PVAc 3 cc/kg daily for 6 months. H-E stain. Original magnification ×200

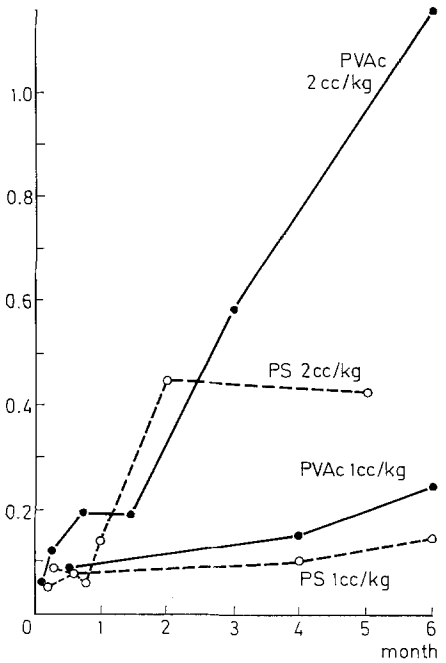

Fig. 11. Percent by weight of the spleen (mean values). 5% PVAc 1 cc/kg was injected intravenously daily for 2 weeks (6 rabbits); 4 months (2 rabbits); and 6 months (2 rabbits). 5% PVAc 2 cc/kg was injected intravenously daily for 3 days (3 rabbits); 1 week (5 rabbits); 2 weeks (2 rabbits); 3 weeks (6 rabbits); 6 weeks (5 rabbits); 3 months (9 rabbits); and 6 months (2 rabbits). 5% PS 1 cc/kg was injected intravenously daily for 1 week (2 rabbits); 2 weeks (10 rabbits); 4 months (3 rabbits); and 5 months (1 rabbit). 5% PS 2 cc/kg was injected intravenously daily for 3 days (3 rabbits); 1 week (5 rabbits); 3 weeks (6 rabbits); 1 month (4 rabbits); 2 months (1 rabbit); and 5 months (3 rabbits)

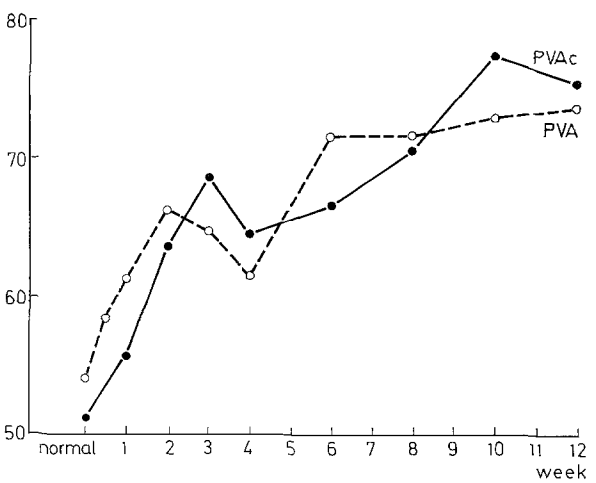

Fig. 12. Congo red index (mean values). 5% PVA 2 cc/kg was injected intravenously daily for 12 weeks (9 rabbits). 5% PVAc 2 cc/kg was injected intravenously daily for 12 weeks (11 rabbits)

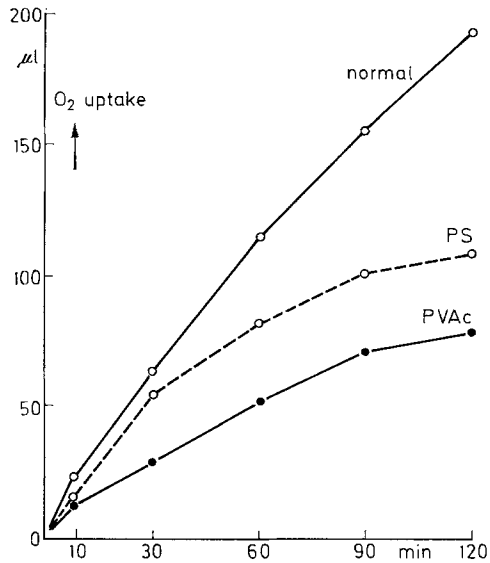

Fig. 13. Succinic oxydase system in the spleen using Warburg's manometric apparatus. 2 cc/kg of 5% PVAc or 5% PS was injected intravenously daily for 3 months (6 rabbits)

