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Pyridoxine and Atherosclerosis: Role of Pyridoxine in the Metabolism of Lipids and Glycosaminoglycans in Rats Fed Normal and High Fat, High Cholesterol Diets Containing 16% Casein

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Abstract

The effect of administration of low and high doses of pyridoxine on the metabolism of lipids and glycosaminoglycans has been studied in rats fed normal and high fat, high cholesterol diets. Low doses of pyridoxine (0·005 mg/100 g body weight) caused increased concentrations of cholesterol and triglycerides in the serum and aorta in animals fed normal and high fat, high cholesterol diets. Administration of high doses of pyridoxine (5·0 mg/100 g body weight) caused decrease in the concentration of these lipids in these tissues except in the case of the aorta in the animals fed a normal diet.

Low doses of pyridoxine generally caused a decrease in the concentration of many glycosaminoglycan fractions in the aorta in rats fed normal and high fat, high cholesterol diets, whilst high doses caused an increase. The activity of glucosaminephosphate isomerase (glutamine-forming) and UDPglucose dehydrogenase, both key enzymes in the biosynthetic pathway of glycosaminoglycans, decreased in rats given low doses of pyridoxine and increased in rats given high doses. The activity of many enzymes concerned with the degradation of glycosaminoglycans—hyaluronoglucosidase, β-glucuronidase, β-*N*-acetylglucosaminidase, aryl sulphatase, and cathepsin D—generally increased in rats fed low doses of the pyridoxine and decreased in those given high doses. The concentration of hepatic 3′-phosphoadenosine-5′-phosphosulphate, and the activity of the sulphate-activating system and of aryl sulphotransferase decreased when the dose of pyridoxine was low and increased when the dose was high.

Introduction

The hypocholesterolaemic and anti-atherosclerotic action of vitamin A and ascorbic acid in experimental animals have been reported previously from this laboratory (Bala Nambisan and Kurup 1974; Sudhakaran and Kurup 1974). It has also been reported that vitamin D, on the other hand, increased the severity of atherosclerotic lesions in rats fed a high fat, high cholesterol diet (Vijayakumar and Kurup 1974). Pyridoxine is another vitamin whose effect in atherosclerosis has been the subject of some investigation, but results reported have not been in agreement. Many of these reports generally indicate increased cholesterol in the serum and aorta in pyridoxine-deficient animals (Goswami and Sadhu 1960; Seronde 1960; Shah *et al.* 1960; Daghir and Belloun 1963; D'Andrea and Mastrovilli 1965; Ghosal and Sadhu 1966; Bonollo and Nicoles 1967) and decreased serum cholesterol in atherosclerotic patients on administration of pyridoxine (Fujii and Motoyasu 1963; Motoyasu 1963; Zubova 1964; Bobkova 1965; Ghosal and Sadhu 1966; Khasanova and Chernaya 1967). There are also other reports that pyridoxine administration did not produce any hypocholesterolaemic effect (Fidanza *et al.* 1962, 1963; Lupien 1968).

It is known that the metabolism of glycosaminoglycans is also deranged in atherosclerosis in addition to that of lipids. A decrease in the concentration of sulphated glycosaminoglycans has been reported in the aorta of atheromatous rats from this laboratory (Vijayakumar *et al.* 1975) and this decrease has further been shown to be due to decreased synthesis and increased degradation of these macromolecules (Vijayakumar and Kurup 1975). Vitamins are known to have an effect on the metabolism of glycosaminoglycans and the roles of vitamins A, D, and C have been reported previously from this laboratory (Bala Nambisan and Kurup 1974; Sudhakaran and Kurup 1974; Vijayakumar and Kurup 1974). Very little information is available on the role of pyridoxine in this connection, in spite of the reports on its anti-atherogenic action. The only information available is an early report by Bombardella and Marin (1960) that administration of pyridoxine resulted in considerable increase in acid mucopolysaccharides in the arterial wall of rats.

In view of the above findings the effect of administration of low and high doses of pyridoxine on the concentration of different glycosaminoglycan fractions of the aorta has been studied in rats fed normal and high fat, high cholesterol diets. Some of the enzymes concerned with biosynthesis of precursors of glycosaminoglycans and degradation of glycosaminoglycans as well as those involved in biological sulphation have also been studied. In addition, the concentration of lipids in the tissues has been investigated.

Materials and Methods

Male albino rats (Sprague–Dawley strain, weight 110–115 g) were divided into six groups of 20 rats each as follows:

Normal Diet

Group I. Adequate dose of pyridoxine (0·5 mg/100 g body weight)
Group II. Low dose of pyridoxine (0·005 mg/100 g body weight)
Group III. High dose of pyridoxine (5·0 mg/100 g body weight)

High Fat, High Cholesterol Diet

Group IV. Adequate dose of pyridoxine (0·5 mg/100 g body weight)
Group V. Low dose of pyridoxine (0·005 mg/100 g body weight)
Group VI. High dose of pyridoxine (5·0 mg/100 g body weight)

Diets

The normal diet used had the following composition (g/100 g): dextrin, 68; casein (vitamin- and fat-free), 16; ground nut oil, 8; salt mixture, 4; vitamin mixture, 1; cellulose, 3.

