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Title: Vitamin B-6 and Pyrimidine Deoxynucleoside Metabolism in the Rat

Abstract approved _____
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Serine transhydroxymethylase (STHM), a pyridoxal 5'-phosphate requiring enzyme is indirectly involved in pyrimidine deoxynucleotide metabolism. A decrease in the activity of this enzyme could lead to altered deoxycytidine (dC) metabolism. This study was undertaken to determine if a vitamin B-6 deficiency affects dC metabolism. The effect of a vitamin B-6 deficiency on the activity of STHM in liver, thymus, spleen and bone marrow was examined. In addition, the effect of a vitamin B-6 deficiency on urinary excretion of dC was examined. The effect of a vitamin B-6 deficiency on the urinary excretion and tissue retention of ^{3}H label from ip injected ^{3}H-dC was monitored.

Rats were assigned in groups of six to one of four treatment groups: ad libitum control (ALC), pair fed control (PFC), ad libitum deficient (ALD) or meal fed deficient (MFD). At the end of weeks 2 and 6, rats from each treatment group received an ip injection of ^{3}H-dC. Urines were collected for 24 hours following the ip

inhibited due to lack of cofactor, then dTMP levels would fall. In an attempt to increase the concentration of dTMP, enzymes active in the conversion of dC and dCMP to dUMP would be expected to increase. Thus, dC salvage pathways would increase and dC synthesis would decrease as metabolism shifts toward production of deoxythymidine triphosphate (dTTP). The result would be lower urinary dC excretion.

The present study was undertaken to explore the relationship between vitamin B-6 and pyrimidine deoxynucleotide metabolism. There were four hypothesis tested: Vitamin B-6 deficient rats will excrete less urinary dC than either ad libitum or pair fed controls; vitamin B-6 deficient rats will excrete a lower percentage of labeled dC in urine than control rats; vitamin B-6 deficient rats will incorporate less labeled dC into DNA than control rats but may retain more label in tissues as dC metabolites; activity of STHM from tissues of vitamin B-6 deficient rats will be lower than that from the control rats.

Vitamin B-6 and Pyrimidine Deoxynucleoside Metabolism in the Rat

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CHAPTER 1

INTRODUCTION

In a previous study (Jensen, 1979) we quantitated a ten-fold decrease in urinary deoxycytidine (dC) excretion by the third week of a vitamin B-6 deficiency when ad libitum fed vitamin B-6 deficient rats were compared to ad libitum fed control rats. The present study was undertaken to verify this observation, to compare the urinary excretion of dC when deficient rats are compared to pair fed control rats and to explore the mechanism for the decreased urinary dC excretion.

A vitamin B-6 deficiency is known to inhibit the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) (Robson, 1975). The pyridoxal 5'phosphate (PLP) requiring enzyme, serine transhydroymethylase (STHM) (EC 2.1.2.1.) (Blakley, 1955), is suspected of being the controlling factor which results in the reduced converion of dUMP to dTMP in vitamin B-6 deficiency. Reduced activity of STHM would result in a decrease in synthesis of 5,10-methylenetetrahydrofolate (5,10-MTHF). The 5,10-MTHF formed by STHM is required by thymidylate synthase, the enzyme which catalyzes the conversion of dUMP to dTMP. If thymidylate synthase is

inhibited due to lack of cofactor, then dTMP levels would fall. In an attempt to increase the concentration of dTMP, enzymes active in the conversion of dC and dCMP to dUMP would be expected to increase. Thus, dC salvage pathways would increase and dC synthesis would decrease as metabolism shifts toward production of deoxythymidine triphosphate (dTTP). The result would be lower urinary dC excretion.

The present study was undertaken to explore the relationship between vitamin B-6 and pyrimidine deoxynucleotide metabolism. There were four hypothesis tested: Vitamin B-6 deficient rats will excrete less urinary dC than either ad libitum or pair fed controls; vitamin B-6 deficient rats will excrete a lower percentage of labeled dC in urine than control rats; vitamin B-6 deficient rats will incorporate less labeled dC into DNA than control rats but may retain more label in tissues as dC metabolites; activity of STHM from tissues of vitamin B-6 deficient rats will be lower than that from the control rats.

CHAPTER 2

REVIEW OF LITERATURE

VITAMIN B-6

History and Chemistry: In the mid to late 1930's, vitamin B-6 was first named (Gygory, 1934), isolated (Gygory, 1938, Lepkovsky, 1938) and identified (Harris and Folker, 1939). During the suceeding, other forms of the vitamin were identified and subsequently named (Snell, 1942, 1944). In 1973, the IUPAC-IUC Commission on Biochemical Nomenclature published nomenclature for the six 3-hydroxy-2-methylpyridine derivatives which by that time had collectively been termed vitamin B-6. Vitamin B-6 exists as an alcohol, an aldehyde and an amine. The six forms of vitamin B-6 are pyridoxal (PL), pyridoxine (PN), pyridoxamine and the corresponding phosphorylated compounds pyridoxal 5'phosphate (PLP), pyridoxine 5'phosphate (PNP) and pyridoxamine 5'phosphate (PMP) The structure, common names, and abbreviations for these six major forms of vitamin B-6 and the major metabolite 4-pyridoxic acid (4-PA) can be seen in Figure 1. Beginning in the late 1970's, glycosylated conjugates of vitamin B-6 were isolated and quantitated from plants (Yasumoto et al., 1977; Kabir et al., 1983; Suzuki et al., 1986; Tadera et al., 1985). These included a 4'glycoside pyridoxine, several 5'O-(*B*-D-glucopyranosyl) (Suzuki et al., 1986; Tadera et al., 1985) and other as yet unidentified conjugates (Tadera et al., 1986).

Food Sources: Pyridoxal (PL) and PM and their phosphorylated forms PLP and PMP are the major forms found in animal foods (Orr, 1969). The phosphorylated vitamers predominate in many foods. Several glycosylated-pyridoxine conjugates also exist in plant foods and may constitute a significant portion of the total vitamin B-6 content in specific foods (Leklem, 1989; Kabir, 1983).

Absorption: Vitamin B-6 absorption has been studied extensively in the rat using whole animals (Booth, 1962), everted sacs (Middleton, 1977), isolated loops (Middleton, 1979a,b) luminal perfusion studies (Middleton, 1985) and intestinal rings (Tsuji et al., 1973). The nonphosphorylated, nonconjugated forms of vitamin B-6 are passively absorbed throughout the intestine decreasing from proximal to distal segments of the intestine (Middleton, 1977, 1982, 1985; Tsuji, 1973). Studies in humans (Wozenski et al., 1980) suggests that PL and PN are more rapidly absorbed than PM. However, in an in vitro system, Henderson (1984) found that PM was more rapidly absorbed than PN. In rats, PN absorption is enhanced at lower concentrations (Middleton, 1985) Limited absorption of the phosphorylated forms can occur (Mehensho, et al., 1979; Middleton et al., 1985). At normal physiological doses, phosphorylated forms appear to be dephosphorylated by membrane bound intraluminal phosphatases (Middleton, 1982; Hamm, 1979) prior to absorption (Lumeng and Li, 1975). After absorption, the vitamers are found in the circulation in the

nonphosphorylated form in which they were administered (Mehansho, 1979; Hamm,1979). This suggests they are not further metabolized by intestinal mucosal cells prior to transport into the circulation.

Bioavailability: Bioavailability has been defined as the percent of vitamin B-6 in food (as determined by Saccharomyces uvarum bioassay) which is available to the experimental animal as a source of vitamin B-6 (Ink, 1984). Food processing and storage, other components of the diet and the form of the vitamin ingested are known to influence the bioavailability of this vitamin.

In heat processed, dehydrated foods, bioavailability of vitamin B-6 decreased by 40-50% (Gygory and Kirk, 1978). The decrease is believed to be due to a reduction of the Schiff base formed between PLP and the amino group in the lysine residue in the protein. These *E*-pyridoxyl lysine residues thus formed can account for 50% of the PLP degradation in the dehydrated foods. Growth of rats and bacteria were decreased when this food was provided as a source of vitamin B-6. Furthermore, Gregory (1980) reported that bovine serum albumin bound phosphopyridoxyllysine has about 50% of the molar activity of PLP for rat growth, food efficiency and maintenance of liver PLP concentration. In these rats, this phosphopyridoxyl protein was found to accelerate a vitamin B-6 deficiency when low doses were administered. Coursin (1959) postulated that this compound

may have led to the convulsive seizures observed in infants fed heat sterilized, nonfortified canned infant formula.

In humans, dietary fiber has been found to slightly reduce bioiavailability of vitamin B-6 (Leklem et al., 1980; Lindberg et al., 1982; Kies et al., 1984). In rats, fiber was found to have little effect on bioavailability (Nguyen et al , 1981,1983b). Studies in humans (Kabir et al., 1981) and rats (Nguyen et al., 1983a) indicate vitmain B-6 from plant sources is less efficiently utilized than vitamin B-6 from animal sources. The poor utilization of vitamin B-6 from plant sources has been attributed to the presence of B-glucoside conjugates of the vitamin. In humans, an inverse relationship has been found between vitamin B-6 bioavailibility and glycosylated vitamin B-6 content of a particular food (Bills et al., 1979; Kabir, 1983). Trumbo and Gregory, (1988a,b, 1989) reported *B*-glucoside of PN had 10-30% of the molar response of PN for rat growth. Furthermore, they reported the intestine was the primary site of conversion of glycoside PN to PN (Trumbo and Gregory, 1988b).

Metabolism: Various nonphosphorylated forms of vitamin B-6 are delivered to the circulation via intestinal absorption and liver metabolism. The metabolic pathway for vitmain B-6 is depicted in Figure 1. Vitamin B-6 metabolism is carried out throughout the body. However, different tissues have varying ability to metabolize various forms of the vitamin. The liver is thought to be responsible for

forming the PLP present in the plasma (Lumeng et al., 1974). The enzyme PL kinase is present in most mammalian tissues including liver, kidney, brain, spleen, blood cells and small and large intestine (McCormick et al., 1961). In contrast, PNP oxidase, which is present in high concentration in the liver, is absent in other tissues including the heart, lung, pancreas and skeletal muscle (Pogell, 1958). Thus, while most tissues can phosphorylate B-6 vitamers, they are unable to convert PNP to PLP. For this reason, the liver is believed to be the primary organ involved in conversion of B-6 vitamers to the PL and PLP found in the circulation and for use by various tissues throughout the body.

In rat liver perfusion studies, Mehensho et al. (1980), demonstrated PN was passively absorbed and trapped as PNP. Using isolated rat liver cells, Lumeng et al. (1980), found that only about 20% of endogenous liver PLP is exchanged with exogenous PLP. Lumeng and coworkers (1980) reported that when a 3.33 umolar solution of PN was incubated for 2 hours in a solution containing 1 g isolated liver cells, 42% of the PN was metabolized and released from the cell as PLP, PL and 4-PA. Endogenous PLP and PMP levels remained constant when PN was present in the media but decreased at the rate of 6 nmoles/2 hours when PN was not present.

Using ^{3}H-PN in rat liver perfusion studies, Mehansho et al. (1980), found that PL was the primary metabolite released into the circulation. Since PLP in plasma is bound

to albumin, it may not be available for absorption into cells. Thus, Anderson (1974), proposed that PL is the form of the vitamin most readily available to most tissues and organs. The charged phosphate group on PLP would hinder its passage through the membrane (Snell and Haskell, 1971). Membrane bound phosphatases functioning in most tissues can hydrolyze PLP to PL for absorption. Once in the tissue, PL can be phosphorylated by cytosolic kinase and trapped as PLP bound to proteins.

In many species erythrocytes are believed to be important in vitamin B-6 metabolism. However, since erythrocytes from rats lack PMP (PNP) oxidase (Mehansho et al., 1980b), any PNP formed can only be hydrolysed back to PN and returned to plasma. Furthermore, erythrocytes do not have the ability to release phosphorylated vitamers from the cells (Anderson, 1971).

Vitamin B-6 Metabolites: Isotope studies in normal rats indicate that 4-PA represents 10-30% of the daily excretion of vitamin B-6 metabolites (Johannson et al., 1966; Bernett and Pearson, 1968). Using labeled PN, Bernett and Pearson (1968) reported there were more than 9 labeled metabolites in urine of rats given 50 or 100 ug PN/day. At these intakes, 4-PA represented 10 and 19% of the urinary label, respectively, while PN represented 13 and 22% of the urinary label, respectively. No PN or 4-PA was found when rats received 10 ug PN/day. Other studies in rats receiving a 20 mg ip injected dose of PN indicated 50-70% of label

from PN was excreted unchanged in urine and while relatively small amounts of 4-PA were excreted (Cox, 1956). The large ratio of PN/4-PA excreted by these rats may be related to the sizable dose these rats were given.

Urinary 4-PA is widely used in metabolic studies to help assess vitamin B-6 status. Urinary 4-PA excretion is considered indicative of recent dietary vitamin B-6 status. This compound is formed in an irreversible reaction from PL by the action of either an FAD-dependent aldehyde oxidase (Schwartz and Kjelgaard, 1951) or an NAD dependent dehydrogenase (Stanulovic et al., 1976). Evidence suggests that while the aldehyde oxidase functions in human liver (Merrill, 1984), the dehydrogenase functions in rat tissues (Stanulovic et al., 1976). Any PLP not bound to enzymes is dephosphorylated and subsequently irreversibly oxidized to 4-PA (Li et al, 1974; Merrill, 1984).

Storage: Data from isotope studies in mice indicate that after an initial equilibrium period of a few days, 80-90% of the vitamin B-6 in the body is in the form of PLP and PMP while the remaining 10% is present as PL (Johansson et al., 1974). Furthermore, the turnover rate of an equilibrated pool of labeled vitamin B-6 was estimated to be 1.2-1.5%/day in normal mice. Most of the total PLP and PMP in the body is associated with enzymes. The ratio of PLP/PMP varies between tissues but remains constant within a tissue.

The liver is a rich source of vitamin B-6, but the muscle contains the largest pool of vitamin B-6 in the body (Li and Lumeng, 1981). Krebs and Fisher (1964) estimated 60% of the total vitamin B-6 in the rat is associated with muscle due to the large mass and the high concentration of PLP associated with muscle glycogen phosphorylase.

There appear to be at least two labile pools of vitamin B-6 in the body. One is a highly mobile pool exhausted soon after vitamin B-6 is removed from the diet. The depletion of this highly mobile pool is easily monitored by a rapid decrease in 4-PA and PLP levels in weanling rats (Cho, 1987). Urinary 4-PA excretion in rats placed on a vitamin B-6 deficient diet dropped to 33% of the pre-deficiency levels by the end of the first week of deficiency and continued to fall to undetectable levels by the end of week 2 (Cho, 1987; Jensen, 1979). Plasma levels drop to 2-3% of control levels as early as one week into a vitamin B-6 deficiency then decline little through at least the sixth week of deficiency (Cho, 1987). Similarly, tissue levels of PLP drop most rapidly in the first 2 weeks of a deficiency then drop more slowly as the deficiency progresses (Cho, 1987; Wachstein and Moore, 1958). In contrast, the PLP associated with muscle phosphorylase is not mobilized as a result of a vitamin B-6 deficiency (Black, 1978). However, it is mobilized as a result of acute caloric deprivation such as fasting (Black, 1978), exercise (Leklem and Shultz, 1983; Manore, 1987) and trauma (Turkki et al., 1989).

Function: PLP and PMP are the active forms of vitamin B-6 and are required for over 100 enzymes involving many aspects of metabolism (Sauberlich, 1985). Most of these enzymes are involved in amino acid metabolism. There are over 50 known PLP dependent aminotransferases (Braustein, 1973). Other types of reactions include decarboxylation, alpha-beta and beta-gamma elimination, desulfuration, side arm cleavage and racemization reactions (Metzler, 1977). PLP is known to be bound to a large number of enzymes, but the effect of this binding is unknown (Metzler, 1977). PLP also has a regulatory role as a specific inhibitor or perhaps as allosteric effector for a number of enzymes including aldolase, glutamate dehydrogenase (Metzler, 1977), fructose 1,6-diphosphatase (Marcus, 1975) and thymidylate synthase (Tryfiates, 1980). PLP is required by glycogen phosphorylase (Krebs and Fisher, 1964).

