

VITAMIN B6 AND ARTERIOSCLEROSIS

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ABSTRACT

In 1949, Rinehart and Greenberg reported that marked arteriosclerosis occurs in vitamin-B6-deficient monkeys. The present study investigates the relationship between vitamin B6 and arteriosclerosis and summarizes the results. I found that thrombogenesis, disorder of collagen metabolism and production of free radicals may be the processes that cause arteriosclerosis in human and experimental animals with vitamin B6 deficiency.

VITAMIN B6 DEFICIENCY AND ARTERIOSCLEROSIS IN MONKEYS

Rinehart and Greenberg¹⁾ found by chance during studies on vitamin deficiency using rhesus monkeys that marked arteriosclerosis occurs in vitamin-B6-deficient monkeys. The following are some of the interesting findings that they obtained from their experiment. One finding was a marked increase in acid mucopolysaccharides stained by toluidine blue in the hypertrophic tunica intima. A second finding was the very small amount of lipid present in fibrous plaques (no high lipid diet was given). A third finding was that cell proliferation and fibroplasia in the tunica intima occurred in almost all of the arteries in the organs, especially the coronary arteries, the aorta and the kidneys.

We started our research to supplement the work of Rinehart and Greenberg. We used Japanese monkeys instead of rhesus monkeys. The results are shown in Table 1. We observed arteriosclerosis in the brain, pancreas and liver, a new finding not seen by Rinehart and Greenberg. Vascular wall changes similar to those in the arteries were also seen in the arterioles. New findings were obtained in fields not previously reported (Photos 1–10). Therefore, we took our research one step further by planning a regression experiment. This study was performed by rearing young Japanese monkeys (2–3 years of age) on a vitamin-B6-deficient diet for 1 to 2 years, followed by 1.5 to 2 years on a controlled vitamin B6 supplemented diet (Table 1). The results indicated clear regression on arteriosclerosis in the various organs, especially the coronary and splenic arteries. This was the first regression experiment ever performed using monkeys and the results have been quoted in international journals and publications, including *Beyond Cholesterol*²⁾ and *Atherosclerosis Review*³⁾.

WHY DOES ARTERIOSCLEROSIS OCCUR IN B6 DEFICIENT MONKEYS?

We also attempted to answer the question, why arteriosclerosis occurs in B6 deficient monkeys, but there was a difficult barrier to overcome. Naturally, the serum cholesterol of the monkeys did not increase only because of vitamin B6 deficiency (Table 2).^{4,5,6)} Since it was thought at the time that thrombi were formed in various arteries, it appeared that there was a

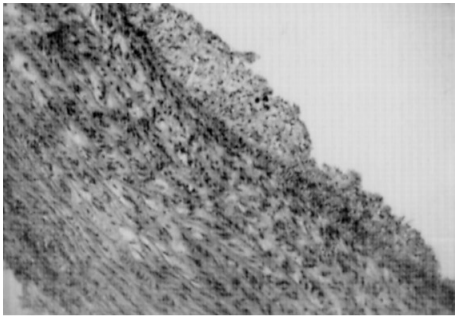


Photo 1. Lipid staining of the thoracic aorta (Very little lipid)

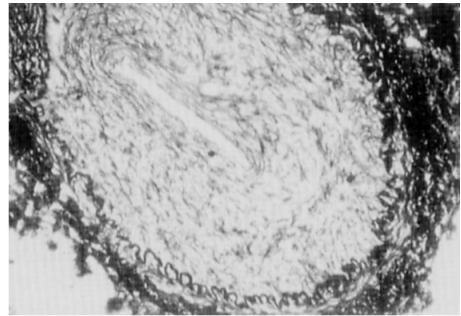


Photo 2. Intraperitoneal artery (Elastica-van Gieson staining)