The high fat, high cholesterol diet used contained (g/100 g): dextrin, 59; casein (vitamin- and fat-free), 16; coconut oil, 15; cholesterol, 2; salt mixture, 4; vitamin mixture, 1; cellulose, 2·5; sodium cholate, 0·5.

The salt mixture used had the following composition (g/kg diet): NaCl, 105; KCl, 120; KH_2PO_4, 310; $Ca_3(PO_4)_2$, 149; $CaCO_3$, 210; $MnSO_4$ (anhydrous), 0·20; $K_2Al_2(SO_4)_4.24H_2O$, 0·09; $MgSO_4$ (anhydrous), 90; $FePO_4.4H_2O$, 14·7; $CuSO_4.5H_2O$, 0·39; NaF, 0·57; KI, 0·05. In addition, the following trace elements were also added (mg/kg diet): $ZnCl_2$, 15; $CoCl_2.6H_2O$, 0·15.

The vitamin mixture used contained (mg/100 g diet): thiamine, 0·8; riboflavin, 0·8; niacin, 5·0; calcium pantothenate, 4·0; inositol, 20·0; choline chloride, 200; folic acid, 0·4; B_{12}, 2·0 μg; biotin, 20 μg; retinyl acetate, 1000 i.u.; ergocalciferol, 150 i.u.; α-tocopherol, 12; and menadione, 0·3.

The protein intake in the diet was kept at a suboptimum, constant level (16%) for the rats of all the groups. Pyridoxine was administered orally by tube. The animals were fed the respective diets for 3 months. At the end of this period the animals in each group were stunned by a blow at the back of the neck and killed by decapitation. The tissues were quickly removed to ice-cold containers for analyses.

Estimations of lipids

Extraction of the tissues for lipid estimation and estimation of total cholesterol, phospholipids, and triglycerides in the lipid extract were carried out as described previously (Sudhakaran and Kurup 1974).

Estimation of Glycosaminoglycan Fractions in the Aorta and Liver

The procedure for the estimation of glycosaminoglycan fractions in the aorta and liver has been described previously (Sudhakaran and Kurup 1974). Papain digest of the dry, defatted tissue was passed through a column of cellulose (microcrystalline, chromatography grade, E. Merck, Germany) previously washed with 1% cetyl pyridinium chloride (CPC) solution. The different glycosaminoglycan fractions—1, hyaluronic acid (HA); 2, heparin sulphate (HS); 3, chondroitin 4-sulphate (Ch 4-S); 4, chondroitin 6-sulphate (Ch 6-S); 5, dermatan sulphate (DS); and 6, heparin (H)—were eluted according to the procedure of Svejcar and Robertson (1967). The individual fractions were quantitated by the estimation of uronic acid by the modified carbazole reaction of Bitter and Muir (1962). Further analysis of the fractions by the enzymic method of Murata and Yoshima (1971) in a previous experiment has shown that fraction 2 contained mostly HS contaminated with small quantities of Ch 4-S; fraction 3 had Ch 4-S as the major fraction with traces of Ch 6-S; fraction 4 contained Ch 6-S with small quantities of DS; and fraction 5 was mostly DS with traces of H. HA and H were found, by cellulose acetate electrophoresis, to be mostly uncontaminated. Since these procedures are too tedious for routine analysis of a large number of samples, the analysis was restricted to the chromatography of the CPC complex over cellulose. The designation of the fractions as HS, Ch 4-S, Ch 6-S and DS means that these are the major components of the respective fractions. The identity of the major glycosaminoglycans in each fraction was confirmed by comparison with standard glycosaminoglycan preparations.

Estimation of Enzyme Activities

The activity of glucosaminephosphate isomerase (glutamine-forming) (EC 5.3.1.19) in the liver and aorta was estimated according to the procedure of Pogell and Gryder (1957). The activity of UDPglucose dehydrogenase (EC 1.1.1.22) was determined according to the procedure of Strominger *et al.* (1957).

For the estimation of degrading enzymes, the tissue was homogenized in aqueous 1% Brij 35 solution and the supernatant diluted with an equal volume of appropriate double strength buffer. The activities of β-glucuronidase (EC 3.2.1.31) and β-*N*-acetylglucosaminidase (EC 3.2.1.30) were estimated according to the procedure described by Kawai and Anno (1971) using *p*-nitrophenyl β-D-glucuronide and *p*-nitrophenyl β-*N*-acetylglucosaminide respectively as substrates with the modification that citrate buffer (0·1 M, pH 4·5) was used in place of acetate buffer for β-*N*-acetylglucosaminidase. The activity of arylsulphatase (EC 3.1.6.1) was estimated according to the procedure described by Roy (1953) using 4-nitrocatechol sulphate as substrate. Hyaluronoglucosidase (EC 3.2.1.35) was assayed as described by Kawai and Anno (1971) using hyaluronic acid (sodium salt) as substrate and estimating the *N*-acetylhexosamine liberated by the method of Reissig *et al.* (1955) (pH of the acetate buffer was 3·8 in the case of the enzyme activity of liver). The activity of cathepsin D (EC 3.4.23.5) was estimated by using 4% haemoglobin in 0·1 M acetate buffer (pH 4·5) as the substrate and determining the amount of tyrosine liberated by the method of Folin and Ciocalteu (1927).

