Biochem. J. (1985) **229**, 153–160
Printed in Great Britain

Effect of vitamin B-6 (pyridoxine) deficiency on lung elastin cross-linking in perinatal and weanling rat pups

Barbara A. MYERS,* Michael A. DUBICK,* Robert D. REYNOLDS† and Robert B. RUCKER*
**Department of Nutrition, University of California, Davis, CA 95616, U.S.A., and †United States Department of Agriculture, Human Nutrition Research Center, Beltsville, MD 20705, U.S.A.*

(*Received 14 January 1985/4 March 1985; accepted 7 March 1985*)

Weanling and perinatal rats were rendered vitamin B-6 (pyridoxine)-deficient. The rat pups were nursed from vitamin B-6-deficient or -sufficient dams and were killed at day 15 after parturition. The weanling rats were fed vitamin B-6-deficient or -sufficient diets and were killed after 5 weeks of treatment. Lung elastin from the groups of rats was then studied with respect to its content of lysine-derived cross-linking amino acids. Lung lysyl oxidase activity was also measured. B-6 deficiency decreased the number of lysine residues in elastin that were converted into the cross-linking amino acid precursor allysine. However, a more significant defect in cross-link formation was an apparent block in the condensation steps leading to the formation of desmosine. Desmosine was decreased, with an increase in the amounts of aldol condensation products (aldol CP) in elastin. It is proposed that the elevation in aldol CP results from the formation of thiazines, which are produced from the reaction between aldehyde and homocysteine. The concentration of homocysteine is significantly elevated in vitamin B-6-deficient rats.

Elastin is a major contribution to the patency and mechanical properties of lung (Rucker & Dubick, 1984). It is first synthesized as a soluble protein, of M_r about 70000, and subsequently is cross-linked covalently to form insoluble fibres. The cross-links are derived from lysine residues, which are oxidatively deaminated to peptidyl-allysine residues (Siegel, 1979). This process may be manipulated by diet. Lysyl oxidase, responsible for the oxidation, is a copper-dependent enzyme associated with the extracellular matrix, and is sensitive to changes in dietary copper (Rucker & Dubick, 1984).

It has also been observed that vitamin B-6 deficiency influences the cross-linking of elastin and collagen (Hill & Kim, 1967; Starcher, 1969; Levene & Murray, 1977; Murray *et al.*, 1978; Fujii *et al.*, 1979; Bird & Levene, 1982, 1983). Specifically, pyridoxal 5'-phosphate is proposed as an additional cofactor for lysyl oxidase, since vitamin B-6 deficiency results in decreased lysyl oxidase activity and cross-linking.

Abbreviations used: aldol CP, aldol condensation products; PLP, pyridoxal 5'-phosphate; phosphate-buffered saline, 0.01 M-sodium phosphate (pH 7.6)/0.9% NaCl; OH-Nle, hydroxynorleucine.

The relationship between vitamin B-6 deficiency and cross-linking has also been proposed as important to the mechanistic understanding of the role of vitamin B-6 in atherosclerosis and related vascular diseases. Levene & Murray (1977) have suggested that vitamin B-6 deficiency early in life may predispose to vascular defects, because of altered elastin metabolism at key stages in vascular tissue development. For example, atherosclerotic lesions have been observed in young monkeys rendered vitamin B-6-deficient (Greenberg *et al.*, 1971).

Likewise, there has been an interest in the role of vitamin B-6 in lung development, since it has been demonstrated that selected features of lung development are correlated with the net production of elastin (Powell & Whitney, 1980; Dubick *et al.*, 1981; Nardell & Brody, 1982; Myers *et al.*, 1983*a*,*b*). Moreover, inhibition of elastin cross-linking as well as destruction of elastic fibres during early lung development in rodents results in emphysematous changes in lung (Kida & Thurlbeck, 1980; Kuhn & Starcher, 1980; O'Dell *et al.*, 1978).

In the present paper we consider possible mechanisms related to lung elastin cross-linking and vitamin B-6 deficiency. We propose that, in

vitamin B-6 deficiency, defects in cross-linking occur primarily at condensation steps involving allysine and related aldol CP. In many respects these cross-linking defects in lung elastin from vitamin B-6-deficient rats appear similar to those reported in homocystinuria (Kang & Trelstad, 1973) or penicillamine intoxication (Pinnell *et al.*, 1968). Both cystathionine β-synthase and cystathionase require pyridoxal 5'-phosphate (PLP) as a cofactor (Sturman, 1981; Finkelstein, 1974). Vitamin B-6 deficiency results in altered sulphur-amino-acid metabolism such that homocysteine is elevated.