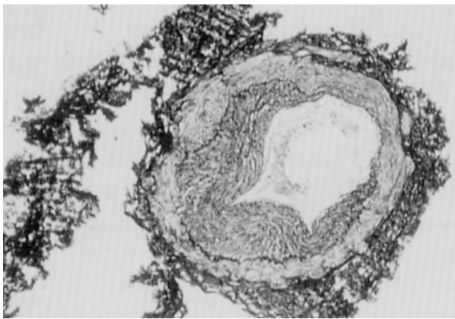


Photo 3. Intraperitoneal artery (Elastica-van Gieson staining)

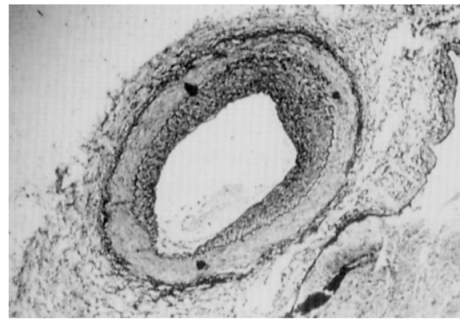


Photo 4. Iliac artery (Elastica-van Gieson staining)

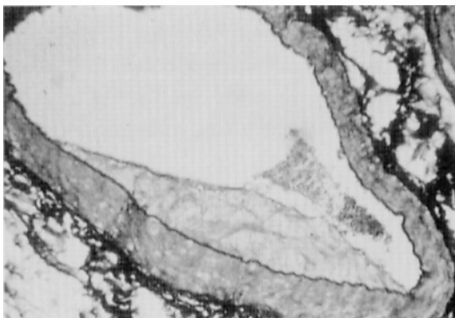


Photo 5. Coronary artery (Elastica-van Gieson staining)

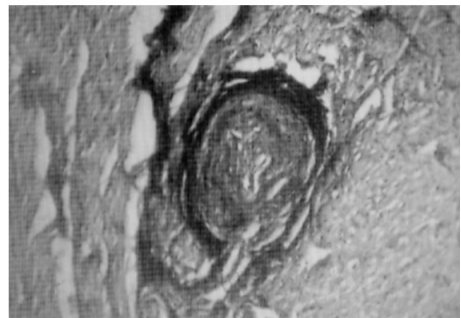


Photo 6. Coronary artery in the myocardium (Elastica-van Gieson staining)

VITAMIN B6 AND ARTERIOSCLEROSIS

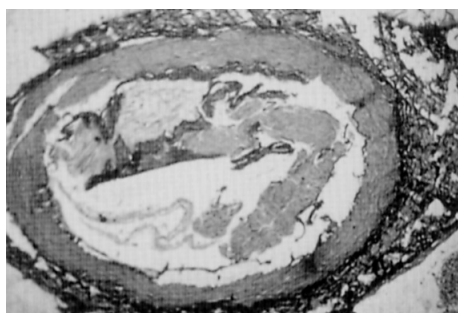


Photo 7. Thrombus in the common iliac artery (Elastica-van Gieson staining)

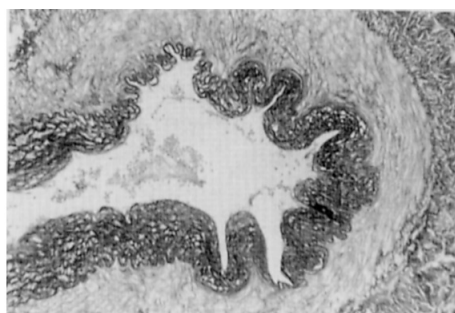


Photo 8. Renal artery (Elastica-van Gieson staining)

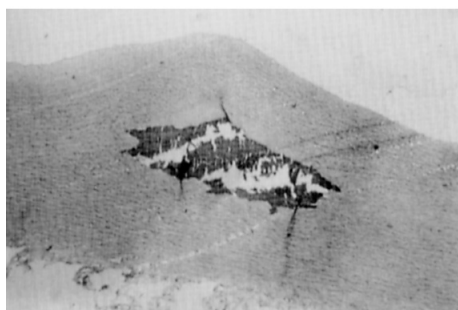


Photo 9. Thoracic aorta (Toluidine blue staining) (calcification)