Sulphate Metabolism

The concentration of PAPS (3′-phosphoadenosine-5′-phosphosulphate), and the activity of the sulphate-activating system, which includes sulphate adenylyltransferase (EC 2.7.7.4), adenylylsulphate kinase (EC 2.7.1.25) and aryl sulphotransferase (EC 2.8.2.1) of the liver were estimated by the method of Vankempen and Jansen (1972, 1973) using methyl umbelliferone. Details of the procedure have been described previously (Sudhakaran and Kurup 1974). Protein was estimated after trichloroacetic acid precipitation by the method of Lowry *et al.* (1952).

Statistical Analysis

The data given in the tables are the means for the number of animals used in each case ± s.e.m. Statistical significance was calculated using Student's *t*-test (Bennett and Franklin 1967).

Results

Weight Gain

Data on body weight gain and liver weight in the animals of the various groups are given in Table 1. The lower weight gain observed is due to the suboptimum protein level in the diet. Thus the results discussed in this paper pertain to the effect of variation in dietary pyridoxine under conditions of suboptimum protein intake. A low dose of pyridoxine showed a significantly lower gain in weight only in rats fed the normal diet, and not in rats fed the high fat, high cholesterol diet.

The diet consumption was comparable in the animals of groups I, II and III ($10 \pm 0 \cdot 8$ g/day) and also in animals of groups IV, V and VI ($10 \pm 1 \cdot 2$ g/day).

Table 1. Body weight gain and liver weight in the six groups of rats

Rats were maintained on the respective diets for 3 months. Values given are the means for 20 rats in each group ±s.e.m.

Group	Weight gain (g)	Liver weight (g)	Group	Weight gain (g)	Liver weight (g)
I	$83 \cdot 0 \pm 5 \cdot 8$	$6 \cdot 2 \pm 0 \cdot 3$	IV	$127 \cdot 0 \pm 6 \cdot 7$	$7 \cdot 1 \pm 0 \cdot 3$
II	$57 \cdot 0 \pm 3 \cdot 4$	$5 \cdot 3 \pm 0 \cdot 4$	V	$120 \cdot 0 \pm 8 \cdot 2$	$6 \cdot 7 \pm 0 \cdot 4$
III	$87 \cdot 0 \pm 4 \cdot 3$	$6 \cdot 4 \pm 0 \cdot 3$	VI	$132 \cdot 0 \pm 8 \cdot 1$	$7 \cdot 9 \pm 0 \cdot 5$

Effect of Diet and Pyridoxine Dose on Serum and Tissue Lipid Content

Results are given in Table 2. Administration of a dose of pyridoxine 100 times lower than normal to rats fed the normal diet caused a significant increase in total cholesterol only in the serum, aorta, and lungs; a decrease in phospholipids only in the serum, aorta, kidney, lungs, and skin; and a significant increase in triglycerides in the serum and all tissues studied except the skin. Administration of a high dose of pyridoxine 10 times the normal dose resulted in a significant decrease in total cholesterol in the serum, liver, heart, and skin; a significant increase in phospholipids only in the liver; and a significant decrease in triglycerides only in the serum, liver, lungs, brain, and skin.

Feeding the rats a high fat, high cholesterol diet for 3 months resulted in a significant increase in total cholesterol and triglyceride in the serum and the other tissues while phospholipids increased in the serum, liver and aorta and decreased significantly in the kidney, lungs and brain. Administration of the low dose of pyridoxine to those rats fed the high fat, high cholesterol diet caused a further increase in cholesterol in the serum, liver, aorta, kidney, lungs, and skin; a significant decrease in phospholipids in the serum, liver, aorta, and heart, and a further decrease in the kidney and brain; and a further increase in triglycerides in the serum and other tissues except the skin. Administration of the high dose of pyridoxine caused a significant decrease in cholesterol in the serum, liver, aorta, heart, kidney, and lungs, and a significant decrease in triglycerides only in the serum, aorta, brain, and skin. On the other hand, there was a further increase in phospholipids in the liver and aorta and, in contrast with the effect of the high fat, high cholesterol diet, also an increase in the kidney and lungs. There was also an increase in phospholipids in the skin.