Experimental

Materials

Time-mated pregnant rats (chronic-respiratory-disease-free) were purchased from Charles River Laboratories, Portage, MI, U.S.A. L-[G-^{3}H]Valine (sp. radioactivity, 12Ci/mmol), L-[U-^{14}C]lysine (sp. radioactivity 450mCi/mmol), L-[4,5-^{3}H]lysine (sp. radioactivity 70Ci/mmol) and NaB^3H_4 (sp. radioactivity 616mCi/mmol) were purchased from ICN Chemical and Radioisotope Division, Irvine, CA, U.S.A. L-[1-^{14}C]Tyrosine (sp. radioactivity 50mCi/mmol) was obtained from New England Nuclear Corp. (Boston, MA, U.S.A.). Tissue-culture supplies were from Grand Island Biochemical Co. (Grand Island, NY, U.S.A.). Reagents used for amino acid and desmosine analysis were obtained from Bio-Rad Laboratories (Richmond, CA, U.S.A.) or Pierce Chemical Co. (Rockford, IL, U.S.A.). Desmosine and isodesmosine are products of Elastin Products (St. Louis, MO, U.S.A.). All other reagents were of the highest grade commercially available.

Animals and diets

After parturition, lactating dams and their pups were randomly assigned (three to four each) to either the vitamin B-6-deficient or -sufficient dietary groups. The basal diet containing no added vitamin B-6 was based on vitamin-free casein (American Institute of Nutrition, 1977). The basal diet or the vitamin B-6-supplemented diet (as 7mg of B-6 hydrochloride/kg) was fed *ad libitum* and introduced 24h after parturition. A third group also consisting of three lactating dams and their pups were fed the supplemented diet, but in an amount daily no greater than that consumed by vitamin B-6-deficient lactating dams. Likewise, the diets were fed for 5 weeks to three groups of 4-week-old weanling rats consisting of 20 rats each (i.e. vitamin B-6-deficient; vitamin B-6-supplemented; vitamin B-6-supplemented but food-restricted).

The pups were killed 15 days after parturition by decapitation; adult rats were killed by injection of sodium pentobarbitol. Lungs were divided into five lobes and either used immediately or stored (−70°C). Blood was collected by heart puncture, dispersed into heparinized tubes and centrifuged at 3000*g* for 10min at 4°C. The plasma was then stored at −70°C until assayed.

Determination and isolation of insoluble elastin

Insoluble lung elastin was determined after extraction of lungs with phosphate-buffered saline, followed by 0.1M-NaOH (O'Dell *et al.*, 1978). Lungs were first homogenized in phosphate-buffered saline by using a high-speed tissue homogenizer for 30s (Tekmar Co., Cincinnati, OH, U.S.A.). The homogenate was then centrifuged (20min; 20000*g*; 4°C). The insoluble residue was extracted at 90°C for a total of 60min in 0.1M-NaOH. The resulting alkali-insoluble fraction was subsequently taken to represent elastin. To estimate elastin content, the alkali-insoluble fraction was hydrolysed under N_2 in 6M-HCl (72h; 104°C). Ninhydrin-reactive nitrogen was then quantified by using bovine ligamentum elastin as a standard (Lefevre & Rucker, 1980). Selected hydrolysates were also subjected to amino acid analysis to confirm the purity of the residue as insoluble elastin.

Assay for new insoluble-elastin accumulation

The rate of newly accumulated elastin was assayed by a method adapted from Collins *et al.* (1981). Left lobes from lungs of pups were minced into flasks containing Medium 199 with Hanks salts containing human α_1-antiproteinase inhibitor (25μg/ml), gentamycin sulphate (50μg/ml), streptomycin (100units/ml) and penicillin (100units/ml). A 9ml portion of medium was used/g of tissue. The minces were first incubated for 30min [37°C under O_2/CO_2 (19:1)] to adapt to the medium. The medium was then aspirated and replaced with medium of the same composition, but containing 20μCi of L-[G-^{3}H]valine/ml and incubated further for 3h. A portion (one-half of the lung mince) was then removed, washed in phosphate-buffered saline, and homogenized. It was determined in preliminary experiments that total protein synthesis was linear for up to 3–5h of incubation and that the free L-valine specific radioactivity reached equilibration after 20–30min. For these estimates, protein was precipitated in homogenates by the addition of sulphosalicylic acid to 6% (w/v). The precipitates were then washed and radioactivity determined. L-Valine and its associated radioactivity were also determined in the supernatant fraction (Myers *et al.*, 1983*b*).