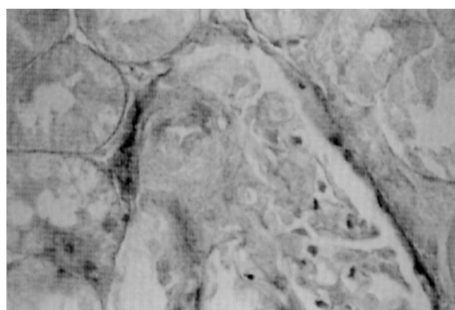


Photo 10. Renal glomerulus (Elastica-van Gieson staining)

Table 1. Arterial Lesions in Pyridoxine-Deficient Monkeys and Their Recovery

	No.	Sex	Exper. Period (months)	Serum Cholest mg/dl	A/G	Aorta		Common iliac	Coronary	Kidney		Spleen		Cerebrals	
						Thorac	Abd			Large Artery	V.aff V.eff	Large Artery	Central	Basiral	Intra-cereb.
Deficiency	1	♂	10			+	+	+	+	+	-	+	+	+	-
	2	♂	10			-	+	+	+	+	-	-	+	-	-
	3	♀	12	192	0.39	+	+	+	+	+	+	+	+	-	-
	4	♂	12	89	0.63	-	+	+	+	+	-	-	+	-	-
	5	♂	12	116	0.70	-	+	+	+	+	-	-	+	-	-
	6	♂	14	113	0.68	-	+	+	+	+	+	+	+	+	+
	7	♂	15	141	0.67	-	+	+	+	+	+	+	+	-	+
	8	♀	16	100	0.71	+	+	+	+	+	+	+	+	+	+
Control	1	♂	12	121	1.09	-	-	-	-	-	-	-	-	-	-
	2	♀	12	154	1.05	-	-	-	-	-	-	-	-	-	-
	3	♀	15	134	1.00	-	-	-	-	-	-	-	-	-	-
	4	♀	15	153	0.91	-	-	-	-	-	-	-	-	-	-
Recovery			Def.	Cont.											
	1	♂	12	2	121	1.00	-	+	+	-	+	-	-	+	+
	2	♂	12	6	114	0.99	-	+	+	-	+	-	-	+	-
	3	♂	12	12	97	2.69	-	+	-	-	+	-	-	-	-
	4	♀	14	24	92	1.10	-	+	+	-	+	-	-	-	-

+ : Lesion - : No lesion A/G : Albumin/globulin

relationship between vitamin B6 deficiency and the blood coagulation-fibrinolysis system. Therefore, it was evident that the plasma fibrinolytic system was reduced in vitamin-B6-deficient monkeys (Figs. 1 and 2). This led to the discovery of a relationship between thrombin and vitamin B6, i.e., it was found that vitamin B6 has antithrombin activity.⁷⁾ At the time, the same problem was also being studied in the United States. McCully reported that when rabbits were given large doses of homocysteine and methionine, many thrombi were formed in various arteries, especially those in the lungs, and that such thrombosis could be prevented by vitamin B6.⁸⁾ We confirmed these results in ensuing experiments.⁹⁾ It was originally reported that arteriosclerosis appeared at an early stage in patients with homocysteinuria, and this causal relationship has been clarified. Therefore, international competition in this field of research has become more heated. We found that homocysteine alone can cause platelet aggregation.⁹⁾ This was the first research of its type in the world. We also discovered that vitamin B6 blocks platelet aggregation by thrombin.¹⁰⁾

Table 2. Serum Lipogram of Pyridoxine-Deficient Monkeys

	Sex	Experi. Period months	Serum Total Cholest. mg/dl	Serum Free Cholest. mg/dl	Serum Lipoid -P mg/dl	C/P	β -Lipo. Index	Trigly. mg/dl
Deficiency	♀	12	192		282.2	0.68		
	♂	12	89	16.3	128.4	0.71	0.25	142.7
	♂	12	116	15.4	94.0	1.23	0.28	374.1
	♂	14	113	20.2	151.4	0.75		
	♂	15	141	25.8	153.7	0.92		
Control	♂	12	121	18.8	147.1	0.82		
	♀	12	154	39.3	108.7	1.42	0.24	101.3
	♀	12	134	19.1	112.2	1.19	0.27	112.5
	♀	15	153	18.8	154.1	0.99		