PLP is involved in carbohydrate metabolism through gluconeogenesis, glycogenolysis, glycogenesis and TCA derived energy metabolism through transamination reactions. Many alpha keto acids produced from transamination reactions are tricarboxylic acid cycle intermediates. In addition, the carbon skeletons of alanine and aspartate produced from aspartate and alanine transaminases are used in gluconeogenesis. Alanine aminotransferase is of particular importance in recycling pyruvate. Anaerobic glucose catabolism produces pyruvate which is transported to liver for regeneration of glucose.

PLP is involved in glycogenolysis through its function as a component of glycogen phosphorylase (Krebs and Fisher, 1964). PLP forms a Schiff base with lysine-679 in rabbit muscle phosphorylase resulting in a 5'phosphate group adjacent to the substrate binding site. This provides evidence that the phosphate is involved in the catalytic reaction (Sygusch et al., 1977; Titani et al., 1977).

Vitamin B-6 is also required for lipid metabolism. Although specific PLP requiring enzymes have not been identified, vitamin B-6 deficiency has long been known to influence lipid metabolism. Halliday (1938) observed two to three times the fat accumulation in livers of vitamin B-6 deficient rats compared to control rats. Accumulation of the lipid was primarily as triglycerides and cholesterol (Okada and Iwane, 1977; Gomikawa and Okada, 1978; Suzuki and Okada, 1982a,b). Oxidation of palmitate and linoleate are decreased in vitamin B-6 deficient rats (Dussault and Lepage, 1979). More recently, Cunnane et al. (1984) observed a decrease in turnover of essential fatty acids, triglycerides and phospholipids in vitamin B-6 deficient rats. In addition, carnitine synthesis has been shown to be influenced by vitamin B-6 deficiency (Cho, 1989). Furthermore, serine transhydroxymethylase (STHM) may be identical to 3-OH-6N trimethyllysine aldolase an enzyme required for carnitine synthesis (Hulse et al., 1978).

Vitamin B-6 is vital to the function of brain biochemistry because it is required for synthesis of a

number of neurotransmitters including dopamine, norepinephrine, serotonin, gama-aminobutyric acid, histamine and taurine (Metzler, 1977). Many of these neurotransmitters are amines and are produced via decarboxylation reactions from amino acids (Metzler, 1977). The enzyme L-DOPA decarboxylase is involved in the metabolic pathway which converts tyrosine to dopamine and subsequently epinephrine and norepinephrine. This enzyme also catalyzes the decarboxylation of 5-hydroxytryptophan to serotonin (5-hydroxytryptamine) (Lowenberg et al., 1962). Another PLP dependent decarboxylase, L-glutamic acid decarboxylase (Haber et al., 1970), is required for GABA synthesis from L-glutamic acid. Furthermore, GABA is degraded by the PLP requiring enzyme GABA transaminase (Baxter and Rober, 1958). PLP is required for the decarboxylation of histidine to form histamine and for the synthesis of taurine (Metzler, 1977).

A vitamin B-6 deficiency has been shown to inhibit the normal expression of the immune function. The inhibition is generally attributed to inhibition of DNA synthesis (Axelrod, 1971, 1964). A vitamin B-6 deficiency has also been shown to inhibit RNA synthesis (Axelrod, 1971; Montjar, 1965; Pandit and Chakrabarti, 1970). Vitamin B-6 deficiency is believed to inhibit DNA synthesis via the role of PLP in formation of folate intermediates, which are required for purine and pyrimidine deoxyribonucleotide synthesis

(Axelrod, 1964, 1971). This will be discussed in greater detail in a later section

Other functions for vitamin B-6 include its involvement in porphyrin and, therefore, heme synthesis. PLP is a cofactor for delta-aminolevulinate synthase (Richert and Schulman, 1959). This enzyme catalyzes a side arm cleavage reaction. In addition, vitamin B-6 has a role as a modulator or regulator of enzymes and hormones. Litwack (1979), suggests PLP modulates hormones by interacting with receptor sites and thus decreasing the ability of hormones to bind. Kondo (1985) reported an increase in tryptophan oxygenase in vitamin B-6 deficient rats. This enzyme is known to be induced by glucocorticoids. Thus, Kondo suggests PLP may either decrease or modulate function of glucocorticoids. Bender (1987,1988) found increased nuclear accumulation of steroid hormones in the uteri and prostrate glands of vitamin B-6 deficient rats. The accumulation suggests a role for PLP in regulation of the concentration of steroid hormone receptors and other steroid-binding proteins in target tissues. Work by Cidlowski and Tanassi (1981) suggests PLP is involved in releasing steroid hormone-receptor-complexes from nuclear binding.

VITAMIN B-6 DEFICIENCY

Requirements: Beaton and Chaney (1965) reported rats fed a 20% protein diet had maximal weight gain and erythrocyte transaminase activity when consuming 40-80 ug PN HCl/day. They therefore recommended an intake of 100 ug PN

HCl/day or 60-70 ug/ 10 g diet. Similarly, Driskel et al. (1973) found 45 ug PN/day resulted in maximal weight gain, brain weight, DNA and protein and erythrocyte transaminase activity. In contrast, Lumeng et al. (1978) reported maximal weight gain and liver and brain PLP and PMP levels on an intake of only 24 ug PN/day (or 1-2 ug/10 g diet). However, they found muscle and plasma PLP levels did not saturate at levels up to 100 ug PN/day. Based on these and other reports, the Nutrition Research Council recommends between 5 and 7 mg PN/kg diet (50-70 ug/10 g) for the rat to maintain normal vitmin B-6 status (AIN, 1977,1980).

A number of physiological abnormalities have been reported as a result of vitamin B-6 deficiencies. These include anorexia, poor growth, scaling dermatitis of the tail, paws, face and ears (Sherman, 1954), neurological disturbances (Chick, 1940) and hypochromic, microcytic anemia (Kornberg et al., 1945). Due to the wide spread involvment of vitamin B-6 in many types of enzymes a deficiency impacts many body functions.

Amino Acid Metabolism: A vitamin B-6 deficiency in rats has a profound effect on amino acid metabolism. A deficiency has been associated with decreased rate of amino acid absorption and abnormal concenatration of amino acids in tissues (Akedo, 1960). The absorption rate of at least 18 amino acids is depressed as a result of a vitamin B-6 deficiency (Astoor et al., 1972). Activity of a number of PLP dependent enzymes involved in amino acid metabolism is

also decreased. These include 5-hydroxytryptophan decarboxylase (Buzzard, 1957), serine and threonine dehydratase, cystathionase, alanine and aspartate aminotransferase (Babcock, 1958; Takami, 1968), kynureninase and kynurenine transaminase (Takami, 1968). A vitamin B-6 deficiency does not influence all PLP requiring enzymes equivilently. Shibuya and Okada (1980) reported mitochondrial aspartate aminotransferase activity from rat liver was not affected by a vitamin B-6 deficiency. Similarly, Takami et al. (1968) studied the effect of a vitamin B-6 deficiency on nine PLP dependent enzymes from cytoplasm and mitochondria in rat liver. Activity of all but one of these 9 enzymes, STHM was found to be decreased as a result of the deficiency.

PLP is required for transamination of amino acids whose carbon skeletons are converted to citric acid cycle intermediates. Aspartate and alanine transaminases are known to be decreased as a result of vitamin B-6 deficiency (Babcock, 1958; Takami, 1968; Angel, 1975). Thus, a vitamin B-6 deficiency could result in a decreased ability to derive energy from amino acid catabolism. The impact of the reduced ability to catabolize protein is especially critical when a high (70%) protein diet is fed in conjunction with a vitamin B-6 deficiency. Evidence for this is reflected by the abnormal urea metabolism observed under these conditions. Okada and Suzuki (1974) reported a significant decrease of 28% in urea nitrogen excretion in rats fed a

high (70%) protein, vitamin B-6 deficient diet. Urinary free ammonia was also decreased, while excretion of free amino acids increased. Total urinary nitrogen and creatinine excretion were not altered. Results from our laboratory are consistant with these findings. We observed a decrease in urinary urea nitrogen in vitamin B-6 deficient rats fed a 20% protein diet (Cho, 1987).

Glucose and Energy Metabolism: Glucose metabolism is affected by a vitamin B-6 deficiency. PLP is required by several transaminases which produce substrates for gluconeogenesis. In vitamin B-6 deficient rats, Angel et al. (1975) found a significant decrease in the activity of liver alanine and aspartate aminotransferase. They also reported no significant decrease in incorporation of labeled alanine into glucose yet fasting glucose levels were not influenced by the vitamin B-6 deficiency. This is in contradiction to results reported by Beaton (1954a) and Huber (1964) who reported a significant decrease in blood glucose levels as a result of a vitamin B-6 deficiency. Angel et al. (1975) reported impaired glucose clearance following glucose administration in ad libitum vitamin B-6 deficient rats as compared to pair fed controls. Differences between these reports may be related to the method of feeding used in the various studies. Another way in which vitamin B-6 deficiency can influence glucose metabolism is through its involvement in glycogenolysis. PLP is an integral part of liver and muscle glycogen

phosphorylase. There is evidence that glycogen accumulates in liver from vitamin B-6 deficient rats (Cho, 1987), suggesting a deficiency may impair glycogenolysis. This is in contrast to results by Angel (1975) and Beaton (1954b) who reported a decrease in liver glycogen as a result of a vitamin B-6 deficiency. Fasting studies reveal that glycogen content is decreased in vitamin B-6 deficient rats suggesting the liver phosphorylase was active in severely deficient rats, (Cho, 1987). However, the liver glycogen in the vitamin B-6 deficient rats was not completely exhausted after a one day fast but was completely exhausted after a three day fast.

Lipid Metabolism: Lipid metabolism is also altered by vitamin B-6 deficiency. Body fat has been reported to be decreased in vitamin B-6 deficient rats (McHenry and Gauvin, 1938; Angel and Song, 1973). Liver lipids, primarily triglycerides, have also been reported to be lower in vitamin B-6 deficient rats (Audet and Lupien, 1974). Fatty livers and high liver cholesterol levels were reported in rats fed a 70% protein, vitamin B-6 deficient diet (Okada and Iwama, 1977). Fat synthesis has been reported to be normal (Desikacharm and McHenry, 1954: Angel, 1975) decreased (Angel, 1973) or increased (Sabo, 1971) in the deficient rats when compared to controls. The differences in these reports may be related to different feeding patterns used in the studies.

Fatty acid metabolism is also affected by vitamin B-6 deficiency. In particular, there is evidence of impaired conversion of linolenic acid to arachidonic acid (Witten and Holman, 1952; Cunnane et al., 1984). Evidence suggests the role of vitamin B-6 deficiency in modified fatty acid metabolism is indirect, through altered methionine metabolism and its effect on carnitine synthesis (Loo and Smith, 1986). In particular, carnitine is required for fatty acid metabolism. There is evidence that PLP is required for carnitine synthesis. Hulse et al. (1978), reported STHM has the capacity to cleave 3-hydroxy-6-N-trimethyllysine. From this observation arose the hypothesis that STHM, and thus PLP, is involved in carnitine synthesis. Further evidence to support this hypothesis is provided by Dunn et al. (1982). They found the vitamin B-6 antagonist 1-amino-O-proline inhibited carnitine synthesis in a perfused liver system. Furthermore, Loo and Smith (1986) demonstrated a vitamin B-6 deficiency decreased the activity of lysine methyl transferase which catalyzes the initial reaction in carnitine synthesis. The effect of the deficiency was postulated to be due to an altered hepatic ratio of s-adenosylmethionine to S-adenosylhomocystein (SAM:SAD) (Loo and Smith, 1986b). Finally, Cho et al. (1989), has demonstrated reduced tissue and circulation and excretion of carnitine in deficient compared to ad libitum and pair fed control rats.

Results from in vitro (Lupein et al., 1969) and in vivo (Hinse and Lupien, 1971) studies in vitamin B-6 deficient rats revealed increased incorporation of labeled acetate into hepatic cholesterol although serum and hepatic cholesterol levels remained normal. Gomikawa and Okada (1978, 1980) reported a vitamin B-6 deficiency resulted in an increase in activity of 3-hydroxy 3-methylgluteryl CoA reductase (HMG), the rate limiting step in cholesterol synthesis. In addition, catabolism and secretion into bile of labeled cholesterol was elevated by a vitamin B-6 deficiency (Iwani and Okada, 1982).

Erythrocyte Function: Vitamin B-6 is involved in erythrocyte function in two ways. One way is through its involvement in heme synthesis. A deficiency can result in hypochromic, microcytic anemia in man (Rabb et al., 1961) and rats (Kornberg et al., 1945). The second way vitamin B-6 deficiency is involved in erythrocyte function is by affecting O_2 binding to hemoglobin. The O_2 binding capacity is influenced by binding of PL and PLP to the alpha and beta chains of hemoglobin, respectively (Kark et al., 1982). PL binding to the alpha chain increases O_2 affinity for hemoglobin while the PLP binding to the beta chain decreased O_2 affinity (Maeda et al., 1976).

Niacin, Folic Acid and Vitamin B-12: Vitamin B-6 is involved with metabolism of at least three other vitamins. PLP is required for the synthesis of niacin from tryptophan. However, it is unlikely that niacin deficiency would result

secondary to a vitamin B-6 deficiency. A deficiency of vitamin B-6 does not completely inhibit conversion of tryptophan to niacin (Leklem et al, 1975) and most diets contain other sources of niacin. The primary interaction between vitamin B-6 and folic acid is in the interconversion of tetrahydrofolate and 5,10-MTHF. There is evidence that this can impact nucleic acid synthesis (Robson, 1975) but a specific effect on folate status has not been assessed. Absorption of vitamin B-12 has been reported to be inhibited in vitamin B-6 deficient animals (Hsu, 1963; Hsu and Chow, 1957; Chow and Hsu, 1958). In addition, low levels of vitamin B-12 have been reported in liver and serum of vitamin B-6 deficient rats (Ranke et al., 1960).

Immune Function: Vitamin B-6 deficiency impairs immune function (Axelrod and Trakatellis, 1964). The impairment is generally considered to impact tissues of the immune system by interfering with nucleic acid synthesis via 1-carbon metabolism involving 5,10-methylene tetrahydrofolate. Since adequate immune function requires cell proliferation, any factor which would inhibit this would also impact the immune function. This will be discussed in more detail later.

Tissue Levels of PLP: Table I summarizes PLP values in a variety of rat tissues from several studies. Results from control and vitamin B-6 deficient rats were included when available. Means were converted from ug/g to nmoles/g when appropriate. All but one study used a tyrosine decarboxylase assay to determine the amount of PLP. The

study by Buchin (1976) used tryptophanase. Values from Wachstein and Moore (1957) appear lower than those from more recent studies. Data presented by Buchin (1976) are most consistant with liver and brain values reported in more recent years (Li and Lumeng, 1981; Vanderslice et al., 1981; Yang et al., 1981) and with values we have generated in our laboratory in recent years (Cho, 1987, unpublished data). This may be a reflection of improved methodology and perhaps diets that more adequately meet the needs of the rat.

PYRIMIDINE DEOXYNUCLEOTIDE METABOLISM

Deoxycytidine triphosphate (dCTP) is one of the two pyrimidine precursors for DNA synthesis. Deoxycytidine is the deoxyriboxyl compound that occurs in the highest concentration in rat blood, tissues (Rotherham et al., 1965) and urine (Rotherham et al., 1960). A 160 g male Wistar rat can be expected to excrete in the neighborhood of 2.7 umol of dC/day under normal conditions (Chen et al., 1968). The major anabolic and catabolic pathways for pyrimidine deoxynucleotides in mammalian tissues are shown in Figure 2-2.