For the remaining portion of minced lung, the radiochemically labelled medium was aspirated

and medium containing unlabelled 5mM-L-valine was added. This tissue was incubated for 24h to allow newly synthesized elastin to become cross-linked as insoluble elastin. To determine an elastin accumulation rate, the tissue was washed again in phosphate-buffered saline and homogenized. However, at this point the homogenate was chemically reduced with $NaBH_4$ to stabilize cross-links (detailed below) before isolation of insoluble elastin. Relative protein synthesis or the rate of new elastin accumulation was expressed as nmol of L-valine incorporated/g of lung per assay.

Radiochemical labelling of lung elastin cross-links with [^{14}C]lysine and NaB^3H_4

Whole lungs from the rat pups were incubated as described above, except lungs were incubated continuously in the presence of 25μCi of [U-^{14}C]lysine per ml of medium for 24h. After incubation the lungs were homogenized, centrifuged (10000***g***, 30min) and the resulting pellet washed twice in phosphate-buffered saline. The residue was then reduced with NaB^3H_4 to stabilize reversible cross-links and to label radiochemically reducible lysine-derived cross-links with ^{3}H for eventual quantification and identification (Paz *et al.*, 1976; Snider *et al.*, 1981). Briefly, each lung sample (approx. 400mg) was suspended in 4ml of phosphate-buffered saline. NaB^3H_4 was then added (60μCi/mg per ml of 1mM-NaOH) in four increments over a 30-min period. The mixture was maintained at pH7.0–9.0 and the reduction allowed to proceed for 90min at 4°C with occasional shaking. The reaction was stopped by acidification to pH5.0 with 50% (v/v) acetic acid and the samples were washed exhaustively with distilled water.

Quantification of radioactivity in [^{14}C]lysine- and NaB^3H_4-labelled lungs

Insoluble elastin was isolated and hydrolysed in 2M-KOH at 105°C for 22h. $HClO_4$ was added dropwise to pH3.0 at 4°C and the $KClO_4$ precipitate removed by centrifugation. The hydrolysates were applied to a Glenco amino acid analyzer (Spectrum Co., Houston, TX, U.S.A.) and the reduced cross-linking amino acids were separated by ion-exchange chromatography as described by Dubick *et al.* (1985). The column (0.325cm × 50cm) was packed with Bio-Rad Aminex A-9 resin. The amino acids were eluted by using a stepwise gradient consisting of 0.2M-sodium citrate, pH3.19, 0.2M-sodium citrate, pH4.25, 0.35M-sodium citrate, pH6.7, for 15min each followed by 0.35M-sodium citrate, pH7.9, plus 1.0M-NaCl for 70min at 50°C (flow rate 0.2ml/min). Fractions (1min) were collected for the various assays.

Lysyl oxidase activity

Lung lysyl oxidase activity was assayed as described by Chichester *et al.* (1981). Embryonic-chick aortae were used as the starting material for the lysyl oxidase substrate (Pinnell *et al.*, 1968). Each incubation mixture contained approx. 300000d.p.m. as L-[4,5-^{3}H]lysine incorporated into chick aorta matrix proteins. The reactions were stopped by the addition of trichloroacetic acid to 10% (w/v, final concn.). Trichloroacetic acid-soluble products were then passed through columns (1cm × 5cm) of Bio-Rad AG50W-X8 resin, and the effluent containing ^3H_2O was collected and radioactivity determined (Peterkofsky & DiBlasio, 1975).

PLP and plasma sulphur-amino-acid determinations

The plasma PLP concentration was determined as a measure of vitamin B-6 status in response to the diets. The apo-(tyrosine decarboxylase) method as modified by Sloger & Reynolds (1980) was used. For plasma amino acids, samples were deproteinized by the addition of sulphosalicylic acid to 6%. Plasma amino acids were then determined by using a standard program for physiological fluids on a Beckman amino acid analyser.

Desmosine and isodesmosine

The desmosine and isodesmosine in insoluble elastin fractions were measured by using the Glenco amino acid analyser after hydrolysis of elastin in 6M-HCl at 110°C for 72h (Lefevre & Rucker, 1980; Lefevre *et al.*, 1982).

Statistics

Data were analysed by using the Rummage II data-analysis system's one-way-analysis-of-variance model, and multiple comparisons between groups were made by using Tukey's *w* procedure (Steel & Torre, 1980).

Results

Vitamin-B-6 status in perinatal and young rats

Body and lung weights were decreased in both vitamin B-6-deficient and food-restricted (F.R.) rats compared with controls (Table 1). In the 15-day-old pups, decreased body weight was observed only in the F.R. group. However, plasma PLP concentrations were significantly decreased in B-6-deficient pups to less than one-tenth of normal values. Plasma PLP concentrations were less than 5% of normal values in weanling rats after 5 weeks of deprivation (also cf. Lumeng *et al.*, 1978; Li & Lumeng, 1981).