C/P: Serum total cholesterol/serum total phospholipid

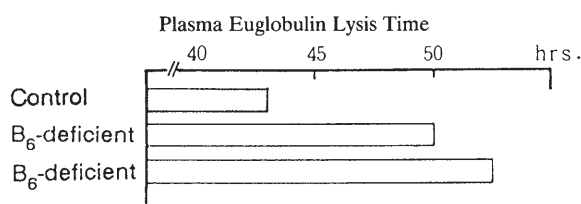


Fig. 1. Fibrinolytic activity of pyridoxine-deficient monkeys

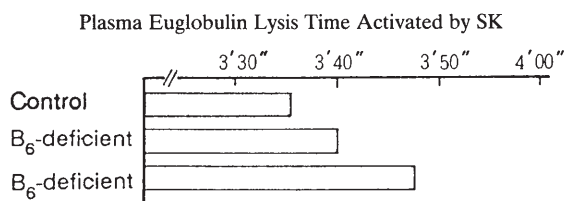


Fig. 2. Fibrinolytic activity of pyridoxine-deficient monkeys

RELATIONSHIP BETWEEN THE FREE RADICAL THEORY OF AGING AND ARTERIOSCLEROSIS IN VITAMIN B6 DEFICIENCY

In the United States and Europe, the lipid or cholesterol theory of arteriosclerosis, which emphasizes the epidemiological relationship between serum cholesterol and arteriosclerosis, is still widely accepted. However, attention is now focused on the relationship between denatured LDL, especially oxidized LDL, and the arteriosclerosis theory of Steinberg et al¹¹⁾, and the Beyond Cholesterol Theory¹²⁾ is again being considered. Native LDL directly contributes very little to atherosclerosis. Interest is directed instead toward denatured LDL, i.e., native LDL is not part of the scavenger pathway and it has been discovered that denatured LDL or VLDL plays the main role in the development of arteriosclerosis. Therefore, the type of denatured LDL actually present at the arteriosclerotic site is the problem, and at present, oxidized LDL is in the limelight.¹³⁾ There is also evidence that LDL is oxidized merely by contact with vascular endothelial cells.¹⁴⁾ The presence of oxidized LDL in atherosclerotic foci has been immunologically confirmed.¹³⁾ We became very interested in the relationship between oxidized LDL, homocysteine and vitamin B6. Vitamin E and probucol have been used as antioxidants, but it has also been found that vitamin B6 has antioxidant action.¹⁵⁾ Recently, Mino et al. reported that homocysteine produces free radicals when conjugated with metal ions.¹⁶⁾ Therefore, it is possible that the action of homocysteine on platelets, which we previously reported, can be explained by free radicals. In 1972, we revealed that H₂O₂ itself causes platelet aggregation.¹⁷⁾ These results were able to explain the Beyond Cholesterol Theory.

COLLAGEN METABOLISM AND VITAMIN B6

Vitamin B6 may be related to arteriosclerosis by means of some mechanism of action on collagen metabolism. With the recent progress made in biochemical research on connective tissue, we have obtained some interesting findings. These include the results that vitamin B6 is a leading activator of lysyl oxidase, an essential enzyme in the cross-linking of collagen and elastin; dissecting aortic aneurysms caused by inhibition of this enzyme activity by β -aminopropionitrile have been seen in rats. With respect to the above-mentioned enzyme inhibition, it has been found that β -aminopropionitrile forms a conjugate with pyridoxal-5'-phosphate (PAL-P), the active form of vitamin B6, and its action is inhibited by PAL-P.¹⁸⁾