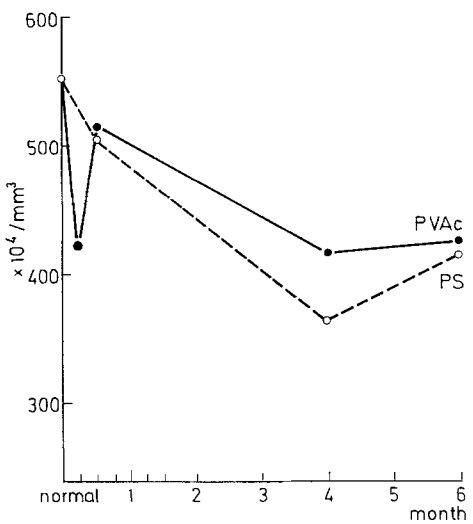

Fig. 14. Erythrocytes in the peripheral blood (mean values). 5% PVAc 1 cc/kg was injected intravenously daily for 1 week, 2 weeks, 4 months or 6 months into 20 rabbits in all. 5% PS 1 cc/kg was injected intravenously daily for 2 weeks, 4 months or 5 months into 15 rabbits in all

Metachromatic leukodystrophy is considered to be a disease caused by sulfatidase defect (Austin *et al.*, 1963; Mehl and Jatzkewitz, 1965; Percy and Brady, 1968), and in this disease sulfatide is increasingly stored not only in the brain but also in the kidneys, the gall bladder, the liver, the endocrine glands and the other organs. As sulfatide seems to be a chemically

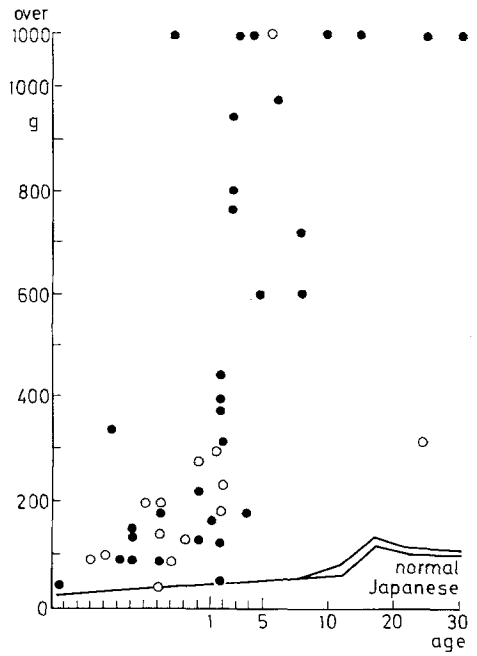

Fig. 15. Spleen weight in Gaucher and Niemann-Pick diseases in Japan. • Gaucher disease, ○ Niemann-Pick disease

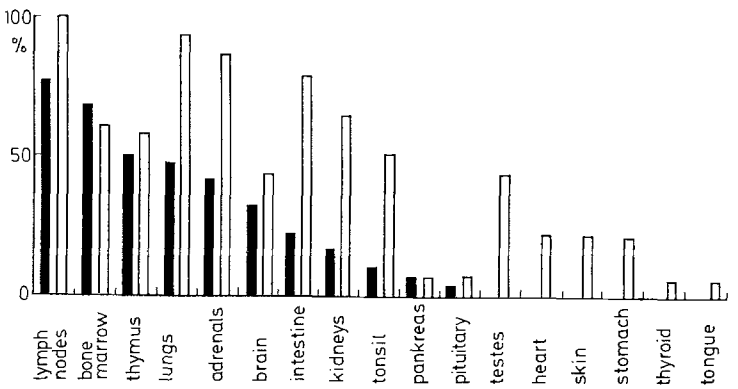

Fig. 16. Foam cells in each organ in Gaucher and Niemann-Pick diseases in Japan. • Gaucher disease, ○ Niemann-Pick disease

considerably stable substance, it is estimated that the cells related with sulfatide metabolism exist in the other organs as well as in the brain and that the increased sulfatide is rarely released out of the cells except the brain. If sulfatide is a chemically considerably stable substance and released into the blood, the cells in the reticulo-endothelial system may well form foam cells in metachromatic leucodystrophy. However, there is no evidence enough to discuss the pathological development of metachromatic leucodystrophy in our experimental results.