Table 1. *Dietary vitamin B-6 and measures of vitamin B-6 status in perinatal and young rats*
Lactating dams or weaning rats were fed vitamin B-6-deficient (B-6, def.) or -supplemented (B-6, suppl.) diets. Pups being suckled by the dams were killed at 15 days after parturition. F.R. (food-restricted) is a group fed *ad libitum* the vitamin B-6-supplemented diet, but at an amount daily equal to that consumed by the vitamin B-6-deficient group. Values represent means ± S.E.M. (nos. of observations). * and ** indicate values significantly different from those obtained for the vitamin B-6-supplemented group at $P<0.05$ and $P<0.01$ respectively. For details of plasma PLP assay, see the Experimental section.

Diet	Rat age	Body weight (g)	Lung weight (g)	Serum [PLP] (nM)
B-6, suppl.	15 days	27 ± 2 (13)	0.37 ± 0.03 (13)	484 ± 26 (5)
B-6, def.	15 days	24 ± 2 (14)	0.36 ± 0.03 (14)	30 ± 2 (5)**
F.R.	15 days	17 ± 1 (17)*	0.32 ± 0.01 (17)	239 ± 21 (6)**
B-6, suppl.	9 weeks	376 ± 25 (6)	1.55 ± 0.17 (6)	562 ± 45 (6)
B-6, def.	9 weeks	226 ± 12 (6)**	1.16 ± 0.11 (6)**	8 ± 2 (6)**
F.R.	9 weeks	208 ± 11 (6)**	1.01 ± 0.09 (6)**	289 ± 92 (6)**

Table 2. L-*Valine incorporation into lung protein and newly accumulated insoluble elastin*
Left lung lobes from 15-day-old rat pups were incubated for 3 h in the presence of L-[^{3}H]valine. After this initial pulse, portions of the lung lobes were chased in medium containing unlabelled L-valine for 24 h. The free L-valine specific radioactivity was determined after the 3 h incubation. These values were then used in estimating lung and new elastin accumulation. For details, see the Experimental section. Values represent means ± S.E.M. (nos. of observations). * and ** indicate values significantly different from those obtained for the vitamin B-6-supplemented group at $P<0.05$ and $P<0.01$ respectively. Treatment abbreviations are as in Table 1.

Treatment	10^{-4} × L-Valine radioactivity (d.p.m./nmol)	Net protein synthesis (μmol/3 h per lobe per assay)	New elastin accumulation (nmol/3 h pulse + 24 h chase per lobe per assay)	Elastin (% of total protein accumulation)
B-6 suppl.	3.46 ± 0.25 (5)	0.12 ± 0.01 (4)	0.78 ± 0.03 (11)	1.6
B-6 def.	3.63 ± 0.13 (6)	0.11 ± 0.01 (6)	0.29 ± 0.03 (12)**	0.7**
F.R.	3.91 ± 0.45 (6)	0.10 ± 0.01 (5)	0.57 ± 0.13 (10)	1.4

Insoluble-elastin accumulation

The concentration of elastin was not significantly influenced by vitamin B-6 intake (Fig. 1). The values for the three treatment groups were in keeping with previously reported normal values during different stages of rat lung development (Dubick *et al.*, 1981; Myers *et al.*, 1983*a*,*b*). In contrast, in lung explants from 15-day-old pups, new-insoluble-elastin accumulation (expressed as nmol of valine incorporated per left lobe), was decreased to 30% of the control value in the vitamin B-6-deficient group (Table 2). Note that there were no observed differences in the values for lung L-valine specific radioactivity or valine incorporation into total protein synthesis with respect to treatment (see the Discussion section).

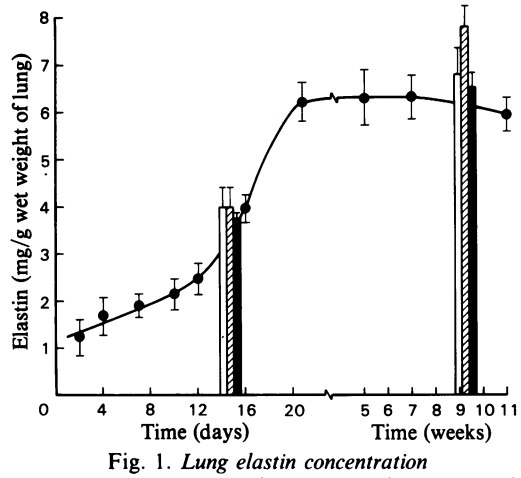

Fig. 1. *Lung elastin concentration*
●——●, Insoluble elastin concentration expressed as mg/g of wet lung (mean ± S.E.M.). ▨, Values for perinatal rat pups and young rats (9 weeks old) rendered vitamin B-6-deficient. □■, Values for supplemented rats fed *ad libitum* (□) or for which food was restricted (■).