Some of the enzymes which are important in the regulation of pyrimidine deoxynucleotide metabolism are depicted in Figure 2-2. Deoxycytidylate deaminase (deoxycytidylate aminohydrolase, 2'deoxyribosyl 4-aminopyrimidine 2,5'-phosphate aminohydrolase:EC 3.5.4.12), catalyzes the deamination of deoxycytidine monophosphate (dCMP) to dUMP (reaction 5). Allosteric control of this

reaction allows for the regulation of the pyrimidine deoxynucleotide pool (Greenberg, 1970). This enzyme is subject to allosteric activation and inhibition by both purine and pyrimidine deoxynucleotides. Deoxycytidiylate deaminase has been shown to be inhibited in rat embryo mince by high concentrations of dTTP and deoxyguanosine monophosphate and activated by low concentrations of dTTP and high concentrations of dCTP (Greenberg, 1970; Fiala, 1965). Indirect support for this regulation includes observations of a temporal seven-fold increase in the dCTP pool in cultured Chinese hamster cells associated with removal of thymidine (dT) from the media (Walters et al., 1975). Removal of dT also correlated with lengthening of the DNA synthetic cycle and increased de novo dTTP synthesis via the ribonucleotide reductase system (reaction 1). Furthermore, Zaharko et al. (1983) demonstrated a four-fold increase in the plasma half life of ^{3}H-dC following the prior administraion of 3600 mg dT/kg body weight to mice, presumably due to feedback inhibition of deoxycytidylate deaminase. In those mice receiving this large dose of dT, there was an increased incorporation of the ^{3}H-dC into DNA of spleen, duodenum and femur marrow. The increases resulted because more substrate was available for incorporation into DNA and less was shunted through alternate pathways (ie deaminated to dUMP).

As a result of deamination, dCMP can be reused via salvage pathways. The dUMP formed from the deamination of

dCMP is catalyzed by thymidylate synthase, an enzyme which requires 5,10-MTHF. Figure 2-3 shows the manner in which 5,10-MTHF is involved in this reaction and how it is regenerated. The 5,10-MTHF acts as a source of reducing power and a one-carbon source for the methylation of dUMP.

The nucleosides dC, dT and dU can be reused via salvage pathways. They can be phosphorylated and re-enter the deoxynucleotide pools. Thus, dC radioisotopes can be used to label DNA (Greenberg et al., 1969). The enzyme thymidine kinase (ATP-thymidine 5'phosphotransferase; EC 2.7.1.7.4) catalyzes the phosphorylation of dT (Figure 2-2, reaction 7). The enzyme deoxycytidine kinase (NTP:deoxycytidine 5'phosphotransferase, EC 2.7.1.7.4) catalyzes the phosphorylation of dC (Figure 2-2, reaction 8). Studies in mice have shown that lymphocytes at different stages of maturation have different abilities to utilize these salvage pathways (Staub et al., 1983). Data suggest more mature lymphocytes have a high ratio of thymidine kinase activity to deoxycytidine kinase activity, whereas more immature T and B cells have higher deoxycytidine kinase activity. In addition, activities of both enzymes were 3-5 times lower in mature T cell populations than in mixed cell or in immature T cell populations.

The major points of catabolism for pyrimidine deoxynucleotides begin with the dephosphorylation of dUMP, dTMP and dCMP (Figure 2-2, reaction 3). Rats are not able to further degrade dC. Thus, there is an accumulation in

tissues and subsequent excretion of this nucleoside in urine. Of the deoxyribosyl compounds found in rat urine, dC occurs in the highest concentration, representing about 60% of the total excreted. This is followed by 5-methyldeoxycytidine and dU representing 20 and 10-15% of the total excreted, respectively (Rotherham, 1960).

Since animals have varying levels of the enzymes required for degradation of these compounds, the level of these deoxynucleosides excreted in urine is species dependent. In particular, hamster, calf and humans have higher concentrations of liver nucleoside aminohydrolase than the rat (Zicha, 1969; Buric, 1969). The activity of this enzyme in the liver of humans, calf, golden hamster and rat has been reported to be 583$\pm$842, 80$\pm$155, 190$\pm$38 and 2.6$\pm$2.2 nmoles/g/min, respectively (Buric, 1969). Further degradation of the compound to CO_2 occurs in hamster (Zicha, 1969; Hill, 1975). The high aminohydrolase activity and the extremely low urinary excretion of dC found in humans suggests a similar fate for dC in humans (Chen, 1968). In contrast, a urinary excretion of 2.7 umoles/day has been reported for normal male Wistar rats weighing 160 g (Chen, 1968).

In a previous study, we quantitated a decrease in dC excretion in vitamin B-6 deficient rats (Jensen, 1979). Levels fell from 1.26 umoles/day and plateaued at 0.17-0.13 umoles/day by the third week of a six week vitamin B-6 deficiency. Furthermore, levels quickly rebounded when

vitamin B-6 was added back to the diet. We postulated that the influence of a pyridoxine deficiency on pyrimidine deoxynucleoside metabolism was through its effect on folate metabolism.

PYRIMIDINE NUCLEOTIDES, FOLIC ACID AND VITAMIN B-6

The interrelationship between folic acid and vitamin B-6 metabolism and pyrimidine deoxynucleotide synthesis can be seen in Figure 2-3. Reaction 3 is catalyzed by thymidylate synthase and converts dUMP to dTMP. The reaction requires the folate vitamer 5,10-MTHF. The primary pathway for the generation of 5,10-MTHF is via reaction 1 and is catalyzed by serine transhydroxymethylase (STHM) (EC 2.1.2.1), an enzyme which requires PLP.

Axelrod and co-workers (1964, 1971) have proposed that a deficiency of vitamin B-6 affects deoxynucleotide synthesis by interfering with the production of one-carbon fragments from serine via the reaction catalyzed by STHM. These researchers reported that in vitamin B-6 deficient rats, there was a decreased incorporation of serine-3-C^{14} and an increased incorporation of formate-C^{14} into RNA and DNA of rat liver and spleen (Axelrod, 1964). Takami et al. (1968) also reported a decreased incorporation of serine-3-C^{14} into DNA of regenerating liver in pryidoxine deficient rats. However, they later attributed the decreased incorporation to isotope (serine) dilution, due to elevated endogenous serine pools. Vitamin B-6 deficiency was not

found to interfere with liver regeneration (Takami, 1968), a process which involves rapid cell proliferation.

A vitamin B-6 deficiency has been shown to impair uptake of ^{3}H-dU into small thoracic duct lymphocytes in vitro from rats fed vitamin B-6 deficient diets and the vitamin B-6 analogue 4-deoxypyridoxine for 2 weeks (Robson, 1975). The reduced uptake may reflect a shift in T and B cell populations and/or impaired ability for the lymphocytes to utilize dU for DNA synthesis. In this study, the actual activity of thymidylate synthase and STHM, levels of PLP and 5,10-MTHF were not measured.

There are a number of chemotherapy drugs which work on the principle of inhibiting thymidylate synthase either directly or indirectly. Because a deficiency of vitamin B-6 may indirectly inhibit this same enzyme, a review of the literature in this area is important. One such thymidylate inhibitor is the folate antagonist amethopterin, which inhibits the conversion of dihydrofolate to tetrahydrofolate by competing with the substrate for dihydrofolate reductase. This then presumably reduces the availability of the cofactor 5,10-MTHF. Amethopterin, like a vitamin B-6 deficiency (Takami, 1968), was found to have no affect on liver regeneration (Bertino, 1965). However, activity of dihydrofolate reductase (Bertino, 1962, 1963, 1965), thymidylate synthase (Roberts, 1969) and thymidine kinase (Wilmans, 1971) were all elevated in lymphocytes of patients treated with amethopterin. Maley and Maley (1971) reported

methsquin and quinazoline produce an amethoptrin like elevation in thymidylate synthase in regenerating liver. Dihydrofolate reductase and thymidylate synthase were elevated in cultured mammalian cells (human lymphoblasts) exposed to three different folate antagonists (Chello, 1976). These investigators also found dUMP stabilized thymidylate synthase. They postulated that if the enzyme remains elevated, it could effectively trap 5,10-MTHF, thus preventing its conversion to other folate coenzymes. STHM, 5,10-MTHF dehydrogenase, 10-formyltetrahydrofolate synthetase and thymidylate kinase were not elevated by these antagonists.

Another set of conditions which can affect thymidylate synthesis is a folate deficiency. During a frank folate deficiency or a functional folate deficiency, secondary to a vitamin B-12 deficiency, the conversion of dUMP to dTMP via thymidylate synthase is blocked (Figure 4, reaction 3). The reduction in activity of this reaction is measured by the dU suppression test. This test measures the inhibition of ^{3}H-dT uptake by lymphocytes in the presence of dU. If this pathway is intact, there is a supression of ^{3}H-dT uptake in the presence of exogenous dU. In folate or B-12 deficiency, this suppression is decreased. In a B-12 deficiency, the decrease can be partially corrected by addition of B_{12} to the reaction media. Such a correction will not occur if the decrease is due a folate deficiency (Hall, 1983). There is a possibility that the uridine suppression test could be

modified to detect a vitamin B-6 deficiency by determining the ability of added PLP to suppress ^{3}H-dT uptake.

SERINE TRANSHYDROXYMETHYLASE

Studies on STHM began in the early 1950's and continue through the present time. In the early 1950's, Blakley (1954) and Kisllink and Sakami (1954) demonstrated the enzyme required tetrahydrofolate. Results from in vivo experiments (Deodgi and Sakami, 1953) provided evidence to suggest that PLP was required for the activity of STHM. Subsequently, employing in vitro techniques, Blakley (1955) demonstrated PLP was required for STHM activity.

Early studies focused on the role of the enzyme in the interconversion of serine and glycine along with its ability to transfer hydroxymethyl groups from serine to THF. In the late 1960's, STHM was found to be identical to threonine aldolase (Schirk and Gross, 1968). The enzyme has the ability to cleave B-hydroxy amino acids to glycine and the corresponding aldeheic product. More recently, studies have provided evidence to suggest that this enzyme may be involved in carnitine synthesis via its aldolase activity (Hochalter and Henderson, 1976; Hulse et al., 1978). In vitro studies have demonstrated the enzyme can catalyze the transamination of alanine to pyruvate (Schirk. 1964). The significance of this in vivo is uncertain.

There have been two studies which have explored the impact of a vitamin B-6 deficiency on the activity of this

enzyme. Deodha and Sakami (1953) demonstrated that liver homogenates from vitamin B-6 deficient pigeons had a reduced ability to incorporate formate into serine. A second study by Takami et al. (1968) found no decrease in soluble or mitochondrial STHM activity from liver when comparing vitamin B-6 deficient and control rats. Furthermore, there was no stimulation in activity when PLP was added to the assay media. Still, this is the enzyme postulated to be the limiting factor in reduced immune function associated with a vitamin B-6 deficiency and thus deserves further exploration.

Another possible point of interaction between vitamin B-6 and pyrimidine deoxynucleotide metabolism is through the proposed modulation of thymidylate synthase by PLP. Tryfiates (1980) reported that PLP is required for maximal in vitro activity of thymidylate synthase partially purified from liver of control and vitamin B-6 deficient rats.

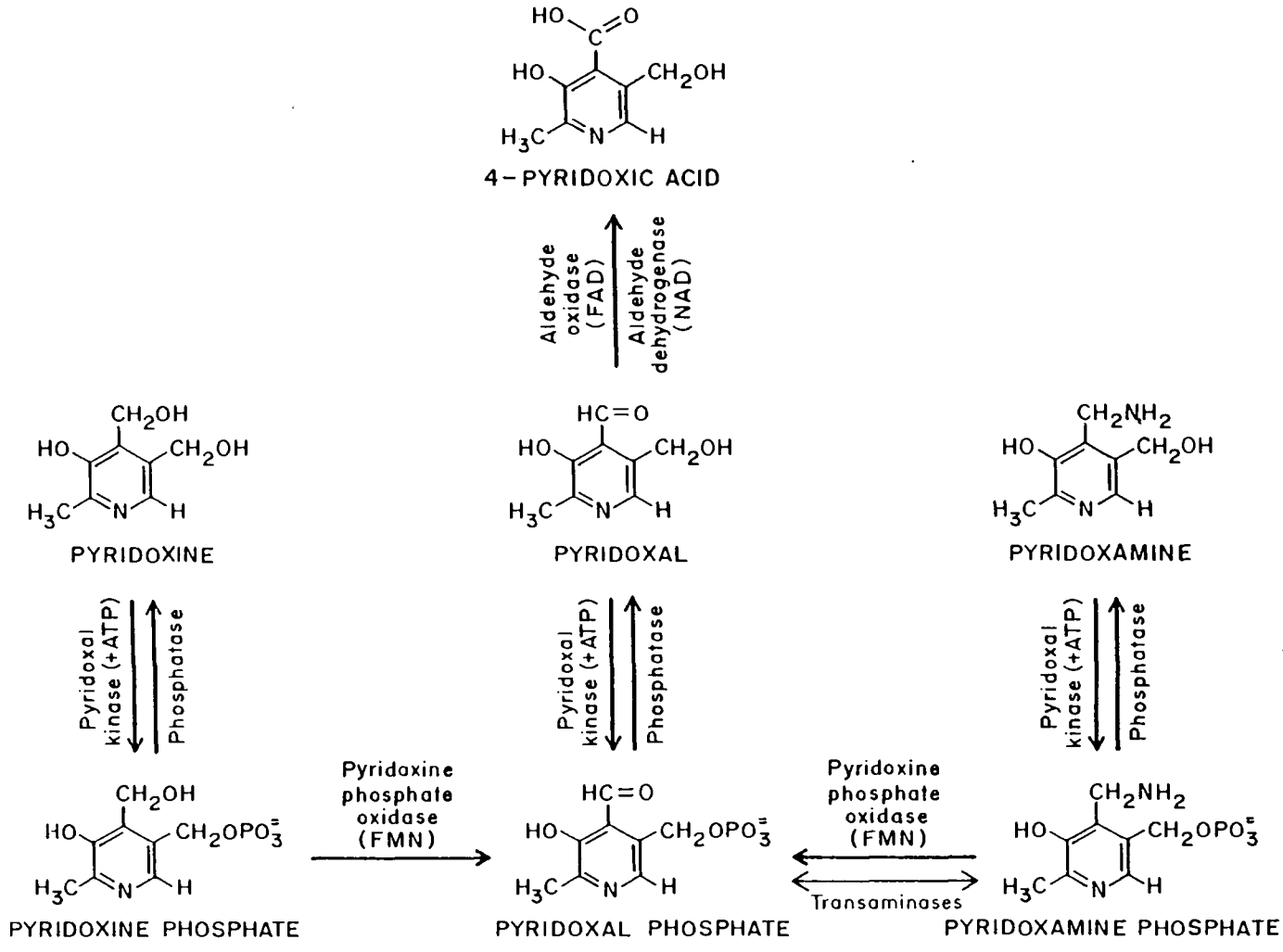
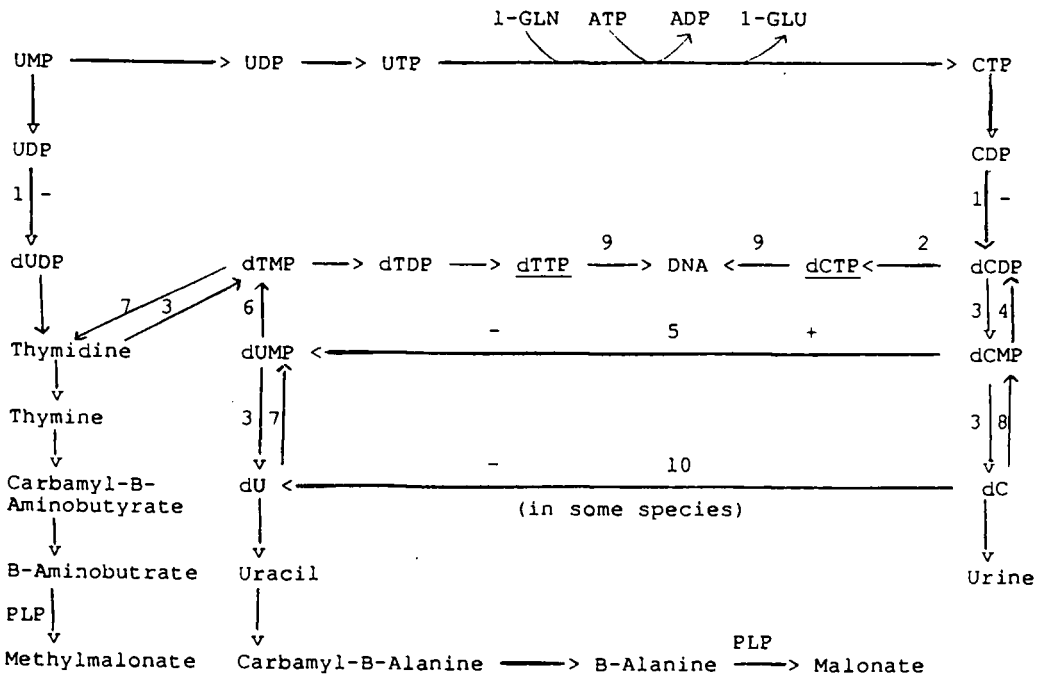