Administration of a dose of pyridoxine 10 times higher than normal when compared to a dose 100 times lower than normal significantly decreased the levels of

Table 2. Concentrations of cholesterol, phospholipids and triglycerides in various tissues of rats fed various doses of pyridoxine

Values for serum are expressed as mg/100 ml; values for all other tissues are expressed as mg/100 g wet weight of tissue. In the case of the triglycerides, values for tissues are expressed as mg glycerol/100 g wet weight of tissue. Each value is the mean for 10 rats in each group ±s.e.m. Groups II and III have been compared with group I, and groups V and VI have been compared with group IV: A, $P < 0{\cdot}01$; B, $0{\cdot}01 < P < 0{\cdot}05$. Group II has also been compared with group III, and group V with group VI: a, $P < 0{\cdot}01$; b, $0{\cdot}01 < P < 0{\cdot}05$. No symbol indicates no significant difference

Group	Serum	Liver	Aorta	Heart	Kidney	Lungs	Brain	Skin
				(*a*) Cholesterol				
I	74·3±1·6	389±19	181±6	220·7±5·7	360·0±15·0	380·0±10·0	1220±57	310±13
II	97·5±3·8^{A}	374±23	241±12^{A}	231·6±9·6	386·0±12·0	613·8±15·2^{A}	1134±36	306±7
III	65·1±3·0^{B,a}	200±6^{A,a}	163±10^{a}	160·7±5·9^{A,a}	352·6±10·0	401·5±16·4^{a}	1114±46	218±8^{A}
IV	192·0±8·1	4039±109	325±14	411·8±8·2	485·2±17·9	559·0±21·0	1805±87	431±10
V	276·3±6·7^{A}	5869±269^{A}	699±18^{A}	405·6±11·0	576·2±24·7^{B}	778·0±22·0^{A}	1923±56	769±34^{A}
VI	164·3±4·8^{A,a}	2290±84^{A,a}	279±8^{B,a}	210·9±7·9 ^{A,a}	378·6± 7·9^{A,a}	477·4±20·0^{B,a}	1722±64^{b}	424±13^{a}
				(*b*) Phospholipids				
I	132·6±4·2	2180±109	1190±30	1920±56	2210±66	2150±67	3480±129	414·8±15·7
II	110·5±5·9^{B}	2094±67	710±32^{A}	1817±62	1740±61^{A}	1757±46^{A}	3162±79	301·4±8·1^{A}
III	136·8±5·1^{A}	2899±130^{A,a}	1320±76^{a}	1850±91	2275±91^{a}	2006±92^{b}	3620±156^{b}	435·0±17·8^{a}
IV	195·0±4·4	4255±106	2843±113	1819±52	1756±81	1619±81	2961±70	399·0±18·3
V	177·1±5·2^{B}	2888±118^{A}	2059±51^{A}	1590±68^{B}	1062±33^{A}	1589±45	2370±111^{A}	381·3±9·5
VI	200·9±7·63^{b}	5509±181^{A,a}	5259±184^{A,a}	1921±63^{a}	2006±52^{B,a}	2120±83^{A,a}	3130±97^{a}	463·7±17·9^{B,a}
				(*c*) Triglycerides				
I	7·1±0·2	420±20	905±56	42·8±1·4	70·0±2·5	410·0± 9·2	117·2±5·3	352·0±9·5
II	10·6±0·5^{A}	590±23^{A}	1389±74^{A}	53·4±1·4^{A}	106·0±4·3^{A}	478·6±17·0^{A}	186·0±7·3^{A}	369·9±9·5
III	3·1±0·2^{A,a}	242±7^{A,a}	890±37^{a}	38·7±1·7^{a}	66·9±2·1^{a}	370·1±11·1^{B,a}	97·9±3·1^{B,a}	209·4±8·6^{A,a}
IV	10·9±0·3	1375±67	2520±85	111·8±2·5	101·0±3·1	630·2±25·0	192·7±7·9	563·0±20·3
V	14·2±0·4^{A}	1792±50^{A}	4634±97^{A}	212·3±7·2^{A}	146·9±6·9^{A}	729·0±23·0^{B}	230·1±5·1^{A}	616·0±25·9
VI	6·8±0·3^{A,a}	1254±39^{a}	1680±79^{A,a}	111·2±5·3^{a}	97·0±2·6^{a}	586·0±15·2^{a}	163·2±5·4^{B,a}	422·0±10·1^{A,a}

cholesterol and triglycerides in the serum and the other tissues studied in rats fed both the normal and the high fat, high cholesterol diet. On the other hand, the concentration of phospholipids was higher in the serum and other tissues in both diet groups (except in the heart in rats fed the normal diet).

Effect of Diet and Pyridoxine Dose on Aorta and Liver Glycosaminoglycans

Results are given in Table 3. Administration of a dose of pyridoxine 100 times lower than normal to rats fed the normal diet caused a significant decrease in the concentration of HS, Ch 6-S, and H in the aorta, but none of the glycosaminoglycans fraction was significantly altered in the liver. On the other hand, administration of a dose of pyridoxine 10 times higher than normal caused a significant increase in the concentration of HA, Ch 6-S, and DS in the aorta and in the concentration of HA, HS, Ch 6-S, and DS in the liver. Rats maintained on the high fat, high cholesterol diet for 3 months showed an increase in HA, Ch 4-S, DS, and H (which, however, was significant only in the case of the former two), a decrease in HS and Ch 6-S in the aorta, and an increase in all the fractions in the liver (which, however, was not significant in the case of HS and H). Administration of the low dose of pyridoxine to the rats fed the high fat, high cholesterol diet caused a further decrease in the concentration of HS and Ch 6-S and counteracted the increase in the other fractions in the aorta, causing a decrease (except in the case of H). In the liver all glycosaminoglycan fractions showed a further decrease. On the other hand, administration of a dose of pyridoxine 10 times higher than normal caused a further increase in the concentration of HA, Ch 4-S, DS, and H and counteracted the decrease in Ch 6-S, causing an increase in Ch 6-S in the aorta. In the liver the high dose of pyridoxine caused a further increase only in HA, Ch 6-S, and DS.