Reduced elastin cross-link profiles and elastin desmosine content

Lung elastin aldol CP was increased twofold in the vitamin B-6-deficient 9-week-old rats relative to both the control and F.R. groups (Table 3). Consequently, the aldol CP/hydroxynorleucine (OH-Nle) ratio was significantly higher than normal in elastin samples from vitamin B-6-deficient rats. Total desmosine and lysine, expressed as residues/1000 total residues, were also significantly lower and higher respectively for vitamin B-6-deficient rats relative to the supplemented rats. This resulted in a significantly lower

Table 3. *Reduced elastin cross-link profiles and elastin desmosine content*

After reduction of lung homogenates with NaB^3H_4, elastin was isolated and hydrolysed. OH-Nle (hydroxynorleucine, i.e. reduced allysine) and reduced aldol CP were then separated by ion-exchange chromatography and radioactivity was determined. Total desmosine (Des) and lysine (Lys) residues were determined from acid hydrolysates of elastin. Values represent means ± S.E.M. (nos. of observations). * and ** indicate values significantly different from those obtained for the vitamin B-6-supplemented group at $P<0.05$ and $P<0.01$ respectively. For treatment abbreviations, see Table 1.

Animals	Treatment	$10^{-3}\times$OH-Nle (d.p.m./mg of elastin)	$10^{-3}\times$Aldol CP (d.p.m./mg of elastin)	Aldol CP/OH-Nle	Des/Lys
9-week-old rats	B-6, suppl.	2.52±0.31 (10)	3.02±0.72 (10)	0.86±0.23 (10)	0.54±0.09 (5)
	B-6, def.	2.76±0.57 (10)	6.10±1.37 (10)*	2.13±0.25** (10)	0.19±0.02 (4)*
	F.R.	2.55±0.66 (10)	3.11±0.79 (10)	1.10±0.29 (10)	–
15-day-old rat pups	B-6, suppl.	1.06±0.57 (4)	0.82±0.13 (4)	0.66±0.41 (4)	0.50±0.16 (5)
	B-6, def.	0.62±0.17 (6)	0.88±0.14 (6)	1.20±0.32 (6)	0.27±0.02 (4)*
	F.R.	0.87±0.10 (4)	1.09±0.22 (4)	0.89±0.30 (4)	–

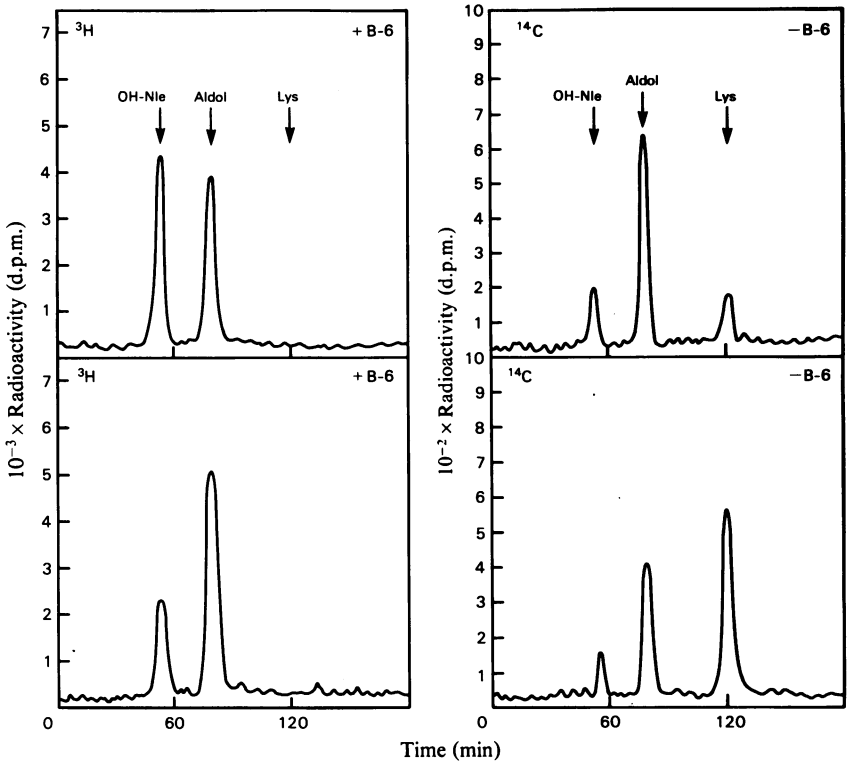

Fig. 2. *Profiles of the major cross-linking amino acids in elastin obtained after reduction with NaB^3H_4, base hydrolysis and ion-exchange chromatography*

The panels labelled '^{3}H' represent elastin samples reduced with NaB^3H_4 before hydrolysis. Those labelled '^{14}C' were obtained from data for lung explants cultured in medium containing [^{14}C]lysine before reduction. The designations '+B-6' or '−B-6' represent data for rats fed vitamin B-6-supplemented or -deficient diets respectively. For details, see the Experimental section.

desmosine/lysine ratio for elastin samples from the vitamin B-6-deficient rats (Table 3).