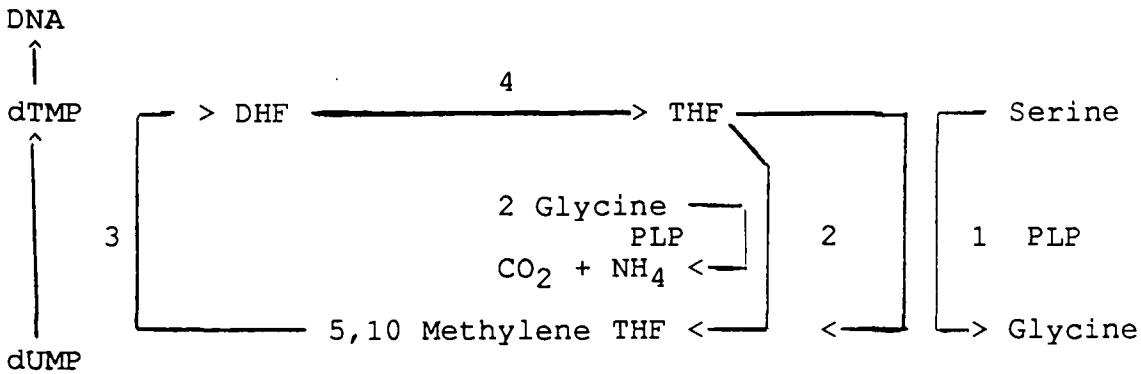
Figure 2-1. Metabolism, structure and common names of the 6 major forms of vitamin B-6 and the major metabolite 4-pyridoxic acid.



ENZYMES	MODULATOR dTTP	dCTP
1. Ribonucleoside diphosphate reductase	-	
2. Nucleoside diphosphokinase		
3. Nucleoside phosphorylase		
4. Deoxycytidine kinase		
5. Deoxycytidylate deaminase	-	+
6. Thymidylate synthase		
7. Nucleoside kinase	-	
8. Deoxycytidne kinase	-	-
9. DNA polymerase		
10. Deoxycytidine deaminase	-	

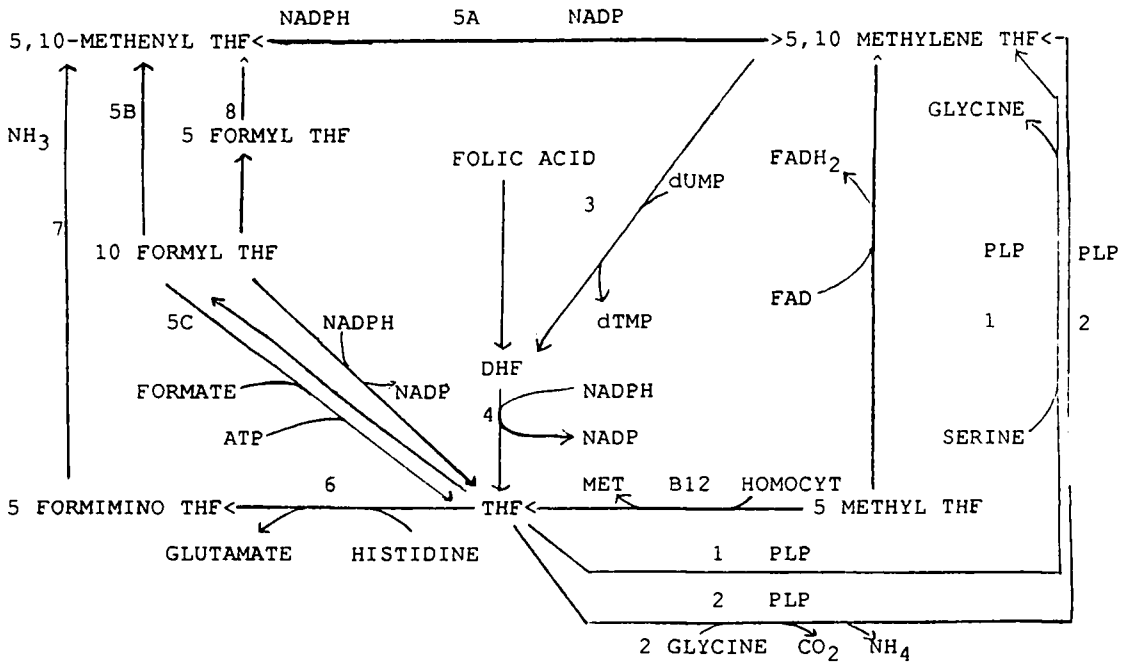
(- = inhibition, + = stimulation)
Adapted from: Zaharko, (1983)

Figure 2-2. Biochemical pathways involved in pyrimidine deoxynucleotide metabolism.



Enzyme	Cofactor
1. Serine Transhydroxymethylase (EC 2.1.2.1)	PLP
2. Glycine Cleavage Complex (Glycine Synthetase) (EC 2.1.2.10)	PLP
3. Thymidylate Synthase (EC 2.1.1.45)	5,10,MTHF
4. Dihydrofolate Reductase (EC 1.5.1.3)	

Figure 2-3. Interrelationship between Folate, Pyridoxal 5'-phosphate and Pyrimidine Deoxynucleotide Synthesis.



ENZYME	COFACTOR
1. Serine transhydroxymethylase (EC 2.1.2.1)	PLP
2. Glycine cleavage complex (Glycine synthase) (EC 2.1.2.10)	PLP
3. Thymidylate synthase (EC 2.1.1.45)	5,10-MTHF
4. Dihydrofolate reductase (EC 1.5.1.3)	NADPH
5. Trifunctional protein:	
A. 10-formyltetrahydrofolate synthase/	
B. 5,10 methenyltetrahydrofolate cyclohydrolase/	
C. 5,10-methylenetetrahydrofolate dehydrogenase.	
6. Glutamate formiminotransferase (Formiminoglutamate: THF-formiminotransferse) (EC 2.1.2.5)	
7. Formiminotetrahydrofolate Cyclodeaminase (EC 4.3.1.4)	
8. Methenyltetrahydrofolate synthetase (5 formyltetrahydrofolatecyclodehydrase) (EC 6.3.3.2)	

Figure 2-4. Folate Metabolism

Table 2-1. Literature values of pyridoxal 5'phosphate concentration for a number of tissues.

Tissue		Buchin[1]	Wachstein[2]	Coburn[3]	Bhagavan[4]
Adrenals	C[5]	---	---	2.95±0.49	2.87±0.61
	D[5]	---	---	0.73±0.24	0.35±0.10
Brain	C	9.02±0.89	---	---	---
	D	---	---	---	---
Heart	C	8.96±2.59	2.5(2.3-2.8)	---	---
	D	---	0.6(0.3-0.8)	---	3.52±0.10
Intestine	C	5.34±0.65	---	---	---
	D	(Mucosa)	---	0.81±0.04[6]	---
Kidney	C	22.3±2.3	2.6(2.3-2.8)	---	7.8±0.39
	D	---	0.4(0.04-0.08)	---	8.9±0.08
Liver	C	53.9±1.3	2.7(2.6-2.8)	15.4±1.2	---
	D	---	0.6(0.2-0.8)	9.7±0.4	---
Lung	C	1.42±0.77	---	---	1.23±0.10
	D	---	---	---	0.18±0.01
Muscle	C	25.6±7.6	2.9(2.7-3.1)	23.5±1.6	---
	D	---	0.6(0.2-0.7)	14.2±2.4	---
Pancrease	C	14.0±1.0	---	---	---
	D	---	---	---	---
Spleen	C	4.09±0.36	---	---	---
	D	---	---	---	---
Thymus	C	---	---	0.57±0.16	---
	D	---	---	0.49±0.10	---
Blood	C	---	478(365-637)	---	---
	D	---	67(15-116)	---	---
Plasma	C	---	495(419-556)	353±53	301±99
	D	---	84(61-98)	15.4±2.8	18.0±2.4
RBC	C	---	---	---	---
	D	---	---	23.7±7.7[6]	---
Leucocytes	C	---	4.8(3.5-5.8)	---	---
	D	---	1.0(0.5-1.9)	---	---

1. Buchin (1976): Ad libitum, 130-150 g male Wistar rats, 1 mg PN/100 g diet, apotryptophanase, non-deficient.
2. Wachstein (1958): Ad libitum, 120 g male Wistar rats, 2.2 mg PN HCl/100 g diet, tyrosine decarboxylase, 6 week deficiency.
3. Coburn (1981): Ad libitum, 300-400 g male Wistar rats, 2.2 mg PN HCl/100 g diet, tyrosine decarboxylase, 5 week deficiency.
4. Bhagavan (1976): Ad libitum, male weanling Sprague-Dawley rats, 3.0 mg PN HCl/100 g diet, tyrosine decarboxylase, 8 week deficiency.
5. C = control; D = deficient rats.
6. These data were preliminary unpublished data from the Coburn study and rats were maintained on the deficient diet for less time than the other rats.

CHAPTER 3

CHAPTER 3

EFFECT OF VITAMIN B-6 DEFICIENCY ON SPECIFIC ACTIVITY OF SERINE TRANSHYDROXYMETHYLASE IN RAT THYMUS, SPLEEN, BONE MARROW AND LIVER

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Abstract

The purpose of this study was to determine the effect of a vitamin B-6 (B-6) deficiency on the activity of serine transhydroxymethylase (STHM) in rat spleen, thymus, bone marrow and liver. Four experimental groups were studied: ad libitum control (ALC), pair fed control (PFC), ad libitum B-6 deficient (ALD) and meal fed B-6 deficient (MFD). Tissues were collected at the end of weeks 2 and 6 and at the end of a 2 week B-6 repletion period (feeding of the control diet). Thymus, spleen and bone marrow STHM activity was significantly lower ($p \leq 0.05$) in the B-6 deficient rats by the end of week 2. In the liver STHM activity was significantly lower in the ALD than in the ALC but not MFD or ALD compared to PFC until the end of week 6 of the deficiency. Addition of PLP to the enzyme assay did not restore enzyme activity to the control levels although the % stimulation was generally significantly greater in the B-6 deficient rats. STHM activity in all tissues of B-6 repleted rats increased to levels comparable to or greater than that of control rats. Feeding patterns influenced STHM activity differently in various tissues. In liver, the mean STHM activity of PFC rats was about 50% lower than that of the activity of ALC rats. Mean spleen STHM activity of PFC rats was 16-33% higher than that of ALC rats. Similarly, spleen STHM activity of MFD rats was greater than that of ALD rats. Results indicate that activity of STHM was

decreased in a B-6 deficiency and the effect was seen sooner in rapidly proliferating tissues.

Key words: Serine transhydroxymethylase, vitamin B-6 deficiency, pyridoxal 5'phosphate, spleen, thymus, bone marrow, liver.

Introduction

Pyridoxal 5'phosphate (PLP), the active form of vitamin B-6, is a cofactor for serine transhydroxymethylase (STHM) (EC 2.1.2.1) (Blakley, 1955). This enzyme catalyzes the conversion of tetrahydrofolate (THF) to 5,10-methylenetetrahydrofolate (5,10-M-THF) which is required for synthesis of pyrimidine deoxynucleotides and thus DNA synthesis. Decreased activity of STHM would be expected to impair cell proliferation. Takami et al. (1968) found there was no effect of a 60 day vitamin B-6 deficiency on the activity of this enzyme in either the soluble or mitochondrial fraction of rat liver. Furthermore, hepatic regeneration, a condition of rapid cell proliferation, was not impaired as a result of the vitamin B-6 deficiency. In contrast, studies by Axelrod et al. (1964, 1971) and Robson et al. (1975) strongly support the thesis that a vitamin B-6 deficiency inhibits the immune system by interfering with the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP). This reaction can be

affected by STHM activity which gives rise to 5,10-MTHF, the cofactor for thymidylate synthase.

To further explore the apparent discrepancy between the work of Takami and that of Axelrod and Robson, the present study was undertaken. The purpose of the study was to assess the effect of a vitamin B-6 deficiency on the activity of STHM in several tissues, including those associated with the immune system. In addition, the enzyme activity was determined in liver so results could be compared with the study of Takami et al. (1968). Enzyme activity was assessed with and without added PLP following both a short term and a long term vitamin B-6 deficiency. The hypothesis tested was that a vitamin B-6 deficiency would decrease STHM activity and the decrease would be more profound in rapidly proliferating tissues than in liver.

Vitamin B-6 deficiency results in decreased appetite and thus decreased growth rate. Pyrimidine deoxynucleotide metabolism is involved in DNA synthesis and DNA synthesis is essential to growth. Thus, factors which result in reduced rate of growth may be expected to affect DNA synthesis and may affect pyrimidine deoxynucleotide metabolism. To eliminate the reduction in food intake and growth rats as variables, we included a control group of rats which were pair fed to the ad libitum deficient rats. Because pair feeding results in meal feeding, we also included a meal fed deficient group to eliminate meal feeding as a variable. In addition, we conducted fasting studies on both the deficient

and control rats to assess the impact of an acute caloric deprivation on STHM activity. Enzyme levels in spleen, thymus, bone marrow, liver, intestine, and heart were measured.

Materials and Methods

Animals

Weanling male Wistar rats (60 to 80 g) (purchaced from Charles Rivers Laboratory) were randomly assigned to 4 groups: ALC (12 rats with ad libitum access to the control diet), ALD (36 rats with ad libitum access to a B-6 deficient diet), PFC (36 rats pair fed to the ALD group with the control diet) and MFD (12 rats allowed access to the deficient diet for two hours daily). The four groups were subdivided into groups of six for various treatments. The animals were housed individually in wire bottomed stainless steel cages and exposed to a 12 hour light-dark cycle. Water was provided ad libitum at all times. Body weights were taken weekly.

Diet

The animals were fed a semisynthetic diet which conformed to AIN-76 nutrient recommendations for the rat (AIN 1977, 1980) with the exception of vitamin B-6. The diet contained 20% casein, 50% sucrose, 15% cornstarch, 5% corn oil, and 5% fiber as Celufil. The remaining 5% was an AIN vitamin and mineral mix, DL-methionine and choline. Analysis of the two diets prior to the start of the study

indicated that the control diet contained 5.7 mg pyridoxine (PN)/kg diet and the vitamin B-6 deficient diet contained 0.04 mg PN/kg diet. Details on the diet have been reported elsewhere (Cho, 1989).

Feeding

All animals were adapted to the control diet for 1 week prior to being fed their respective diets. Training for the meal fed animals was begun during this period of adaptation during which they had daily access to food for only two hours, from 8:00 to 10:00 a.m. Each of the 4 groups were then fed their respective diets for 2 or 6 weeks. At the end of the 6th week, subgroups of 6 animals each from the PFC and ALD groups were fasted for 1 or 3 days. At the end of week 6, 12 ALD rats were repleted with ad libitum access to the control diet. The remaining 12 PFC rats continued to be pair fed to these 12 remaining ALD rats. By the end of the two week repletion period all animals were consuming control diet ad libitum. After repletion, 6 animals from each group were fasted for 3 days.