Administration of a dose of pyridoxine 10 times higher than normal, when compared to a dose 100 times lower than normal, caused a significant increase in the glycosaminoglycan fractions of the liver and aorta in both normal and high fat, high cholesterol diet groups (except Ch-4S in the aorta and liver, and H in the liver in the normal diet group).

Effect of Diet and Pyridoxine Dose on the Enzymes of Glycosaminoglycan Metabolism

(i) *Activity of some enzymes concerned with the biosynthesis of hexosamine and uronic acid precursors of glycosaminoglycans*

Results are given in Table 4. Administration of a dose of pyridoxine 100 times lower than normal to rats fed the normal diet caused a significant decrease in the activity of glucosaminephosphate isomerase (glutamine-forming) in the aorta but not in the liver. Hepatic UDP-glucose dehydrogenase activity was also significantly decreased. On the other hand, administration of a dose of pyridoxine 10 times higher than normal caused a significant increase in the activity of glucosaminephosphate isomerase in the aorta and liver and in the activity of hepatic UDPglucose dehydrogenase.

Feeding a high fat, high cholesterol diet for 3 months caused a significant decrease in the activity of these enzymes. Administration of the low dose of pyridoxine caused a further decrease in the activity of glucosamine phosphate isomerase in the aorta and in the activity of UDPglucose dehydrogenase in the liver. On the other hand, administration of the high dose of pyridoxine counteracted this decrease in these enzyme activities and caused an increase.

Table 3. Concentrations of glycosaminoglycan fractions in the aorta and liver in rats fed various diets of pyridoxine

Values are expressed as micrograms of uronic acid per gram dry defatted tissue. Each value is the mean for six rats in each group ±s.e.m. Groups were compared as in Table 2

Group	Aorta						Liver					
	HA	HS	Ch 4-S	Ch 6-S	DS	H	HA	HS	Ch 4-S	Ch 6-S	DS	H
I	524·0 ±10·6	1597 ±42	901 ±21	1276 ±40	976 ±36	964 ±25	133·0 ±4·1	280 ±13	163·1 ±7·5	159·0 ±9·1	142 ±7	173 ±10
II	531·4 ±18·0	1210^{A} ±39	893 ±26	952^{A} ±36	8991 ±23	8401^{B} ±29	136·7 ±3·7	269 ±13	160·0 ±7·9	169·7 ±10·4	154 ±9	164 ±10
III	$653·6^{A,a}$ ±18·9	1649^{a} ±64	970 ±28	$1399^{B,a}$ ±29	$1137^{A,a}$ ±34	1031^{a} ±40	$171·5^{A,a}$ ±6·0	$321^{B,a}$ ±9	171·0 ±8·0	$215·0^{A,b}$ ±11·2	$220^{A,a}$ ±8	176 ±9
IV	580·0 ±12·2	1420 ±68	982 ±26	1092 ±48	998 ±23	998 ±44	175·3 ±3·5	300 ±10	220·2 ±5·9	200·3 ±7·0	165 ±6	186 ±4
V	$425·6^{A}$ ±14·0	1082^{A} ±35	676^{A} ±32	816^{A} ±25	802^{A} ±44	988 ±22	$81·5^{A}$ ±2·4	122^{A} ±5	$172·6^{A}$ ±6·7	$126·1^{A}$ ±3·7	142^{B} ±5	138^{A} ±4
VI	$760·8^{A,a}$ ±20·8	1360^{a} ±33	$1260^{A,a}$ ±52	$1296^{A,a}$ ±38	$1262^{A,a}$ ±48	$1232^{A,a}$ ±46	$200·2^{B,a}$ ±7·4	288^{a} ±5	$244·6^{a}$ ±11·3	$234·0^{B,a}$ ±10·5	$323^{A,a}$ ±7	183^{a} ±9

(ii) *Activity of enzymes concerned with degradation of glycosaminoglycans*

Results are given in Table 5. Administration of a dose of pyridoxine 100 times lower than normal to rats fed the normal diet caused a significant increase only in the activity of β-*N*-acetyl glucosaminidase and arylsulphatase in the liver. On the other hand, administration of a dose of pyridoxine 10 times higher than normal caused a significant decrease in the activity of β-glucuronidase, hyaluronoglucosidase and cathepsin D. Compared to the low dose, administration of the high dose caused a significant decrease in the activity of β-*N*-acetylglucosaminidase, β-glucuronidase, hyaluronoglucosidase and cathepsin D.