The data obtained for elastin from 15-day-old rat pups were similar. Also, by using lung explants in culture, it was possible to obtain an indication of recent and past history with respect to cross-link formation. For example, Fig. 2 shows elution profiles of cross-links as chemically reduced [^{14}C]lysine products. Only 50% of [^{14}C]lysine was incorporated into elastin cross-links of lung from

Table 4. *Plasma concentrations of sulphur amino acids*

Values were obtained after 5 weeks of vitamin B-6 deficiency or supplementation using weanling rats. Values represent means ± S.E.M. (nos. of observations). * and ** indicate values significantly different from those obtained for the vitamin B-6-supplemented group at $P<0.05$ and $P<0.01$ respectively. For treatment abbreviations, see Table 1.

Treatment	Concn. (μmol/dl) Methionine	Homocysteine	Cystathionine
B-6, suppl.	5.76 ± 0.40 (6)	0.12 ± 0.02 (6)	0.20 ± 0.01 (6)
B-6, def.	6.95 ± 1.04 (2)	0.35 ± 0.18 (2)*	3.75 ± 0.47 (2)**
F.R.	3.62 ± 0.26 (5)**	0.12 ± 0.02 (5)	0.20 ± 0.01 (5)

vitamin B-6-deficient rat pups, whereas 80% of the [^{14}C]lysine was incorporated into elastin cross-links of lung from vitamin B-6-supplemented rat pups. At 24 h, the ratio of aldol CP (labelled 'Aldol' in Fig. 2) to allysine (as OH-Nle) was greater than 1 (^{14}C data) for samples from both vitamin B-6-deficient and -sufficient rat pups. In contrast, the data for NaB^3H_4-reduced elastin, which reflect continuous and past history with respect to cross-link formation, indicated that, again, the aldol CP/OH-Nle ratio was normally 1 or less. For samples from vitamin B-6-deficient rats, the aldol CP/OH-Nle ratio remained greater than 1, i.e. similar to the situation for newly synthesized or immature elastin.

Lung lysyl oxidase activity

Lysyl oxidase activity was measured using tissue from the weanling rats. Lysyl oxidase activity, expressed per whole lung, was decreased significantly to about 50% of control values in both vitamin B-6-deficient and food-restricted groups. The values per whole lung were: vitamin B-6-supplemented, 6800 ± 2100 d.p.m.; vitamin B-6-deficient, 4200 ± 600 d.p.m.; food-restricted, 3100 ± 1300 d.p.m. When expressed as lysyl oxidase activity/g of lung, there were no differences with respect to dietary treatments.

Sulphur-containing amino acids in plasma

The data in Table 4 demonstrates that, in 9-week-old rats, plasma concentrations of methionine increase in response to vitamin B-6 deficiency and decrease in response to food restriction. Plasma concentrations of homocysteine and cystathionine were elevated to 3 and 15 times control values respectively in the vitamin B-6-deficient rats.

Discussion

Data from experiments using lung explant cultures suggest impaired ability of lungs from vitamin B-6-deficient rats to incorporate L-[^{3}H]-valine into newly synthesized cross-linked elastin. In contrast, there was little change in the net content of insoluble elastin in lung from vitamin B-6-deficient rats compared with controls. Bird & Levene (1983) have also demonstrated less newly synthesized insoluble collagen in chick embryos treated with the vitamin B-6 antagonist deoxypyridoxine. One explanation for such a finding is decreased cross-linking of elastin and collagen, since newly synthesized but poorly cross-linked elastin and collagen fibres should be partially solubilized by using chemically harsh extraction procedures. The cross-linking-amino-acid profiles for elastin isolated from lung explants of vitamin B-6-deficient rats indicated that less than normal amounts of [^{14}C]lysine were incorporated into cross-linking acids. The data for [^{14}C]lysine-derived cross-links in rat lung elastin were similar to those observed previously for the distribution of elastin cross-links in vitamin B-6-supplemented and -deficient chicks (Starcher, 1969). Further, since the distribution of cross-linking amino acids was similar in F.R. rats and rats fed *ad libitum*, it may be stated that mechanisms other than retarded physiological development due to the restriction of energy underlie the defects resulting from vitamin B-6 deficiency.