It was found at the same time that the formation of dissecting aortic aneurysms due to β -aminopropionitrile was inhibited by the administration of large doses of vitamin B6.¹⁹⁾ This crosslinkage impairment presents a very important problem not only in the final stage of arteriosclerosis but also at its onset. It has been reported that the onset of arteriosclerosis is also caused by the administration of allylamine, but it was thought that this phenomenon is caused by formation of the same type of complex between allylamine and pyridoxal-5'-phosphate.²⁰⁾, as well as by the platelet aggregation induced by allylamine itself.¹⁰⁾ This platelet aggregation activity was found at the same time to be inhibited by pyridoxal-5'-phosphate.²⁰⁾

RELATIONSHIP BETWEEN DIABETIC VASCULAR COMPLICATIONS AND VITAMIN B6, ESPECIALLY GLYCATION AND VITAMIN B6

It is clear that vitamin B6 plays an important role in both the progression of arteriosclerosis and in its suppression, but the relationship of vitamin B6 with diabetes, which is often clinically associated with arteriosclerotic lesions, has not been explored. Several mechanisms for the onset

of diabetic vascular complications have been proposed. One of them involves polyol metabolism, and another important mechanism is the nonenzymatic glycosylation of protein. This nonenzymatic glycosylation reaction has been reported to progress freely after it starts and is a cause of the onset of the characteristic complications of diabetes.

With respect to the onset of arteriosclerosis, no glycated LDL receptors have been found. Therefore, taking of it into the liver will be delayed, and will take place only in the peripheries so that its half-life in the body is prolonged; this becomes one of the causes of the onset of arteriosclerosis.²¹⁾ This reaction (glycation) is due to Schiff binding between the lysine of the protein and the aldehyde group of glucose. Fig. 3 shows the changes in optical density that occur in vitro because of the reaction between lysine and glucose, i.e., the browning phenomenon, but this phenomenon was found to be suppressed by the addition of pyridoxal-5'-phosphate as shown in Fig. 4.²²⁾

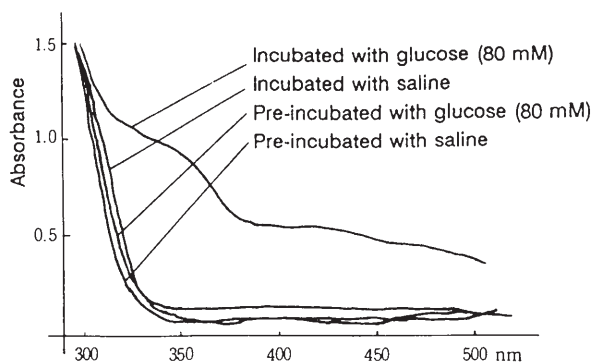
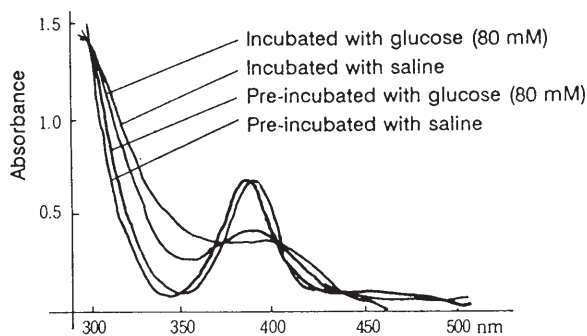


Fig. 3. Absorption spectra of lysine (50 μ M).
Browning phenomenon due to reaction between lysine and glucose



Absorption spectra of lysine (50 μ M) with pyridoxal phosphate (0.1 μ M)

Fig. 4. Inhibition of browning phenomenon by pyridoxal-5'-phosphate

The same inhibition was also found in the glycosylation of lipoprotein when pyridoxal-5'-phosphate was added as shown in Fig. 5.²³⁾ Based on this finding, an in vivo experiment was performed using NSY mice with congenital spontaneous-onset diabetes. When pyridoxal-5'-phosphate was administered daily to the mice (NSY), the amount of glycosylated protein (measured by fructosamine) in animals given pyridoxal-5'-phosphate was reduced to almost the

VITAMIN B6 AND ARTERIOSCLEROSIS

same level as that in the normal control group even though there was no difference in blood sugar level between the pyridoxal-5'-phosphate NSY group and the NSY control group given physiological saline.