Collection of Samples

At the respective time points (2 weeks non-fast, 6 weeks non-fast, 6 week 1 and 3 day fast, repleted non-fast and repleted 3 day fast) animals were anesthetized with CO2 and sacrificed by decapitation. Immediately following decapitation, liver, spleen, and thymus were rapidly excised, weighed, frozen in liquid nitrogen and stored frozen at -34^{o}C until analyzed. The intestine was severed

just below the stomach, cut into 5 cm sections and distributed into various vials for a number of analyses. Bone marrow was obtained by washing the contents of the tibia and the fibula into a vial with a syringe filled with 5 ml of ice cold saline. The mixture was centrifuged for 15 minutes at 2000 X G and saline removed. The resulting pellet was stored frozen at -34^{o}C until analyzed.

Analysis

The vitamin B-6 content of the diet was determined microbiologically, using Saccaromyces uvarum (ATCC No. 9080) as the assay organism (AOAC 1980). STHM was measured with a modification of a radiometric assay (Taylor and Weissbach, 1965). The assay was modified by determining the enzyme activity with and without exogenous PLP (0.1 umoles/incubation tube). Water was added in place of PLP when the enzyme was assessed without added PLP. Measurement with and without PLP allowed us to assess the in vitro stimulation of the enzyme by PLP and gave an estimate of endogenous apo and holoenzyme activity. All samples were assayed in duplicate. The within and between assay coefficient of variation (CV) for liver was 10%, (n=4) and 4%, (n=5), respectively. PLP was determined by a modified radiometric enzyme method (Chabner et al. 1970). Control samples were analyzed with each assay. The within and between assay CV for plasma was 4.2%, (n=6) and 8.5%, (n=21), respectively. Protein was measured colorimetrically using the Bio Rad Protein Assay based on a colorimetric dye

method described by (Bradford, 1976). The within and between assay CV for liver was 5%, (n=8) and 2% (n=6), respectively. Tissue DNA was measured fluorometrically (Labarca et al., 1980). The within and between assay CV for liver was 14%, (n=4) and 6%, (n=5), respectively. Due to the high within assay variation, samples were run in triplicate.

Statistical Analysis

Correlations between STHM activity and tissue PLP levels were determined. In addition, all data were analyzed by variance and tested for significant differences by the least significant difference (LSD) test (Heintz, 1986). A p value ≤ 0.05 was considered to be significant. All values are given as the mean $\pm$ standard deviation.

Results

Effect of Vitamin B-6 Deficiency on Growth

ALD and MFD rats had lower rates of growth than the ALC and PFC counterparts, respectively. Table 3-1 gives mean body weights for each group at the end of week 2, 6 and after the repletion period. The mean body weight of the ALD rats, while consistently less, was not significantly lower than that of the PFC rats until the end of week 6 of the deficiency. This indicates that the deficiency of vitamin B-6, in addition to reduced food intake was responsible for the growth inhibition seen in the ALD rats. By the end of

the vitamin B-6 repletion period, body weight of the repleted ALD (RALD) rats was comparable to that of the repleted PFC (RPFC) rats and growth rates of the two groups were comparable.

Effect of Vitamin B-6 Deficiency on Tissue PLP Concentrations

As reflected in Table 3-2, mean tissue PLP levels of the deficient rats were 30-56% of the control rats by the end of week 2 and decreased to 17-27% of the control level at the end of week 6. Two weeks of vitamin B-6 repletion restored tissue PLP levels to normal. The decreases in tissue PLP levels were independent of meal and pair feeding.

Compared to their non-fasted counterparts, fasting for 1-3 days resulted in a 21-25% decrease ($p<0.05$) in PLP concentration of the liver of PFC, RPFC and RALD rats. Fasting did not result in a significant change in PLP concentration in the spleen from these animals. Similarly, the PLP concentration in thymus was not altered by fasting in the RPFC and RALD rats. The mean PLP concentration in bone marrow was significantly increased by 175% in PFC rats following a three day fast.

In the ALD rats, fasting resulted in a significant increase in spleen and bone marrow PLP concentration. While the mean liver PLP concentration also increased, there was no change in total liver PLP content. The apparent discrepancy between liver PLP concentration and total liver PLP content is related to compositional changes of liver

associated with pair feeding and fasting. Changes in PLP content of thymus from 6 week fasted PFC (FPFC) and ALD (FALD) rats could not be determined because the vitamin B-6 deficiency resulted in atrophy of the thymus tissue, so that by the end of week 6, the tissue was too small to measure remove for assay.

The metabolism and distribution of vitamin B-6 in these rats is discussed elsewhere (Cho, 1987). The influence of the deficiency on PLP distribution was the same regardless of whether the data were expressed per g wet weight, per ug DNA or per mg protein. Results are expressed per mg protein for ease of comparison with the enzyme activity except for bone marrow.

Effect of a Vitamin B-6 Deficiency on STHM Activity

Liver: STHM activity of liver was unique among the tissues measured in response to the vitamin B-6 deficiency. As can be seen in Table 3-1, the mean activity (per mg protein) of STHM in liver of ALD rats was not significantly lower than that of the PFC rats until the end of week 6 of the deficiency. However when activity was expressed on a per tissue basis, the mean STHM activity of ALD rats was not significantly different from that of the PFC rats even after week 6 of the deficiency. This is significant since the mean body weights of these two groups were similar (Table 3-1).

Vitamin B-6 repletion resulted in an increase in total mean liver enzyme activity in both PFC and ALD rats, but

activity never reached 6 week ALC levels. The 3 day fast at 6 weeks and after repletion resulted in a decrease of STHM activity and total liver enzyme content. The decrease in activity was comparable in both PFC and ALD groups. Since the PFC and MFD animals were semi-starved, their liver weights were less than their ad libitum counterparts due to lower levels of glycogen, protein and associated water (reported elsewhere: Cho, 1987). When the ALC and ALD groups were compared, both groups had similar mean liver weight and protein content. In spite of this, mean liver STHM activity was 2.5 times greater in the ALC rats. Similarly, mean liver STHM activity was also 2.5 times greater in PFC than MFD rats.

The in vitro stimulation of STHM by PLP is summarized in Table 3-5. These data suggest that in liver, the vitamin B-6 deficiency per se did not influence the enzyme activity independent of food deprivation until the end of week 6 of the deficiency. This is in agreement with the results on total liver STHM activity. Liver PLP concentration did not parallel changes in the STHM activity.

Spleen: At the end of week 2 of the deficiency, the mean activity of spleen STHM without added PLP in the deficient groups was 52-58% of the mean STHM activity in the control groups (Table 3-4). The STHM activity decreased further in the deficient groups to 20-28% of control values by the end of week 6. Similarly, compared to the mean activity of the control groups, the mean STHM activity with

added PLP was significantly lower in the deficient groups by the end of week 6. When ALD rats were repleted with vitamin B-6, STHM activity without added PLP increased but did not reach levels comparable to RPFC or the STHM activity of the ALC rats at week 6.

The feeding schedule also influenced the STHM activity in the spleen. This was evident by the significantly lower mean activity in both ad libitum groups, compared to the activity of the respective pair and meal fed groups. Furthermore, fasting the ALD rats resulted in an increase in spleen STHM activity to no-fast MFD levels. In RPFC rats, there was a decrease in STHM activity compared to the activity in non-fasted PFC rats. However, the difference was significant only for the in vitro stimulated activity.

The in vitro stimulation of STHM with PLP in the spleen was greater in the deficient groups at all time periods (Table 3-5). This confirmed the results relating activity of the enzyme to vitamin B-6 deficiency. In vitro stimulation in RALD rats reflects a return toward ad libitum control levels in this group. Changes in spleen PLP concentration paralleled changes seen in activity of STHM except following the two week vitamin B-6 repletion period.

Thymus: Mean thymus STHM activity with and without added PLP was significantly lower than that of the control rats. Vitamin B-6 repletion of ALD rats restored the STHM activity to a mean level above that seen in control rats at week 2. Significantly higher in vitro stimulation of STHM

in the deficient rats varifies decreased enzyme activity in the vitamin B-6 deficiency. The change in mean thymus PLP levels at week 2 as a result of the deficiency was consistent with changes in STHM activity.

Bone Marrow: After 2 weeks of deficiency, the mean specific activity of STHM in ALD and MFD rats was significantly lower at 12 and 14% that of the respective control groups. Activity fell to barely detectable levels by the end of week 6 of the deficiency and returned to normal after 2 weeks of vitamin B-6 repletion. Fasting resulted in an increase in enzyme activity both with and without added PLP at 6 weeks and after the repletion period. The in vitro stimulation of STHM with added PLP in bone marrow from the deficient rats was significantly greater than that from the control rats. Changes in PLP concentrations paralleled changes in enzyme activity.

Other Tissues: STHM activity was also measured in heart muscle and intestine. Unstimulated activity in the heart was so low in all groups that it was often undetectable. In the intestine, the effect of the vitamin B-6 deficiency on STHM activity was apparent. Mean intestine enzyme activity levels with and without added PLP were lower in the deficient rats throughout the deficiency. However, in vitro stimulation of the enzyme with added PLP was greater in the deficient rats than the stimulated STHM activity of controls at week 2 but was lower at week 6. This difference was due to the relatively higher

unstimulated enzyme activity in the deficient rats at week 6 compared to week 2. However, due to the large standard deviation in intestineal STHM activity, the differences were not significant.

Discussion

These data demonstrate that the activity of STHM is significantly decreased by a vitamin B-6 deficiency, especially in rapidly proliferating tissues. This was true even before outward physical symptoms of the deficiency such as decreased appetite or rate of growth manifested themselves. Changes in enzyme activity paralleled changes in tissue PLP levels in bone marrow, spleen and thymus. Regression analysis comparing tissue PLP levels to unstimulated STHM activity revealed correlation coffecients of 0.63, 0.81, 0.90 and 0.62 for liver, spleen, thymus and bone marrow respectively. When tissue PLP levels were compared to stimulated STHM activity correlation coeficients for liver, spleen, thymus and bone marrow 0.4,0.41. 0.76 and 0.39, respectively. The low STHM activity in these tissues from the deficient rats may have contributed to impaired cell proliferation by interfering with pyrimidine deoxynucleotide synthesis. Axelrod and coworkers (1964, 1971) and Robson et al. (1975) have presented data strongly supporting the thesis that vitamin B-6 deficiency inhibits conversion of dUMP to dTMP in cells of the immune system.

Our data suggest STHM may be the limiting factor for this inhibition.

The vitamin B-6 deficiency resulted in reduced STHM activity following a relatively short term deficiency of two weeks. This effect was seen slightly before the growth rate and appetite of the deficient rats began to be affected. We observed a significant decrease in the enzyme activity as early as one week into the vitamin B-6 deficiency (data not shown). The significance of the early effect of a deficiency on the activity of STHM is apparent when considering the impact of a marginal vitamin B-6 intake on the rapidly proliferating tissues of the immune system. Vitamin B-6 deficiency in animals results in decreased lymphocyte production (Van den Berg, 1988; Robson and Schwartz, 1975) and antibody response to antigen (Chandra and Puri, 1985). The potential impact of a marginal vitamin B-6 deficiency in humans has been explored by Talbot et al. (1987). They found the immune function in elderly females was impaired and intake of 50 mg PN/day for two months improved the immune function as judged by lymphocyte response. These conclusions in conjunction with results reported here suggest the potential negative impact of a marginal intake of vitamin B-6 on immune function warrants further exploration.

Our conclusions regarding the impact of a vitamin B-6 deficiency on liver STHM activity are not consistent with those of Takami (1968) who found no difference in STHM

activity between ad libitum control and vitamin B-6 deficient rats. Differences between results reported by Takami et al. and our work may relate to differenent methods used. We found enzyme levels to be lower in livers from vitamin B-6 deficient rats after only 2 weeks of deficiency. However, in vitro stimulation was not significantly greater until after week 6. There also appeared to be an effect of reduced food intake as revealed by decreased STHM activity due to pair feeding and to fasting. This decrease appears to be independent of the vitamin B-6 deficiency. The fact that liver regeneration (a condition of rapid cell proliferation) is not impaired in vitamin B-6 deficiency (Takami et al. 1968) may reflect a mobilization of muscle PLP secondary to the catabolic state induced by the trauma of surgery. This hypothesis is strengthened by results of a recent publication on vitamin B-6 status in the postoperative state (Pirkka, 1989).

In summary, our data support the thesis that a vitamin B-6 deficiency decreases the activity of STHM in rapidly proliferating tissues associated with the immune system. This decrease may contribute to the inhibition of the immune function seen in vitamin B-6 deficient animals. The fact that the decrease was seen early in the deficiency suggests that marginal vitamin B-6 status may increase the risk of impaired immune function.

Table 3-1. Effect of a vitamin B-6 deficiency on mean body weight[1].

	2 WEEK RATS[2] (G)	
	WEEK 0	WEEK 2
ALC[3]	120±14[a]	228±19[a4]
PFC	122±8[a]	205±7[a]
ALD	128±11[a]	218±15[a]
MFD	104±16[b]	155±37[b]

	6 WEEK RATS[2] (G)				
	WEEK 0	WEEK 3	WEEK 4	WEEK 5	WEEK 6
ALC	118±14[a]	261±28[a]	308±27[a]	340±25[a]	364±28[a]
PFC	131±7[a]	246±9[ab]	267±9[b]	283±9[b]	289±8[b]
ALD	124±6[a]	223±7[b]	241±11[b]	253±13[c]	254±18[c]
MFD	103±15[a]	155±25[c]	167±33[c]	175±35[d]	179±40[d]
FASTED					
PFC Day 1	---	---	---	---	285±5[b]
Day 3	---	---	---	---	254±20[c]
ALD Day 1	---	---	---	---	249±30[c]
Day 3	---	---	---	---	228±26[c]

	REPLETED RATS[2] (G)		
	WEEK 0	WEEK 6	WEEK 8
ALC[3]	---[5]	---	---
PFC	114±12[a]	270±18[a]	338±36[a]
ALD	118±5[a]	256±26[a]	342±28[a]
MFD	---	---	---
FASTED			
PFC Day 3	121±13[a]	280±[a]	320±8[a]
ALD Day 3	128±[a]	263±39[a]	306±37[a]

1. Values represent mean ± standard deviation of six rats in the respective groups unless otherwise indicated (ie. $=3, #=4, *=5, nd=non-detectable).
2. Week 2 and Week 6 refer to the deficiency period. The deficient and control diets contained 0.04 and 5.7 mg pyridoxine/kg, respectively. Repletion refers to a 2 week refeeding period with control diet at the end of week 6.
3. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.
4. Within a given column, those values with different letters are significantly different ($p<0.05$).
5. --- signifies missing data.

Table 3-2. Effect of vitamin B-6 deficiency on the mean pyridoxal 5'phosphate concentration[1] in selected tissues.

	LIVER	SPLEEN	THYMUS	BONE MARROW
	nmoles per			
	mg protein	mg protein	mg protein	ug DNA
WEEK 2[1]				
ALC[1]	166±22[a2]	27±2[a]	42±4[a]	94±13[a]
PFC	136±10[b]	31±4[a]	40±5[a]	75±32[a]
ALD	51±13[c]	8±2[b]	12±2[b]	30±19[b]
MFD	69±14[d]	9±2[b]	15±1[b]	27±8[b]
WEEK 6[1]				
ALC	156±10[a]	25±3[a]	---[3]	92±8[a]
PFC	158±15[a]	25±4[a]	---	123±24[b]
ALD	42±11[b]	6±1[b]	---	25±7[c]
MFD	55±9[bc]	6±1[b]	---	21±6[c]
FASTED				
PFC Day 1	135±8[d]	30±3[c]	---	164±12[d]
Day 3	130±17[d]	27±2[ac]	---	217±41[e]
ALD Day 1	65±10[c]	8±1[b]	---	80±13[a]
Day 3	65±5[c]	8±1[b]	---	80±13[a]
REPLETION[1]				
RPFC[4]	176±8[a]	28±2[a]	49±8[a]	160±18[a]
RALD[4]	179±13[a]	26±[a]	47±2[a]	136±8[a]
FASTED				
RPFC Day 3	126±12[b]	28±8[a]	36±18[a]	140±35[a]
RALD Day 3	128±25[b]	25±2[a]	44±10[a]	140±28[a]

1. See Table 3-1 for details.
2. Within a given column, within a given time period (ie. week 2, week 6 and repletion), those values with different letters are significantly different ($p<0.05$).
3. Samples not taken or unavailable for analysis (see text for explanation).
4. RPFC = repleted prior pair fed control; RALD = repleted prior ad libitum deficient.