An explanation for decreased collagen and elastin cross-linking in vitamin B-6 deficiency is suggested to be decreased lysyl oxidase activity (Bird & Levene, 1983; Levene & Murray, 1977). Indeed, several indirect lines of evidence suggest that PLP may serve as cofactor for lysyl oxidase (Bird & Levene, 1982, 1983; Levene & Murray, 1977; Murray *et al.*, 1978). However, we were unable to demonstrate a specific relationship between lysyl oxidase activity and the concentration of vitamin B-6 in the diet. Rather, the decrease in desmosine was also accompanied by an increase in aldol CP. A decrease in lysyl oxidase activity usually results in a marked decrease in both allysine and aldol CP formation, e.g. as in nutritional Cu deficiency or β-aminopropionitrile poisoning (Dubick *et al.*, 1985; Pinnell *et al.*, 1968).

In contrast, the cross-link profiles observed in vitamin B-6 deficiency resembled more those observed in D-penicillamine poisoning or homocystinuria. In these conditions, the ability to form aldehydic functions is less impaired, but their condensation to form stable cross-links is impaired (Dubick *et al.*, 1985; Deshmukh & Nimni, 1969; Pinnell *et al.*, 1968; Siegel, 1977). D-Penicillamine blocks cross-link formation after the oxidative deamination of lysine residues in collagen and elastin by forming thiazolidine derivatives with aldehydic functions (Deshmukh & Nimni, 1969; Jackson, 1973; Siegel, 1977). In homocystinuria, elevated concentrations of homocysteine form thiazine derivatives with aldehydes. In particular, homocysteine is reabsorbed by renal tubules so that it may be maintained at high concentrations in the plasma (Jackson, 1973).

Homocystinuria is characterized by numerous deformities and defects in collagen and elastin maturation. Our values for homocysteine in vitamin B-6-deficient rats fed *ad libitum* were three times normal values. Even higher values have been observed when vitamin B-6-deficient rats are starved before drawing blood for sampling (Smolin & Benevenga, 1984; Smolin *et al.*, 1983). Collagen fibril formation is blocked by homocysteine *in vitro* (Jackson, 1973). Thus we propose that vitamin B-6 deficiency affects elastin cross-linking similarly, because of the elevated concentrations of homocysteine. PLP is a cofactor for cystathionine β-synthase, the biochemical basis for the elevation in homocysteine (Finkelstein, 1974; Smolin & Benevenga, 1984).

In the interpretation of these data, an important point is that the full equilibration of lysine to desmosine or isodesmosine occurs slowly over a period of days both *in vitro* and *in vivo* [Gray (1977) and Rucker & Dubick (1984) and references cited therein]. However, once formed, desmosine is stable, whereas the formation of aldol CP from condensation of allysyl residues is reversible. An increase in aldol CP would be expected if thiazine complex-formation blocks the condensation of aldol CP with dehydrolysinonorleucine or allysine and lysine to form desmosine (Gray, 1977). Indeed, data for cross-link formation after reduction with NaB^3H_4 (a measure of past history) indicated an increase in aldol CP, which suggests a more complicated mechanism for decreased elastin cross-linking than merely decreased lysyl oxidase activity.

In summary, changes in elastin cross-linking were observed in response to vitamin B-6 deficiency. However, the cross-link profiles are more in keeping with those observed in penicillamine treatment and homocystinuria than in conditions (Cu deficiency or β-aminopropionitrile poisoning) where lysyl oxidase is inhibited. We were unable to show a relationship between vitamin B-6 deficiency and a decrease in lysyl oxidase activity. Consequently we suggest that desmosine formation in vitamin B-6 deficiency is inhibited primarily because of the block at the step in which aldol CP condense to form desmosine.

We are grateful to Dr. Herbert M. Kagan, Dr. Quinton Rogers and Rebecca Bronson for helpful discussions concerning the assays for amino acids and lysyl oxidase, and to Kim Chonoles and Mark Andon for excellent technical assistance. The work was supported by National Institutes of Health grants HL 15965 and HL 17957.

References

American Institute of Nutrition (1977) *J. Nutr.* **107**, 1340–1348

Bird, T. A. & Levene, C. I. (1982) *Biochem. Biophys. Res. Commun.* **108**, 1172–1180

Bird, T. A. & Levene, C. I. (1983) *Biochem. J.* **210**, 633–638

Chichester, C. O., Palmer, K. C., Hayes, J. A. & Kagan, H. M. (1981) *Am. Rev. Respir. Dis.* **124**, 709–713

Collins, J. F., McCullough, B., Coalson, J. J. & Johanson, W. G. (1981) *Am. Rev. Respir. Dis.* **123**, 305–312