Table 4. Activity of glucosaminephosphate isomerase and UDPglucose dehydrogenase in rats fed various doses of pyridoxine

Values given are the means for 10 rats in each group ±s.e.m. Groups were compared as in Table 2

Group	Isomerase[C]		Dehydrogenase[D]
	Aorta	Liver	
I	27·6±0·8	35·8±1·1	2333±63
II	11·2±0·4[A]	32·3±1·3	1867±118[A]
III	41·6±0·7[A,a]	50·3±2·6[A,a]	3809±225[A,a]
IV	18·6±0·9	23·8±1·0	1980±40
V	9·0±0·35[A]	23·3±0·6	1488±74[A]
VI	22·4±0·52[B,a]	94·3±3·0[A,a]	2764±74[A,a]

[C] Expressed as micromoles of hexosamine per hour per gram of protein.
[D] Expressed as units per gram of protein.

Feeding a high fat, high cholesterol diet caused a significant increase in the activity of these enzymes in the liver. Administration of a dose of pyridoxine 100 times lower than normal caused a further increase in the activity of these enzymes in the liver and a significant increase in the activity of these enzymes in the aorta. On the other hand, administration of a dose 10 times higher than normal caused a significant decrease in the activity of β-glucuronidase, β-*N*-acetylglucosaminidase and cathepsin D in the aorta only, and in the activity of hyaluronoglucosidase in the liver. All these enzyme activities were significantly lower in the rats receiving the high dose of pyridoxine when compared to those receiving the low dose.

(iii) *Sulphate metabolism*

The concentration of PAPS, and the activity of the sulphate-activating system and of aryl sulphotransferase is given in Table 6. Administration of a dose of pyridoxine 100 times lower than normal to rats fed the normal diet caused a significant decrease in the concentration of hepatic PAPS, and a significant decrease in the activity of the sulphate-activating system (which includes sulphate adenylyltransferase and adenylylsulphate kinase) and of aryl sulphotransferase. On the other hand, administration of a dose of pyridoxine 10 times higher than normal caused a significant increase in the concentration of PAPS and in the activity of the sulphate-activating system and of aryl sulphotransferase.

Maintaining the rats on a high fat, high cholesterol diet for 3 months resulted in a significant decrease in the concentration of PAPS and in the activity of the sulphate-activating system and of aryl sulphotransferase in the liver. Administration of the low

Table 5. Activities of hyaluronoglucosidase, β-glucuronidase, β-*N*-acetylglucosaminidase, arylsulphatase and cathepsin D in the liver and aorta of rats fed various doses of pyridoxine

Values given are the means for 20 rats in each group ±s.e.m. Groups were compared as in Table 2

Group	Hyaluronoglucosidase[C]		β-Glucuronidase[D]		β-*N*-Acetylglucosaminidase[D]		Arylsulphatase[E]		Cathepsin D[F]	
	Aorta	Liver	Aorta	Liver	Aorta	Liver	Aorta	Liver	Aorta	Liver
I	—	62·5 ±2·3	—	79·6 ±3·5	—	63·4 ±2·1	—	169·0 ±4·2	—	151·4 ±5·4
II	—	66·7 ±3·2	—	81·2 ±4·3	—	76·5 ±3·1[A]	—	192·4 ±5·7[A]	—	153·0 ±7·0
III	—	43·7 ±2·7[A,a]	—	59·7 ±3·6[A,a]	—	59·9 ±3·1[a]	—	178·1 ±7·1	—	129·0 ±3·7[A,a]
IV	72·1 ±2·5	98·6 ±3·7	123·7 ±6·1	108·3 ±7·7	103·4 ±3·3	147·3 ±4·3	227·0 ±5·2	305·6 ±11·9	122·3 ±3·1	217·2 ±8·7
V	126·9 ±3·6[A]	152·1 ±7·1[A]	208·4 ±8·3[A]	207·1 ±9·7[A]	225·9 ±10·4[A]	344·5 ±14·5[A]	259·9 ±8·1[A]	442·1 ±20·3[A]	236·7 ±7·1[A]	320·7 ±14·4[A]
VI	72·9 ±3·0[a]	85·9 ±2·5[B,a]	102·5 ±3·3[B,a]	98·3 ±3·5[a]	77·5 ±1·8[A,a]	156·4 ±5·8[a]	224·5 ±9·0[b]	286·1 ±8·3[a]	73·3 ±2·6[A,a]	227·1 ±11·4[a]

[C] Expressed as milligrams of *N*-acetyl hexosamine liberated per hour per gram of protein.
[D] Expressed as milligrams of *p*-nitrophenol liberated per hour per gram of protein.
[E] Expressed as milligrams of nitrocatechol liberated per hour per gram of protein.
[F] Expressed as milligrams of tyrosine liberated per hour per gram of protein.

dose of pyridoxine to rats fed the high fat, high cholesterol diet caused a further decrease in the concentration of hepatic PAPS and in the activity of the sulphate-activating system. On the other hand, administration of a dose of pyridoxine 10 times higher than normal caused a significant increase in the concentration of PAPS and in the activity of the sulphate-activating system and of aryl sulphotransferase.