Table 3-3. Effect of vitamin B-6 deficiency on the mean activity of STHM[1] with and without pyridoxal 5'phosphate added to rat liver homogenate.

	LIVER STHM ACTIVITY				LIVER WEIGHT (g)
	U per mg protein[2]		U per LIVER		
	- PLP[4]	+ PLP[4]	- PLP	+ PLP	
WEEK 2[1]					
ALC[1]	256±72[a3]	1104±209[a]	580±230[a]	2380±730[a]	12.7±2.6[a]
PFC	166±57[b]	1058±89[a]	290±110[b]	1890±280[a]	8.9±0.9[b]
ALD	148±27[b]	929±219[ab]	312±70[b]	1980±560[a]	12.7±2.2[a]
MFD	110±36[b]	773±96[b]	150±60[b]	1030±140[b]	6.2±1.4[c]
WEEK 6[1]					
ALC	456±33[a]	1664±90[a]	1110±230[a]	4020±670[a]	13.8±2.1[a]
PFC	264±38[b]	983±217[b]	460±60[b]	1700±360[bc]	8.2±0.3[b]
ALD	191±40[c]	862±189[b]	440±110[b]	1990±540[c]	12.3±1.0[a]
MFD	184±63[c]	923±202[b]	230±50[c]	1160±320[b]	6.1±1.4[c]
FASTED					
PFC					
Day 1	236±43[bc]	1016±169[b]	360±60[bd]	1550±250[bc]	7.1±0.3[d]
Day 3	217±54[bc]	740±211[b]	300±70[d]	1020±270[d]	6.5±0.4[e]
ALD					
Day 1	209±76[bc]	923±179[b]	340±140[d]	1550±450[bc]	8.1±1.3[bf]
Day 3	205±30[bc]	861±226[b]	320±80[d]	1320±320[d]	7.1±0.9[def]
REPLETION[1]					
RPFC	278±25[ab]	1132±162[a]	610±130[a]	2370±440[a]	12.3±2.1[a]
RALD	348±96[b]	1177±147[a]	860±220[b]	2950±270[b]	14.2±1.3[a]
FASTED DAY 3					
RPFC	233±31[a]	798±96[b]	370±60[c]	1260±170[c]	7.5±0.2[b]
RALD	209±44[a]	696±244[b]	320±80[c]	1070±410[c]	7.9±0.6[b]

1. See Table 3-1 and 3-2 for details.
2. U = nmoles formaldehyde formed/hour per mg protein or per liver.
3. Within a given column, within a given time period (ie. week 2, week 6 and repletion), those values with different letters are significantly different ($p<0.05$).
4. - PLP and + PLP refers to the deletion or addition of PLP to the STHM assay, respectively.

Table 3-4. Effect of vitamin B-6 deficiency on the mean activity of STHM[1] with and without pyridoxal 5'phosphate added to the homogenates of rat spleen, thymus and bone marrow.

	SPLEEN (U/mg protein)[2]		THYMUS (U/mg protein)[2]		BONE MARROW (U/ug DNA)	
	- PLP[2]	+ PLP[2]	- PLP	+ PLP	- PLP[3]	+ PLP
WEEK 2[1]						
ALC[1]	50±16[a3]	300±39[a]	176±41[a]	812±143[a]	483±60[a]	1170±110[a]
PFC	72±12[b]	290±33[a]	186±36[a]	851±46[a]	408±70[b]	1125±170[a]
ALD	26±6[c]	283±40[a]	31±19[b]	567±93[b]	58±78[c]	807±330[b]
MFD	42±16[a]	298±45[a]	43±19[b]	661±45[b]	57±56[c]	722±250[b]
WEEK 6[1]						
ALC	65±14[a]	280±28[ab]	---[1]	---	108±54[ab]	591±166[ab]
PFC	76±7[a]	327±74[b]	---	---	212±131[c]	654±258[ab]
ALD	13±10[b]	223±33[acd]	---	---	nd[3]	526±200[a]
MFD	21±11[bc]	188±36[cde]	---	---	37±23[ac]	465±354[a]
FASTED						
PFC						
Day 1	63±8[a]	260±50[ad]	---	---	755±114[d]	1752±212[c]
Day 3	68±20[a]	254±50[adf]	---	---	308±46[e]	853±123[b*]
ALD						
Day 1	22±9[bc]	212±39[fd]	---	---	356±77[e]	1402±153[d]
Day 3	29±10[c]	232±40[acde]		---	---	113±52[a]
687±262[ab]						
REPLETION[1]						
RPFC	72±11[a]	276±36[a]	189±65[a]	953±142[a]	288±72[a]	802±210[a]
RALD	47±18[a]	259±20[ab]	282±27[b]	1088±79[b]	292±26[a#]	650±252[a#]
DAY 3 FASTED						
RPFC	43±18[b#]	181±40[c#]	180±16[a]	830±46[a]	395±72[a]	815±180[a]
RALD	48±23[ab]	223±60[bc]	200±38[a]	904±68[a]	318±80[a]	825±150[a]

1. See Table 3-1 and 3-2 for details.
2. U = nmoles formaldehyde formed/hour/mg protein.
3. Within a given column, within a given time period (ie. week 2, week 6 and repletion), those values with different letters are significantly different ($p<0.05$).

Table 3-5. Mean percent in vitro stimulation[1] of STHM[2] by pyridoxal 5'phosphate added to the assay homogenate of selected tissues from control and vitamin B-6 deficient rats.

	LIVER	SPLEEN	THYMUS	BONE MARROW
WEEK 2[2]				
ALC[2]	77±3[a3]	84±4[a]	78±7[a]	59±4[a]
PFC	85±4[a]	75±5[a]	78±5[a]	61±6[a]
ALD	84±4[a]	91±2[b]	95±4[b]	95±6[b]
MFD	86±5[a]	86±4[b]	95±4[b]	92±8[b]
WEEK 6				
ALC	73±2[a]	77±4[a]	---[2]	83±6[a]
PFC	72±6[a]	76±5[a]	---	79±11[a]
ALD	78±3[b]	94±4[b]	---	100±0[b]
MFD	80±5[b]	88±9[b]	---	92±6[b]
FASTED				
PFC Day 1	76±6[ab]	75±6[a]	---	55±8[c]
Day 3	70±6[ab]	73±8[a]	---	64±10[c]
ALD Day 1	77±5[ab]	89±5[b]	---	75±4[b]
Day 3	75±5[ab]	88±3[b]	---	84±3[a]
REPLETION				
PFC	72±4[a]	74±5[a]	80±6[a]	64±7[a]
ALD	70±7[a]	81±6[a]	80±6[a]	73±4[b]
FASTED				
PFC Day 3	70±6[a]	79±2[a]	78±[a]	51±4[c]
ALD Day 3	68±7[a]	79±7[a]	78±4[a]	61±7[a]

1. Percent stimulation = (activity with PLP- activity without PLP) / (activity without PLP) X 100.
2. See Table 3-1 for details.
3. Within a given column, within a given time period (ie. week 2, week 6 and repletion), those values with different letters are significantly different ($p<0.05$).

CHAPTER 4

CHAPTER 4

EFFECT OF VITAMIN B-6 DEFICIENCY ON URINARY EXCRETION OF DEOXYCYTIDINE AND METABOLISM OF ^{3}H-DEOXYCYTIDINE IN THE RAT

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ABSTRACT

This study was undertaken to examine the effect of vitamin B-6 (B-6) deficiency on deoxycytidine (dC) excretion and metabolism. Four groups of rats were studied: ad libitum control (ALC), pair fed control (PFC), ad libitum deficient (ALD) and meal fed deficient (MFD). At the end of weeks 2 and 6, animals received an ip injection of ^{3}H-dC and urine was collected for 24 hours. Animals were then sacrificed and tissues collected. In addition, four subsets of each of the 6 week PFC and ALD rats were fasted for 3 days, repleted with B-6 for 2 weeks or fasted for 3 days following B-6 repletion. Mean urinary dC in the deficient rats decreased to 40 and 9% of levels excreted by ALC at week 2 and 6, respectively. Excretion of dC increased by 125% in the ALC rats between weeks 2 and 6 whereas excretion decreased by 42% in PFC rats during this time. Thus, dC excretion in the 6 week PFC rats was only 28% of the level excreted by the 6 week ALC rats. Fasting resulted in a decrease of dC excretion which was independent of vitamin B-6 deficiency. Excretion of dC in 2 and 3 day fasted, ad libitum control and deficient rats was 18-24% of pre-fast levels. The excretion of dC after 2 and 3 days of fasting in the PFC rats was 59% of pre-fast levels. The dC excretion increased after B-6 repletion to levels greater than the 2 week and less than the 6 week ALC levels. Deficient rats excreted a lower percentage of the injected label and a lower percentage of the label as dC than did the

control rats. Compared to control rats, B-6 deficient rats retained more label in liver and bone marrow, and less in thymus. Results support the hypothesis that during a vitamin B-6 deficiency, dC metabolism is shunted toward deoxuridine monophosphate in an attempt to synthesize deoxythymidine monophosphate.

Key Words: Urinary deoxycytidine excretion, vitamin B-6 deficiency.

Introduction

Urinary deoxycytidine (dC) is excreted in high concentration by the rat (Rotherham, et al.,1960). In a preliminary study, we quantitated a ten fold decrease in dC excretion by the third week of a six week vitamin B-6 deficiency in ad libitum deficient rats when compared to ad libitum control (Jensen, 1979). We postulated the decrease may have occurred as a result of inhibition of deoxythymidine triphosphate (dTTP) synthesis. A vitamin B-6 deficiency has been shown to inhibit incorporation of deoxyuridine monophosphate (dUMP) into deoxythymidine monophosphate (dTMP) (Robson, 1975). Thus, a deficiency of vitamin B-6 may interfere with synthesis of dTTP. Metabolism of dTTP and deoxycytidine triphosphate (dCTP) are interrelated and the ratio of dTTP/dCTP is tightly regulated (Greenberg,1970). A decrease in the synthesis of one will result in a shift in metabolism of a common pool of precursors toward production of the other. Thus, if dTTP

synthesis is decreased, dCTP and dC metabolism will be shifted toward synthesis of dTTP and less dC would be excreted.

Vitamin B-6 deficiency results in decreased appetite and thus decreased growth rate. Pyrimidine deoxynucleotide metabolism is involved in DNA synthesis and DNA synthesis is essential to growth. Thus, factors which result in reduced rate of growth may be expected to affect DNA synthesis and may affect pyrimidine deoxynucleotide metabolism. To eliminate the reduction in food intake as a variable, we included a control group of rats which were pair fed to the ad libitum deficient rats. Since pair feeding results in meal feeding, we also included a meal fed deficient group to help evaluate the impact of this variable. In addition, we conducted fasting studies on both the deficient and control rats to assess the impact of acute caloric deprivation on dC metabolism.

The purpose of this study was to confirm our earlier observations of a decrease in dC excretion in vitamin B-6 deficient rats and to compare excretion levels from ad libitum and pair fed control rats. The impact of decreased food intake on dC excretion was further explored in fasted rats. Also, the metabolic process which led to the decreased dC excretion was explored by monitoring the retention and excretion of ip administered ^{3}H-dC.

Materials and Methods

Animals

Weanling male Wistar rats (60-80 g) (purchased from Charles Rivers Laboratories) were randomly assigned to one of 4 groups: ALC (12 rats with ad libitum access to the control diet), ALD (36 rats with ad libitum access to a vitamin B-6 deficient diet), PFC (36 rats pair fed to the ALD rats with control diet) and MFD (12 rats allowed access to the deficient diet for two hours daily). The four groups were subdivided into groups of six for various treatments. The animals were housed individually in wire bottomed stainless steel cages and a 12 hour light-dark cycle was employed. Water was provided ad libitum at all times. Body weights were taken weekly.

Diet

The animals were fed a semisynthetic diet which conformed to AIN-76 nutrient recommendations for the rat (AIN 1977, 1980), with the exception of vitamin B-6. The diet composition was 20% casein, 50% sucrose, 15% corn starch, 5% corn oil and 5% fiber as celufil. The remaining 5% was AIN vitamin and mineral mix, DL methionine and choline. Based on analysis prior to the study, the control diet contained 5.7 mg pyridoxine (PN)/kg diet and the vitamin B-6 deficient diet contained 0.04 mg PN/kg diet. Details of the diet have been reported elsewhere (Cho, 1989).

Feeding

All animals were adapted to the control diet for 1 week prior to feeding them their respective diets. Training for the meal fed animals was begun during this period of adaptation. For this group, the rats had daily access to food for two hours, from 8:00 to 10:00 a.m. Each of the 4 groups were then maintained on their respective dietary treatments for 2 or 6 weeks. At the end of the 6th week, subgroups of 6 animals each from the PFC and ALD groups were fasted for 3 days. At the end of week 6, 12 ALD rats were repleted with vitamin B-6 by allowing ad libitum access to the control diet. The remaining 12 PFC rats continued to be pair fed to these 12 ALD rats. By the end of the two week repletion period, all animals were eating control diet ad libitum. After the two weeks of repletion, 6 animals from each group were fasted for 3 days.

^{3}H-Deoxycytidine Administration

At the end of weeks 2 and 6, six rats from each group were given an ip injection of ^{3}H-5'dC in sterile saline (0.1 uCi/g body weight, specific activity 18 Ci/mmole).

Collection of Samples

At the respective time points (2 weeks non-fasted, 6 weeks non-fasted, 6 week 3 day fasted, repleted non-fasted and repleted 3 day fasted) animals were placed in stainless steel metabolic cages and one (four for the fasted rats) 24 hour urine sample was collected. Following this, rats were anesthetized with CO_2 and sacrificed by decapitation.

Immediately following decapitation, liver, spleen, and thymus were rapidly excised, weighed, frozen in liquid nitrogen and stored at -34°C until analyzed. Intestine was excised just below the stomach, cut into 5 cm fractions, distributed to various vials for different analysis and frozen as above. Bone marrow was obtained by washing the contents of the tibia and fibula into a vial with a syringe filled with 5 ml of cold saline. The mixture was centrifuged and saline removed. The pellet was stored frozen at -34°C until analyzed.

Analysis

Diet: The vitamin B-6 content of the diet was determined microbiologically, using Saccharomyces uvarum (ATCC No. 9080) as the assay organism (AOAC, 1980).

Deoxynucleoside separation and quantitation: Deoxynucleosides were separated from ribonucleosides with affinity chromatography. Thirty percent of a 24 hour urine sample was mixed with 300 ul of 2.5 M ammonium acetate buffer/ml of urine. The solution was centrifuged for 5 minutes at 2000 G at 4°C. The precipitate was rinsed with 1 ml of a 0.25 M ammonium acetate, centrifuged for 5 minutes at 2000 X G at 4°C. The combined supernatants were applied to a 1 cm^3 Affigel 601 (BioRad. Chemical Division, Richmond, CA) column conditioned with 0.25 M ammonium acetate (pH 9.5). Ribonucleotides were retained on the column. The bases and deoxyribonucleotides came through in the initial eluent and residual compounds were eluted with an additional

15 ml of 0.25 M ammonium acetate. The column was reconditioned by eluting the ribonucleosides with 10 ml of formic acid, rinsing with 20 ml of water (to avoid bubbles forming when formic acid mixes with the ammonium acetate) and then addition of 25 ml of 0.25 M ammonium acetate (pH 9.5). The deoxynucleoside containing eluent was dried on a rotary evaporator at 24^{o}C, diluted to 1-2 ml with water and stored frozen at -34^{o}C for subsequent separation by HPLC.