Deshmukh, K. & Nimni, M. E. (1969) *J. Biol. Chem.* **244**, 1787–1795

Dubick, M. A., Rucker, R. B., Last, J. A., Lollini, L. O. & Cross, C. E. (1981) *Toxicol. Appl. Pharmacol.* **58**, 203–210

Dubick, M. A., Rucker, R. B. & Keen, C. (1985) *Exp. Lung Res.* in the press

Finkelstein, J. D. (1974) *Metab. Clin. Exp.* **23**, 387–398

Fujii, K., Kajiwara, T. & Kurosu, H. (1979) *FEBS Lett.* **97**, 193–195

Gray, W. R. (1977) *Adv. Exp. Biol. Med.* **79**, 285–286

Greenberg, L. D., Miscouri, L. Y. & Wang, A. C. (1971) *Res. Commun. Chem. Pathol. Pharmacol.* **2**, 869–875

Hill, C. H. & Kim, C. (1967) *Biochem. Biophys. Res. Commun.* **27**, 94–100

Jackson, S. H. (1973) *Biochim. Biophys. Acta* **45**, 215–217

Kang, A. H. & Trelstad, R. L. (1973) *J. Clin. Invest.* **52**, 2571–2577

Kida, K. & Thurlbeck, W. M. (1980) *Am. Rev. Respir. Dis.* **122**, 467–475

Kuhn, C. & Starcher, B. C. (1980) *Am. Rev. Respir. Dis.* **122**, 453–460

Lefevre, M. & Rucker, R. B. (1980) *Biochim. Biophys. Acta* **630**, 519–529

Lefevre, M., Heng, H. & Rucker, R. B. (1982) *J. Nutr.* **112**, 1344–1352

Levene, C. I. & Murray, J. C. (1977) *Lancet* **ii**, 628–631

Li, T. K. & Lumeng, L. (1981) in *Methods in Vitamin B-6 Nutrition* (Leklem, J. E. & Reynolds, R. D., eds.), pp. 289–296, Plenum Press, New York

Lumeng, L., Ryan, M. P. & Li, T. K. (1978) *J. Nutr.* **108**, 545–553

Murray, J. C., Frasher, D. R. & Levene, C. I. (1978) *Exp. Mol. Pathol.* **28(3)**, 301–308

Myers, B. A., Dubick, M. A., Gerriets, J., Rucker, R. B., Jackson, A. C., Reiser, K. M., Williams, S. M. & Last, J. A. (1983*a*) *J. Nutr.* **113**, 2308–2315

Myers, B. A., Dubick, M. A., Last, J. A. & Rucker, R. B. (1983*b*) *Biochim. Biophys. Acta* **761**, 17–22

Nardell, E. A. & Brody, J. S. (1982) *J. Appl. Physiol.* **53**, 140–148

Nimni, M. E. (1968) *J. Biol. Chem.* **243**, 1457–1466

O'Dell, B. L., Kilburn, K. H., McKenzie, W. N. & Thurston, R. J. (1978) *Am. J. Pathol.* **90**, 413–432

Paz, M., Keith, D., Travurso, H. & Gallop, M. (1976) *Biochemistry* **15**, 4912–4917

Peterkofsky, B. & DiBlasio, R. (1975) *Anal. Biochem.* **66**, 279–286

Pinnell, S. R., Martin, G. R. & Miller, E. J. (1968) *Science* **161**, 475–476

Powell, J. T. & Whitney, P. L. (1980) *Biochem. J.* **188**, 1–8

Rucker, R. B. & Dubick, M. A. (1984) *EHP Environ. Health Perspect.* **55**, 179–191

Siegel, R. C. (1977) *J. Biol. Chem.* **252**, 254–259

Siegel, R. C. (1979) *Int. Rev. Connect. Tissue Res.* **8**, 73–118

Sloger, M. S. & Reynolds, R. D. (1980) *J. Nutr.* **110**, 1517–1524

Smolin, L. A. & Benevenga, N. J. (1984) *J. Nutr.* **114**, 103–111

Smolin, L. A., Crenshaw, T. D., Kurtycz, D. & Benevenga, N. J. (1983) *J. Nutr.* **113**, 2122–2133

Snider, R., Faris, B., Verbikki, V., Moscaritolo, R., Salcedo, L. & Franzblau, C. (1981) *Biochemistry* **20**, 2614–2618

Starcher, B. C. (1969) *Proc. Soc. Exp. Biol. Med.* **132**, 379–382

Steele, R. G. D. & Torre, J. H. (1980) *Principles and Procedures of Statistics*, McGraw-Hill, New York

Sturman, J. A. (1981) in *Methods in Vitamin B-6 Nutrition* (Leklem, J. E. & Reynolds, R. D., eds.), pp. 341–371, Plenum Press, New York