Table 6. Sulphate metabolism in rats fed various doses of pyridoxine

Values given are the means for 20 rats in each group ±s.e.m. Groups were compared as in Table 2

Group	Concn of PAPS[C]	Aryl sulpho-transferase[C]	Sulphate-activating system[C]
I	162·0±5·2	21·4±0·8	26·9±1·1
II	102·0±6·0[A]	14·6±0·7[A]	14·7±0·4[A]
III	227·0±10·1[A,a]	32·1±1·8[A,a]	44·1±2·7[A,a]
IV	49·0±1·1	12·2±0·6	16·9±0·6
V	26·6±0·9[A]	11·1±0·3	13·9±0·6[A]
VI	57·1±2·5[A,a]	32·1±1·1[A,a]	19·9±0·4[A,a]

[C] Estimated as micromoles of methyl umbelliferone sulphate produced per hour per gram of protein.

Discussion

Pyridoxine has a definite effect on the metabolsim of lipids and glycosaminoglycans under the conditions of suboptimum protein intake reported here.

While a low dose of pyridoxine increases or has no significant effect on the concentration of total cholesterol and triglycerides in the tissues, the concentration of phospholipids is decreased or not significantly altered. High doses of pyridoxine cause a decrease or no significant alteration in the concentration of cholesterol and triglycerides and an increase or no significant alteration in the concentration of phospholipids. Thus the effect of pyridoxine in some tissues appears to be opposite in the case of cholesterol and triglycerides on the one hand and the phospholipids on the other.

The aorta is particularly important from the point of view of lipid accumulation in atherosclerosis. The increase in the concentration of cholesterol and triglycerides in the aorta as well as in the serum in rats fed a dose of pyridoxine 100 times lower than normal may indicate that pyridoxine deficiency may be one of the factors involved in the pathogenesis of atherosclerosis, particularly along with a high fat, high cholesterol diet. The significant decrease in the concentration of these lipids in the aorta and serum on the administration of a dose of pyridoxine 10 times higher than normal shows that pyridoxine in high doses has a definite anti-atherosclerotic action.

The aorta is also of particular significance because of the high concentration of glycosaminoglycans present. Low doses of pyridoxine generally cause a decrease in the concentration of many of the glycosaminoglycan fractions in this tissue, whilst high doses of pyridoxine cause an increase. This observation is of significance from the point of view of the observed anti-atherosclerotic action of this vitamin. It has been reported from this laboratory that the sulphated glycosaminoglycan fractions of the aorta, after an initial increase, decreased with an increase in the severity of atheromatous lesions in rats maintained on a high fat, high cholesterol diet containing potassium perchlorate for 6 months (Vijayakumar *et al.* 1975). It is believed that

glycosaminoglycans may play an important role in lipid accumulation in tissues. Increased lipid accumulation has generally been reported to coincide with a decrease in the concentration of the glycosaminoglycans (Vijayakumar *et al.* 1975). High doses of pyridoxine appear to correct the decrease in the concentration of glycosaminoglycans in rats fed high fat, high cholesterol diet. Thus the anti-atherosclerotic action of this vitamin may also involve its effect on the concentration of glycosaminoglycans.