Deoxycytidine was separated from other deoxynucleosides and bases by HPLC. The column was a Supelcosil LC-18-S (25 cm X 4.6 mm id with 0.5 um packing). Bases and deoxynucleosides were separated with a gradient as described in Table I.

A 50 ul sample of the deoxynucleoside containing eluent from the Affigel separation was injected onto the column. All samples were done in duplicate. Every 15 seconds, between 1 and 7 minutes, 0.5 ml fractions were collected. One set of fractions was used to quantitate deoxycytidine and any other deoxynucleosides present in detectable levels. The other set of fractions were used to quantitate the H^3 label in the eluent.

Deoxycytidine was quantitated on the eight, 0.5 ml HPLC fractions collected between 2.5 and 4.5 minutes. A colorimetric method described by Chen (1968) was used to measure the deoxyribose moiety of the deoxycytidine. Other deoxyribonucleosides were non-detectable, presumably because they were at levels below the detection limit of the assay.

Urinary and tissue ^{3}H determination: A 0.05 ml fraction of the Affigel 601 eluent containing the deoxyribonucleosides was counted in 10 ml of ANPO (259 g Naphthalene, 18.4 g 2,5-Diphenyloxazole, 2(1-Naphthyl)-5-phenyl-oxazole, 1.4 L xylene, 1.4 L dioxane, 0.84 L absolute ethanol). The 0.5 ml fractions eluted between 1 and 7 minutes were also counted. A 0.2 ml aliquot of urine and an aliquot of the labeled deoxycytidine solution injected into the rats was also counted at this time to account for radioactive decay.

A sample of each tissue was homogenized in 80 mM phosphate buffer (pH 7.4) for use in a number of analyses including PLP (Chabner et al., 1970), cold DNA (Labarca et al., 1980), and protein (Bradford, 1976) determination. Details of these methods are reported elsewhere (Cho, 1989). An aliquot of the tissue homogenate was counted and used to determine retention of label in the tissue. Label incorporated into DNA was determined by the method described by Klubes et al. (1978). A crude DNA extract was hydrolyzed in 90% TCA and quantitated spectrophotometrically. An aliquot of the solution containing the oligopolynucleosides was then counted to determine incorporation of label into DNA in various tissues.

Statistical Analysis: All data were analyzed by variance and tested for significant differences by the least significant difference (LSD) test (Heintz, 1986). A p value

of 0.05 was considered to be significant. All values are reported as the mean ± standard deviation.

Results

Vitamin B-6 Deficiency

Severity of the deficiency was monitored by growth and by tissue and plasma PLP levels. Mean plasma PLP concentration at the end of week 2 for ALC, PFC, ALD and MFD rats were 1184±283, 1141±378, 26±14 and 64±16 nmol/ml respectively. By the end of week 6 the mean plasma PLP concentration for the ALC, PFC, ALD and MFD rats were 1160±195, 676±264, 13±8 and 21±8 nmol/ml, respectively. Mean liver PLP levels for the 2 week ALC, PFC, ALD and MFD rats were 28±3, 27±2, 10±3 and 15±3 nmol/g wet weight, respectively. By the end of week 6 mean liver PLP levels for ALC, PFC, ALD and MFD rats were 27±1, 33±3, 8±2 and 12±2 nmol/g wet weight, respectively.. Growth in the PFC and ALD rats were less than ALC rats after week 3 of the deficiency. Greater details of the effect of the dietary treatments on growth and tissue PLP levels has been reported elsewhere (Cho, 1989).

Effect of a Vitamin B-6 Deficiency on Deoxycytidine Excretion

Table 2 shows that the mean deoxycytidine excretion in the vitamin B-6 deficient rats decreased to 35% of the control level after only 2 weeks of deficiency. Compared to the two week ALD and MFD levels, the excretion decreased an

additional 50% in the deficient rats by the end of the 6th week. The mean dC excretion in PFC rats was significantly lower than that of ALC rats after 6 weeks but not after 2 weeks. In addition, fasting resulted in a significant decrease in mean excretion in 6 week ALD, PFC and repleted ALD and PFC rats. The decrease which resulted from fasting was independent of the decrease which resulted from the vitamin B-6 deficiency.

Effect of a vitamin B-6 Deficiency on Excretion of Label From 3H-dC

Table 3 shows the impact of a vitamin B-6 deficiency on mean urinary excretion of label in the first 24 hours after an ip injection of ^{3}H-dC. After only 2 weeks of the vitamin B-6 deficiency, the mean percentage of injected counts excreted in urine was significantly lower in the vitamin B-6 deficient rats compared to controls. In addition, by the end of week 6, the mean percentage of injected count excreted as ^{3}H-dC was significantly lower in the vitamin B-6 deficient rats. Specific activity of labeled dC tended to be higher in the deficient rats (Table 3b).

Effect of a Vitamin B-6 Deficiency on Retention of Label from 3H-dC

Table 4 shows the effect of a vitamin B-6 deficiency on retention of label in tissues. The percent of label (^{3}H) retained varied with tissue. In the liver and bone marrow, a vitamin B-6 deficiency resulted in a greater retention of label. In the spleen, there was no difference in the amount

of label retained between vitamin B-6 deficient and control rats. In the thymus and intestine, there was less label retained by the vitamin B-6 deficient rats compared to controls. In addition, pair and meal feeding resulted in an increase in retention of label by liver and bone marrow but had no impact on retention by spleen, thymus, and intestine.

Effect of a Vitamin B-6 Deficiency on Incorporation of 3H-dC in the DNA

Incorporation of label into DNA was so low in liver, spleen, and thymus that the label was only marginally detectable with the methods used. There was no apparent effect of the vitamin B-6 deficiency or meal feeding in these tissues. In contrast, there was a significant amount of label incorporated into bone marrow DNA as can be seen in (Table 5). There was a slight effect of deficiency and a significant effect of meal feeding on incorporation of dC into bone marrow DNA. Less label was incorporated into bone marrow from ad libitum fed and meal fed vitamin B-6 deficient rats than into bone marrow of their respective controls. There was no effect of meal feeding in the 2 week deficient rats. However, by the end of week 6, the meal and pair fed rats had incorporated significantly less dC into bone marrow DNA than the ad libitum fed counterparts.

Discussion

This study was undertaken to explore the effect of a vitamin B-6 deficiency on dC metabolism. In a preliminary study we (Jensen, 1979) quantitated a decrease in dC excretion in ad libitum fed rats. In the present study a pair fed control group was included to determine if the effect of the vitamin B-6 deficiency was independent of a decrease in growth rate due to appetite suppression associated with the vitamin B-6 deficiency. Furthermore, retention of ip injected ^{3}H-dC was monitored to explore the metabolic processes which might explain the observed decrease in dC excretion.

The data do verify a decrease in dC excretion due to a vitamin B-6 deficiency and demonstrate the decrease is independent of growth inhibition or caloric restriction. In our previous study (Jensen, 1979), excretion was monitored at intervals over a 6 week deficiency period. These earlier data demonstrated a progressive decrease in urinary dC excretion in the vitamin B-6 deficient rats through the end of the third week of vitamin B-6 deficiency. Urinary dC then plateaued and remained constant through the end of a six week deficiency period. Excretion of dC returned to control levels after 2 but not 1 week of vitamin B-6 repletion. Current data are in agreement with the previous results.

Earlier work has suggested that a vitamin B-6 deficiency may interfere with pyrimidine deoxynucleotide metabolism (Axelrod, 1964, Robson, 1975). Pyridoxal 5'phosphate (PLP) is involved in pyrimidine deoxynucleotide synthesis as a cofactor for STHM (Blakley, 1955) and an apparent modulator for thymidylate synthase (Tryfiates, 1980). Thus a deficiency of this vitamin could result in an inhibition of the conversion of dUMP to dTTP. The ratio of dTTP/dCTP is tightly regulated (Greenberg, 1970). Low levels of one of these deoxynucleotides will result in a shift in metabolism of a common pool of precursors toward production of the other (Greenberg, 1970; Fiala, 1965). Thus, if a vitamin B-6 deficiency resulted in a decrease in dTTP synthesis, enzyme systems would shift toward synthesis of dUMP. Synthesis of dC would be reduced and dC would be salvaged more efficiently.

The low urinary excretion of dC observed here and the decreased STHM activity (Jensen and Leklem, 1989) in the vitamin B-6 deficient rats is consistent with this theory. The decrease in dC excretion due to caloric deprivation may be related to a need to salvage energy producing substrates such as deoxcytidine for energy metabolism and a general decrease in synthesis of deoxynucleotides.

The results of the excretion and retention of label from ^{3}H-dC also support our hypothesis. A lower percent of injected label was excreted in the urine of the vitamin B-6 deficient rats and a lower percent of the injected dose was

excreted as ^{3}H-dC. This strongly supports the concept that dC is salvaged to a greater extent in the vitamin B-6 deficient rats than in the controls. However, the fate of the labeled dC is unclear. Results of the retention of ^{3}H label in tissue homogenates demonstrate that liver and bone marrow from vitamin B-6 deficient rats retained more label than control rats. Compared to the retention of label in the control rats, retention of label in thymus and intestine was less in the deficient rats. Retention of label in spleen was unaffected by the B-6 deficiency.

An attempt was made to determine how much label was incorporated into DNA. Due to limited availability of tissue and low specific activity of the labeled dC, results in liver, spleen and thymus were inconclusive. However, a lower incorporation of labeled dC into bone marrow DNA from deficient rats compared to controls and into meal or pair fed rats compared to ad libitum fed counterparts, strongly suggest there was both a feeding and a deficiency effect on dC incorporation into DNA. There was less label incorporated per ug oligopolynucleosides in ALD and MFD rats compared to the ALC and PFC counterparts, respectively (see Table 5). This could be related to differences in cell proliferation and/or to isotope dilution. Tissue levels of dC and its metabolites were not determined and the amount of label in tissue dC and it's specific metabolites is not known.

In summary, the data confirm that a vitamin B-6 deficiency results in a decrease in dC excretion, and that the decrease is independent of caloric intake or growth inhibition. Furthermore, the results suggest that the dC metabolism is influenced by both a vitamin B-6 deficiency and calorie deprivation. It appears that dC is salvaged to a greater extent in the deficient rats than in the control rats and in the meal or pair fed rats than in the ad libitum counterparts. The exact fate of exogenous dC is not clear from the present study. There is evidence that dC or its metabolites accumulate in some tissues but results regarding incorporation into DNA were inconclusive. The observed alterations in dC metabolism and our reported observation of reduced activity of serine transhydroxymethylase activity (Jensen, 1989) in a variety of tissues from vitamin B-6 deficient rats add support to the hypothesis that vitamin B-6 deficiency inhibits cell proliferation by interfering with deoxyribonucleotide metabolism. This may be of particular importance when considering the potential impact of a frank or marginal vitamin B-6 deficiency on immune function. Our data on two week deficient rats would suggest that the impact would occur early in a deficiency.

Table 4-1. Solvent gradient used to separate deoxynucleosides and bases on Supelcosil LC-18-S (25cm X 4.6 mm id with 0.5 um packing).

TIME (min.)	% Buffer B* (min.)	DURATION (min.)	FLOW RATE (ml/min.)
0	97.5	---	2.0
0	---	---	---
5.1	94.1	5.0	2.0
5.1	---	5.0	---
11.1	85.3	11.0	2.0
11.1	---	11.01-	---
22.1	85.3	20.0	---
42.1	97.4	10.0	---
42.1	---	10.0	2.0
END	---	---	---

* Buffer A was 2.5% methanol, 0.5M KH_2PO_4, pH 4.0.
Buffer B was 12.5% methanol, 0.5M KH_2PO_4, pH 4.0.

Table 4-2. Effect of vitamin B-6 deficiency and of fasting on mean urinary deoxycytidine[1] excretion.

	ALC[2]	PFC[2]	ALD[2]	MFD[2]
		(umoles/day)		
WEEK 2	1.47±0.41[a3]	1.71±1.17[a]	0.58±0.14[b]	0.56±0.18[b]
WEEK 6	3.31±1.21[a]	1.00±0.34[b$]	0.29±0.14[c@]	0.26±0.20[c]
WEEK 6 FASTED				
PREFAST	---[4]	0.78±0.10[a*]	0.34±0.16[a*]	---
Day 1	---	0.80±0.20[a*]	0.27±0.15[a*]	---
Day 2	---	0.50±0.20[b]	0.15±0.04[ab*]	---
Day 3	---	0.46±0.19[b]	0.07±0.04[b*]	---
REPLETION FASTED				
PREFAST	---	2.17±0.72[a]	2.36±1.24[a]	---
Day 1	---	1.94±0.93[a]	2.25±0.89[a]	---
Day 2	---	1.30±0.24[a]	1.18±0.87[ab]	---
Day 3	---	0.39±0.32[c]	0.49±0.41[b]	---

1. Values are reported as the mean ± standard deviation for 6 rats unless otherwise indicated (ie. & n=3, # n=4, * n=5, @ n=10 and $ n=11).
2. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient and MFD = meal fed deficient.
3. For all groups, within a given row, those values with different letters are significantly different ($p<0.05$). For the fasted groups, within a given column and within a given row, those values with different letters are significantly different ($p<0.05$).
4. --- indicates missing data

Table 4-3. Effect of a vitamin B-6 deficiency in rats on the mean urinary excretion of label[1] from ^{3}H-deoxycytidine in the first 24 hours after injection.

Table 4-3a. Percent of radioactivity from ^{3}H-deoxycytidine which was excreted in urine, excreted as deoxycytidine and excreted as unidentified deoxycytidine metabolites.

	% of ^{3}H-dC DOSE WHICH WAS EXCRETED:		
	IN URINE	AS dC	AS dC METAB[3]
WEEK 2			
ALC[1]	16.7±5.6^{a2}	6.6±2.2^{a}	1.7±0.3^{a}
PFC	16.1±4.0$^{a\#1}$	7.0±2.1$^{a\#}$	1.0±0.2$^{a\#}$
ALD	11.9±2.1^{a*}	4.0±0.8^{a*}	1.7±0.3^{a*}
MFD	15.4±3.5$^{a\#}$	5.8±1.5$^{a\#}$	2.6±0.3$^{a\#}$
WEEK 6			
ALC	31.8±3.9^{a}	16.2±4.3^{a}	3.0±0.3^{a}
PFC	19.5±3.3^{b*}	9.9±4.4$^{a\#}$	3.7±1.4$^{a\#}$
ALD	8.7±1.8^{c}	2.4±0.5^{b}	2.7±0.6^{a}
MFD	11.4±2.2^{c*}	3.6±1.6^{b*}	3.2±1.7^{a*}

Table 4-3b. Mean dose, amount excreted and specific activity of excreted ^{3}H-deoxycytidine in control and vitamin B-6 deficient in rats.

	^{3}H-dC DOSE (nmol/rat)	^{3}H-dC (pmol/day)	^{3}H-dC/dC (pmol/umol)
Week 2			
ALC	1.12±0.10	74.5±27.7^{a}	50.1±9.9^{a}
PFC	1.03±0.05	71.4±21.0$^{a\#}$	51.5±4.7$^{a\#}$
ALD	1.10±0.05	44.1±11.0^{a*}	75.6±20.8^{ab*}
MFD	0.80±0.18	46.6±25.0$^{a\#}$	87.2±33.2$^{b\#}$
WEEK 6			
ALC	1.99±0.17	320.7±67.0^{a}	98.9±10.3^{a}
PFC	1.57±0.04	154.2±67.0$^{b\#}$	143.5±15.4$^{b\#}$
ALD	1.36±0.07	30.8±7.7^{c}	132.9±31.9^{ab*}
MFD	0.97±0.2	43.0±20.4$^{c\#}$	139.2±43.0$^{ab\#}$

1. See Table 2 for details.
2. Within a given column, and within a given time period (week 2 and week 6), those values with different letters are significantly different ($p<0.05$).
3. dC Metab. refers to label eluted from HPLC in the first 2.5 minutes after injection of a semifractionated sample of urine. In all but 2 (deficient) rats, all of the radioactivity in the urine preparation which was not present in the dC fraction was accounted for in this fraction.