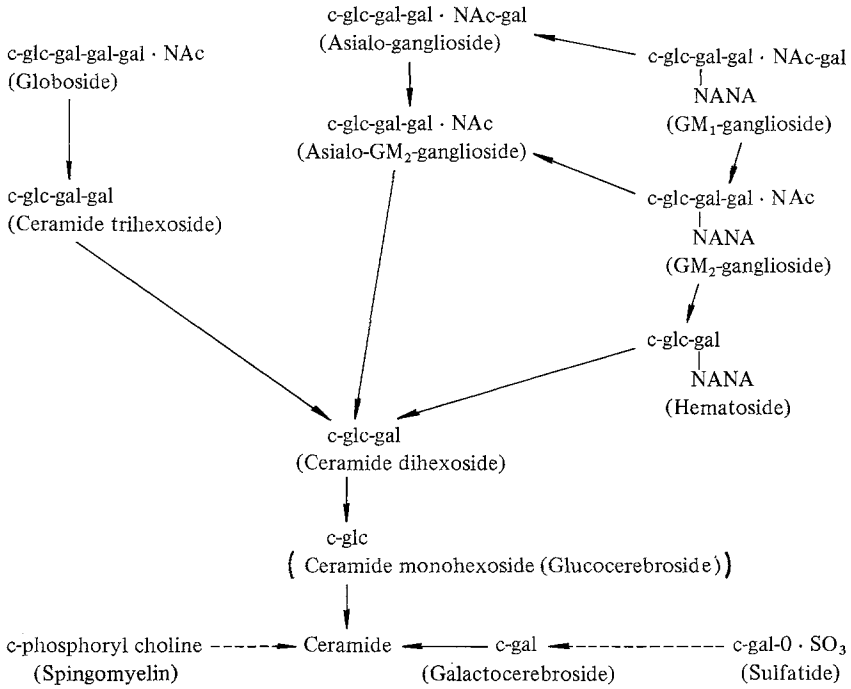

Fig. 17. Metabolic relationship of the sphingolipids

Although pathological findings of lactosyl-ceramidosis with increased ceramide dihexoside (CDH) are not obvious (Dawson and Stein, 1970), in Fabry disease in which β-galactosidase defect is probably present (Brady *et al.*, 1967a, b; Kint, 1970), CDH and ceramide trihexoside (CTH) are increasingly stored in the kidneys and CTH is in the smooth muscle of the blood vessels, the heart, and the cells of the sympathetic nervous cells, however, they are hardly increased in the brain. In the case we experienced, globoside and CTH are markedly stored in the liver and the reticulo-endothelial system in addition to the areas where the substances are usually stored in Fabry disease (Miyasaki and Saito, 1973). As it is generally considered that the solubility of polymer substance in water augments with the increase of the number of sugar chains on chemical structure, CTH and globoside are possibly excreted in the urine even if increased in the body. This is also estimated from the experiment on the administration of PVA, which is easily excreted in the urine. PVA is less stored in the reticulo-endothelial system and forms less foam cells than PVAc and PS even after the massive intravenous injection of PVA for a long period of time, and a considerable amount is excreted in the urine. Water-soluble polymers with different degree of polymerization such as PVA are stored in the body and excreted in the urine at different degrees. PVA with higher polymerization is easily stored in the body, and this is also known in polyvinylpyrrolidone. Although it is not evident whether there are different degrees of polymerization in CHT or globoside, the difference

between the lesion of Fabry disease and that we experienced may be due to the difference in the degree of polymerization as well as the chemical structural difference. Glycosphingolipids are massively stored in the kidneys both in Fabry disease and the case we experienced, and many cases die of uremia particularly in Fabry disease (Miyasaki, 1974). It is possible to consider that CTH increased in the body is stored in the kidneys in the way of excretion for a long time and causes renal lesions in Fabry disease.

In Sandhoff disease (Dolman, Change and Duke, 1973) similarly to Tay-Sachs disease, GM_2-ganglioside and asialo-GM_2-ganglioside are stored in the brain, and in addition globoside is increased in the liver, the kidneys and the spleen. Cerebral lesions are so marked in Sandhoff disease that patients do not seem to be survive for a long period of time. Therefore, it may be difficult to compare the findings and lesions of our case with those of Sandhoff disease pathologically. Furthermore, the disease which shows the increasing systemic storage of sphingolipids in addition to the main lesions of the brain is GM_1-gangliosidosis (O'Brien *et al.*, 1965; Gonatas and Gonatas, 1965; Derry *et al.*, 1968), where the storage of GM_1-ganglioside is observed not only in the brain, but also in the liver and the spleen, and at the same time keratan sulfate are increased in the liver and the spleen (Suzuki, 1968). The findings is patho-histologically resembled to Niemann-Pick disease, except for the cerebral lesions. It is not unreasonable to see that in Fabry disease with increased CTH, the cells in the reticulo-endothelial system rarely form foam cells, while in GM_1-gangliosidosis and in our reported case (Miyasaki and Saito, 1973), in which stored substances have chemically more sugar chain than CTH, foam cells are observed. As mentioned above, even if the polymer is water-soluble, the degree of storage varies with the difference of polymerization, and higher polymerized substance is likely to be stored in the body. Therefore, there is a possibility that globoside and GM_1-ganglioside are fairly highly polymerized.

On the other hand, there exists a concept of mucolipidosis (Spranger and Wiedemann, 1969, 1970a, b), and mucopolysaccharide and sphingolipids may be increasingly stored together in some cases. The condition is really seen in GM_1-gangliosodosis. Metabolism in sugar side chain of mucopolysaccharide and sphingolipids is considered to be same and it is hardly to deny the co-existence of metabolic abnormalities of sphingolipids and those of mucopolysaccharide. In future, metabolisms of sphingolipids, mucopolysaccharides and glycoproteins are to be investigate at the same time.

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Dr. K. Miyasaki
Department of Pathology
Kobe University School of Medicine
Ikuta-ku
Kobe, Japan