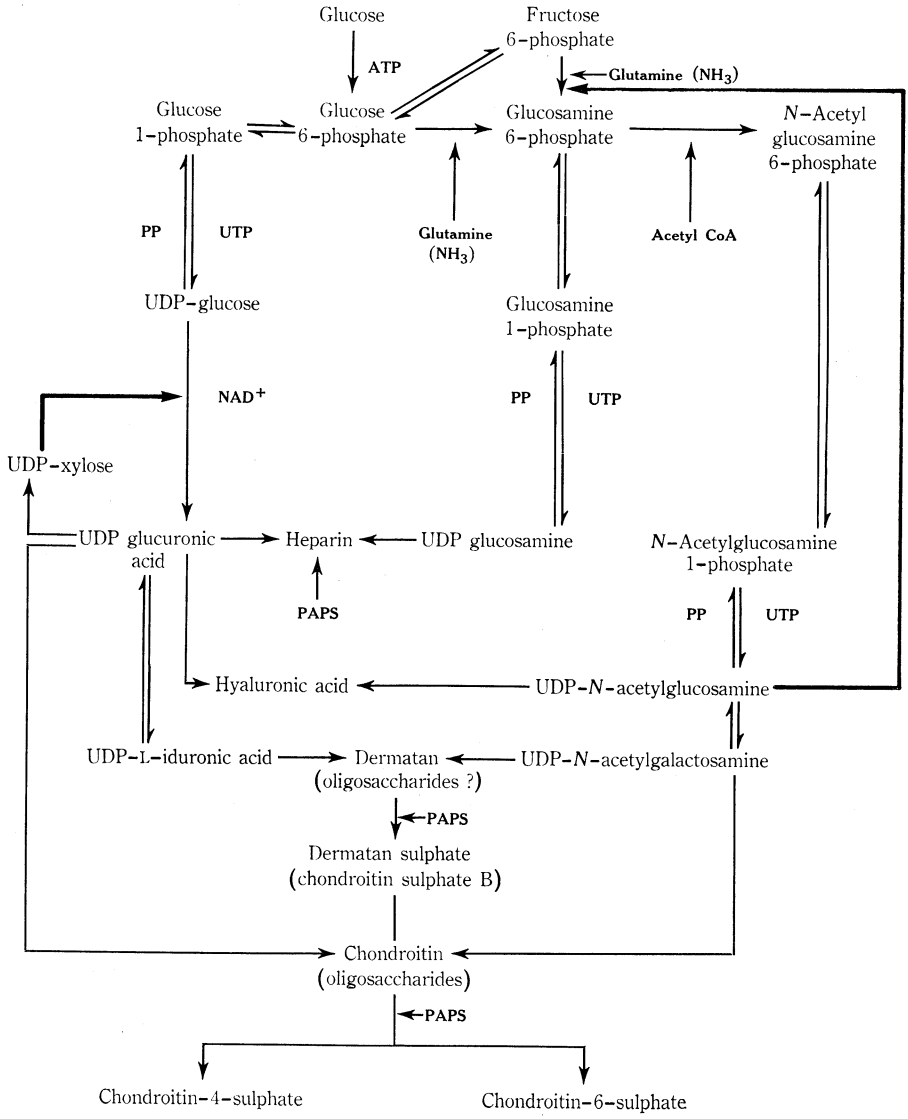

Fig. 1. Metabolic pathways of biosynthesis of glycosaminoglycans. Thick lines indicate feedback inhibition.

The pathway for the synthesis of glycosaminoglycans is given in Fig. 1. Glucosaminephosphate isomerase (glutamine-forming), which forms glucosamine-6-phosphate, is a key enzyme in the biosynthetic pathway of hexosamine precursors of

glycosaminoglycans. It is a site of metabolic regulation, since its activity is inhibited by feedback inhibition by UDP-*N*-acetylglucosamine. UDPglucose dehydrogenase, which forms UDPglucuronic acid, is an important enzyme in the biosynthetic pathway of uronic acid precursors of glycosaminoglycans. It is also considered to be another site of regulation of glycosaminoglycan synthesis, since its activity is inhibited by feedback inhibition by UDPxylose. The decreased activity of these enzymes in rats given a low dose of pyridoxine can result in decreased availability of hexosamine and uronic acid precursors for glycosaminoglycan synthesis whilst in the animals fed a high dose of pyridoxine the increased activity of these enzymes can result in increased availability of the precursors. Thus, synthesis of glycosaminoglycans may be decreased in rats receiving a low dose of pyridoxine and increased in the animals fed a high dose.

The activity of some of the enzymes concerned with degradation of glycosaminoglycans is generally decreased in rats fed a high dose of pyridoxine when compared to those receiving a low dose, resulting in decreased degradation of glycosaminoglycans in the animals of the former group. Hyaluronoglucosidase degrades HA, Ch 4-S and Ch 6-S but is without action on DS, HS and H. β-Glucuronidase and β-*N*-acetylglucosaminidase are both exoenzymes, splitting off glucuronic acid and hexosamine residues from the non-reducing end of glycosaminoglycans. Aryl sulphatases are non-specific enzymes which bring about desulphation whilst cathepsins cleave proteoglycans, the form in which glycosaminoglycans are present in the tissues and liberate the glycosaminoglycans. The concerted action of these enzymes result in the degradation of glycosaminoglycans. Thus the increased concentration of glycosaminoglycans in the animals fed a high dose of pyridoxine may be due to both increased synthesis and decreased degradation. In this connection a decrease in the activity of enzymes concerned with synthesis of glycosaminoglycans and an increase in the activity of enzymes concerned with degradation of glycosaminoglycans have been reported in atheromatous rats (Vijayakumar and Kurup 1975). Pyridoxine thus appears to correct the changes in these enzyme activities occurring in atheromatous rats and contributes towards maintaining normal concentrations of glycosaminoglycans.

Pyridoxine has significant effects on sulphate metabolism. The increased concentration of hepatic PAPS, the biological sulphate donor in rats fed a high dose of pyridoxine, and its decreased concentration in animals given a low dose of pyridoxine, may be due to the increased activity of the sulphate-activating system (sulphate adenylyltransferase and adenylylsulphate kinase which generate PAPS) in the former case and decreased activity in the latter case. The increased activity of aryl sulphotransferase in the livers of rats given a high dose of pyridoxine is either a reflection of increased availability of PAPS or a direct stimulating effect of pyridoxine on this enzyme. Even though the enzyme now studied is aryl sulphotransferase, PAPS is also the sulphate donor for the sulphation of glycosaminoglycans and any change in its concentration may affect sulphation of glycosaminoglycans. It is also possible that pyridoxine as pyridoxal sulphate may be involved in sulphate metabolism. This aspect is being investigated in detail.

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