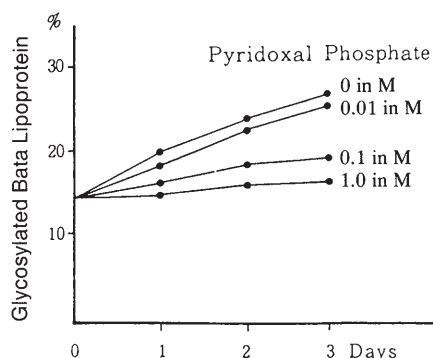


Fig. 5. Inhibition of glycosylation of β -lipoprotein by pyridoxal-5'-phosphate

The basement membrane thickening in the NSY mice administered physiological saline found by electron microscopic examinations of the kidneys as shown in photo 11 was inhibited in the group administered pyridoxal-5'-phosphate, as shown in photo 12. This means that it is possible to prevent vascular complication, mainly inhibition of glycosylation of protein and arteriosclerotic lesions, by administering pyridoxal-5'-phosphate in vivo. Therefore, vitamin B6 is considered to show important in vivo activities for the inhibition of arteriosclerosis via various mechanisms of action.



Photo 11 Electron micrograph of the kidney of a control NSY mouse administered physiological saline (Thickening of the basement membrane and deposits of a uniform structure are seen.)

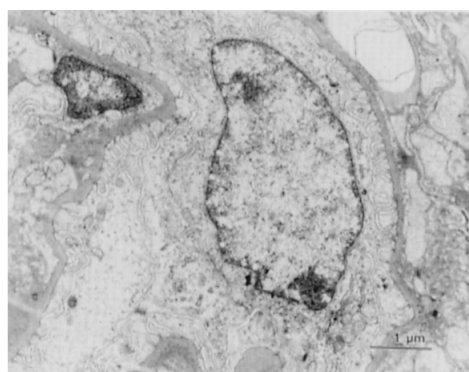


Photo 12 Electron micrograph of the kidney of an NSY mouse administered pyridoxal-5'-phosphate (Thickening of the basement membrane is generally absent.)

It is also known that fibrinogen which is considered to play an important role at the sites of arteriosclerosis, is subject to glycosylation, but it has recently been reported that active oxygen appears during such glycosylation of protein. It has also been found that vitamin B6, in addition to vitamin E and superoxide dismutase (SOD), acts as an inhibitor of active oxygen in the above-mentioned situation. This finding indicates the importance of the antioxidation action of vitamin B6 (Fig. 6.).

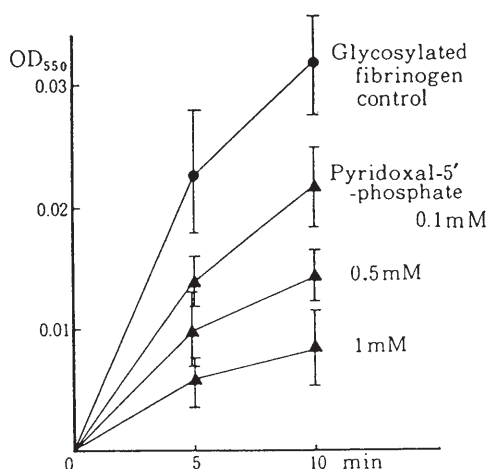


Fig. 6. Inhibitory effects of pyridoxal-5'-phosphate on superoxide production by glycosylated fibrinogen

CONCLUDING REMARKS

This paper outlines my long-term research on the relationship between vitamin B6 and arteriosclerosis. Since reading the report of Rinehart and Greenberg, I have been deeply interested in this work. I met Dr. Greenberg at the San Francisco Medical Center on my way back from studying in the United States. He said that since I was the only one in the world performing such research at the time, I should consider working with him in the United States. I was never able to return to the United States, but I plan to carry on with Dr. Greenberg's wishes in Japan. I do not know who will continue my work in the future, but I hope someone will.

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VITAMIN B6 AND ARTERIOSCLEROSIS

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