Table 4-4. Effect of a vitamin B-6 deficiency on retention of label from ^{3}H-deoxycytidine in tissue homogenates from rats.

	% OF DPM INJECTED/UG DNA				
	LIVER	SPLEEN	THYMUS	BONE MARROW	INTESTINE
WEEK 2					
ALC[1]	0.77±0.05[a2]	0.40±0.05[a]	1.00±0.34[a]	0.7±0.[ab]	0.54±0.28[a]
PFC	0.88±0.10[a]	0.62±0.25[a]	1.04±0.36[a]	0.3±0.3[a]	0.74±0.32[a]
ALD	1.02±0.17[a]	0.39±0.16[a]	0.80±0.29[a]	0.5±0.5[a]	0.63±0.22[a]
MFD	1.38±0.18[a]	0.59±0.37[a]	0.81±0.22[a]	0.8±0.7[b]	0.44±0.17[a]
WEEK 6					
ALC	0.60±0.15[a]	0.57±0.15[a]	---[1]	0.59±0.09[a]	0.74±0.30[a]
PFC	0.72±0.17[a]	0.54±0.21[a]	---	0.77±0.16[ac]	1.15±0.33[b]
ALD	1.11±0.34[b]	0.43±0.21[a]	---	1.14±0.19[b]	0.96±0.17[ab]
MFD	1.33±0.40[b]	0.53±0.15[a]	---	1.07±0.44[cb]	1.25±0.52[b]

1. See Table 2 for details.
2. 2. Within a given column, and within a given time period (week 2 and week 6), those values with different letters are significantly different ($p<0.05$).

Table 4-5. Effect of a vitamin B-6 deficiency on incorporation of ^{3}H-deoxycytidine into DNA of tissues from rats.

	CPM/UG OLIGONUCLEOSIDES		
	SPLEEN	THYMUS	BONE MARROW
WEEK 2			
ALC[1]	2.10±1.05^{a2}	3.84±2.47^{a}	20.5±1.4^{a}
PFC	1.22±0.36$^{ab\#1}$	1.81±0.86^{a}	---
ALD	0.89±0.48^{b}	4.92±1.11$^{a\#}$	24.6±5.1^{a*}
MFD	0.58±0.14$^{b\#}$	4.40±2.60$^{a\&}$	23.8±2.2^{a}
WEEK 6			
ALC	3.39±0.97^{a}	---[1]	40.3±4.0^{a}
PFC	1.48±0.65^{b}	---	33.2±2.4^{ab*}
ALD	2.18±1.09^{b}	---	35.6±7.4^{a*}
MFD	2.22±0.47^{b}	---	24.9±8.4^{b}

1. See Table 2 for details.
2. Within a given column, and within a given time period (week 2 and week 6), those values with different letters are significantly different ($p<0.05$).

CHAPTER 5

SUMMARY AND CONCLUSIONS

Serine transhydroxymethylase is a pyridoxal 5'phosphate requiring enzyme involved in pyrimidine deoxynucleotide metabolism. A decrease in the activity of this enzyme may interfer with pyrimidine deoxynucleotide metabolism. The purpose of the study was to explore the effect of a vitamin B-6 deficiency on the metabolism of the pyrimidine deoxycytidine (dC). This was accomplished in three ways. First the effect of a vitamin B-6 deficiency on serine transhydroxymethylase was determined in liver, thymus, spleen and bone marrow. Second, the effect of a vitamin B-6 deficiency on urinary dC excretion was quantitated. Third, the effect of a vitamin B-6 deficiency on the urinary excretion and tissue retention of ^{3}H from ip injected ^{3}H-dC was determined. Fasting studies as well as pair fed control and meal fed deficient groups were included to account for effects due to caloric deprivation resulting from reduced appetite associated with vitamin B-6 deficiency.

Ninty six rats were assigned to one of four experimental groups: an ad libitum control group, a pair fed control group, an ad libitum deficient group and a meal fed deficient group. Subgroups of 6 rats each received different treatments. Treatments were 2 or 6 weeks of maintenance on the respective diets and feeding regime,

fasting for 1 or 3 days at the end of week 6, repletion for 2 weeks with control diet at the end of the week 6 and fasting for 3 days at the end of the 2 week repletion period. Urines were collected for 24 hours following the ip injection of ^{3}H-dC and just prior to sacrifice in all rats. Urines were also collected prior to and during the 1 and 3 day fast. After sacrifice, plasma and tissues were collected, frozen and stored at -34^{o}C until analyzed.

The results are consistant with other studies (Axelrod, 1968, Robson, 1975) which have suggested that a vitamin B-6 deficiency inhibits cell proliferation by interfering with pyridmidine deoxynucleotide metabolism. Feeding rats a vitamin B-6 deficient diet resulted in reduced activity of STHM. The decrease in STHM activity due to the vitamin B-6 deficiency was greater than the decrease in activity associated with caloric deprivation. Furthermore, the decrease associated with the deficiency manifested itself prior to the onset of growth and appetite changes associated with a vitamin B-6 deficiency. A decrease in the activity of STHM would reduce synthesis of 5,10-methylene tetrahydofolate (5,10-MTHF). This folate derivative is a cofactor for thymidylate synthase which catalyzes the conversion of dUMP to dTMP. If 5,10-MTHF is decreased, the conversion of dUMP to dTMP may be impaired. Since dTMP is in equilibrium with dTTP and dTTP/dCTP, levels are tightly regulated, this would lead to altered dCMP and thus dC metabolism.

Indeed, the vitamin B-6 deficiency led to a significant ($p \leq 0.05$) decrease in dC excretion. The decreased excretion suggests reduced synthesis and/or greater salvage of this compound by the vitamin B-6 deficient rats. As with the STHM activity, the decrease in dC excretion associated with the vitamin B-6 deficiency was greater than, and independent of, the decrease in dC excretion asssociated with reduced food intake and reduced growth. The decrease in dC urinary excretion and STHM activity paralleled changes in tissue PLP levels.

To further explore the impact of a vitamin B-6 deficiency on dC metabolism, rats were given an ip injection of ^{3}H-dC. The vitamin B-6 deficient rats excreted less ^{3}H in the first 24 hours after the injection than did the control rats. Furthermore, retention of the label in the tissue homogenates revealed greater retention of label in the tissues of the vitamin B-6 deficient rat. Attempts to measure incorporation into DNA were limited by specific activity of dC and by tissue availability. However, results suggest little or no effect of dC incorporation into DNA due to vitamin B-6 deficiency in spleen, thymus and bone marrow. In contrast, caloric deprivation decreased incorporation of ^{3}H into DNA of bone marrow from both control and vitamin B-6 deficient rats. The data on retention of ^{3}H suggests the label was retained in the deficient rat because of increased conversion of dC into dC metabolites.

In conclusion, our data strongly support the four hypothese tested in this study. First, the vitamin B-6 deficiency resulted in lower STHM activity in liver, spleen, thymus and bone marrow. Second, the vitamin B-6 deficiency resulted in a reduced urinary excretion of dC and the reduction was independent of decreased appetite and growth associated with a vitamin B-6 deficiency. Third, urinary excretion of ^{3}H from ^{3}H-dC was lower in the vitamin B-6 deficient rats. Fourth, the deficient rats retained a greater percent of the ^{3}H-dC dose in liver and bone marrow than the control rats. However, incorporation of label into DNA did not appear to be affected by the deficiency.

Our results add to the pool of information linking a vitamin B-6 deficiency with reduced cell proliferation and provide additional support to the hypothesis that the inhibition is associated with interference with folate metabolism.

FUTURE STUDIES

Further studies in this area of research need to be conducted to definitively link STHM with the altered pyrimidine deoxynucleotide metabolism in vitamin B-6 deficiency. Evidence exists that thymidylate synthase may be modulated by PLP levels (Tryfiates, 1980), suggesting a more direct link between vitamin B-6 deficiency and pyrimidine deoxynucleotide metabolism. If the activity of thymidylate synthase were inhibited by a vitamin B-6 deficiency, the impact on dC metabolism would be similar to

what we observed. If on the other hand, reduced STHM activity is responsible for the alteration in dC metabolism, it would be of interest to determine how this influences folate status. In addition, the possibility of developing a diagnostic test for vitamin B-6 deficiency based on the thymidine suppression test used to differentiate between a folate and vitamin B-12 deficiency remains an interesting possibility. Work by Robson et al., (1975) has gone far toward demonstrating this as a possibility. They showed decreased incorporation of radioactive labeled dUMP into dTMP in human lymphocytes grown in a vitamin B-6 deficient media. However, these authors did not report the effect of stimulating the reaction with exogenous PLP.

Other areas needing further study include the effects of marginal vitamin B-6 deficiency on dC excretion. A marginal deficiency should be studied since the data suggest the effect is seen as early as 1 week into a vitamin B-6 deficiency. Finally, the effect of a marginal vitamin B-6 deficiency on pyrimidine deoxynucleotide metabolism in particular cell types of the immune system deserves further exploration. A "vitamin B-6 uridine supression test" using lymphocytes may prove valuable in this exploration.

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APPENDICIES

Appendix 1. Composition of the diet.

Components	Percent
Vitafree casein	20.0
DL Methionine	0.3
Cornstarch	15.0
Sucrose	50.0
Celufil (fiber)	5.0
Corn oil	5.0
Choline bitartrate	0.2
AIN mineral mix*	3.5
AIN vitamin mix (without pyridoxine)**	1.0

*AIN mineral mix (3.5% of total diet)	g/kg
Dibasic calcium phosphate ($CaHOP_4$)	500.0
Sodium chloride (NaCl)	74.0
Potassium citrate monohydrate ($HOC(CO_2K)(CH_2CO_2K)_2:H_2O$)	200.0
Potassium sulfate (K_2SO_4)	52.0
Magnesium carbonate (42-48% Mn)	3.5
Ferric carbonate (16-17% Fe)	6.0
Zinc carbonate (70% Zn)	1.6
Cupric carbonate (53-55% Cu)	0.3
Potassium iodate (KIO_3)	0.01
Sodium selenite ($Na_2SeO_3 5H_2O$)	0.01
Chromium potassium sulfate ($CrK(SO_4)_2:12H_2O$	0.55
Finely powdered sucrose	118.0

**AIN-76 vitamin mix	per kg
Thiamin-HCl	600.0 mg
Riboflavin	600.0 mg
Pyridoxine-HCl***	700.0 mg
Nicotinic acid	3.0 g
D-Calcium panthothenate	1.6 g
Folic acid	200.0 mg
D-Biotin	20.0 mg
Cyanocobalamin (B_{12})	1.0 mg
Retinyl palmitate (vitamin A premix)	800.0 mg
DL-D-Tocopheryl acetate	20.0 g
Cholecalciferyl (vitamin D_3)	2.5 g
Menequinine (vitamin K)	5.0 g
Finely powdered sucrose	972.9 g

***Vitamin B-6 in the Diet	mg PN/kg diet
Control diet	5.691
Vitamin B-6 deficient diet	0.039

Appendix 2. Abbreviations.

ALC	ad libitum control
PFC	pair fed control
ALD	ad libitum deficient
MFD	meal fed deficient
RPFC	repleted pair fed control
RALD	repleted ad libitum deficient
B-6	vitamin B-6
PN	pyridoxine (mw=169.18)
PM	pyridoxamine ((mw=168.19)
PL	pyridoxal (mw=167.16)
PNP	pyridoxine 5'phosphate (mw=249.16)
PMP	pyridoxamine 5'phosphate (mw=248.18)
PLP	pyridoxal 5'phosphate (mw=247.14)
4-PA	4-pyridoxic acid (mw=183.2)
dC	deoxycytidine
dU	deoxyuridine
dT	deoxythymidine
dCMP	deoxycytidine monophosphate
dUMP	deoxyuridine monophosphate
dCTP	deoxycytidine triphosphate
dTTP	deoxythymidine triphosphate
THF	tetrahydrofolate
5,10-MTHF	5,10-methylenetetrahyfolate
GABA	gamma amino butyric acid
STHM	serine transhydroxymethylase
LSD	least significant difference
ip	interperitoneal

Appendix 3a. Individual urinary 4-pyridoxic acid excretion (nmoles/24 hours).

	ANIMAL	WEEK 0	WEEK 2	WEEK 4	WEEK 6
ALC	7	169	152	169	195
	8	196	164	250	337
	9	120	55	142	192
	10	139	87	134	172
	11	96	53	141	204
	12	161	89	196	222
MEAN		130	100	172	220
±SD		±49	±48	±44	±60
PFC	19	236	161	160	200
	20	184	169	119	189
	21	222	148	111	201
	22	41	21	17	22
	23	21	16	10	22
	24	75	110	154	184
MEAN		186*	147*	136*	194*
±SD		±81	±26	±25	±8
ALD	55	123	ND	ND	ND
	56	21	ND	ND	ND
	57	138	ND	7	ND
	58	169	ND	ND	ND
	59	149	ND	ND	ND
	60	166	ND	ND	ND
MEAN		149#	ND	ND	ND
±SD		±19			
MFD	91	93	ND	ND	ND
	92	116	ND	ND	ND
	93	134	ND	ND	ND
	94	90	ND	ND	ND
	95	122	ND	ND	ND
	96	138	ND	ND	ND
MEAN		115	ND	ND	ND
±SD		±20			

* n=4, # n=5, ND = nondetectable. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 3b. Individual 4-pyridoxic acid excretion in rats fasted for 1 to three days.

		4-PA (nmoles/day)			4-PA (nmoles/mg creatinine)			
ANIMAL	DAY 0	DAY 1	DAY 2	DAY 3	DAY 0	DAY 1	DAY 2	DAY 3
WEEK 6 PFC								
25	187	258	168	102	4.35	3.55	2.75	1.61
26	212	289	236	226	4.42	3.99	3.44	3.41
27	139	263	198	156	3.40	3.83	3.12	2.51
28	18	42	32	29	0.47	0.63	0.60	0.50
29	177	246	153	132	4.49	3.79	2.67	2.21
30	159	276	201	94	3.76	4.04	2.95	1.63
MEAN*	175*	266*	191*	142*	4.08*	3.84*	2.99*	2.27*
±SD	±28	±17	±32	±53	±0.48	±0.20	±0.31	±0.74
WEEK 6 ALD								
61	nd	22	19	nd	nd	0.42	0.30	nd
62	nd	14	14	nd	nd	0.22	0.21	nd
63	nd	2	nd	nd	nd	0.03	nd	nd
64	nd	nd	nd	nd	nd	nd	nd	nd
65	nd	14	nd	nd	nd	0.25	nd	nd
66	nd	nd	nd	nd	nd	nd	nd	nd
MEAN	nd	9	6	nd	nd	0.15	0.08	nd
±SD		±9	±9			±0.17	±0.13	
		17#	16$					
		+4	+3					
REPLETION PFC								
43	132	170	234	174	2.27	3.27	2.87	1.98
44	264	245	333	206	4.54	5.04	4.52	3.38
45	273	317	263	193	5.22	5.33	3.89	2.53
46	31	43	42	28	0.57	0.52	0.39	0.41
47	197	403	225	191	3.88	6.11	3.13	2.20
48	344	453	294	160	6.35	6.44	3.78	2.11
MEAN	262*	318*	270*	185*	4.45*	5.24*	3.64*	2.44*
SD	±55	±115	±44	±18	±1.52	±1.24	±0.65	±0.59
REPLETION ALD								
79	233	295	255	163	3.86	4.19	3.03	1.87
80	265	246	161	133	5.39	4.34	2.77	2.12
81	171	193	117	86	2.95	2.97	1.91	1.48
82	225	238	150	96	3.73	3.40	2.15	1.42
83	303	326	241	145	5.63	4.87	3.27	1.83
84	276	297	232	149	4.50	4.12	2.94	1.71
MEAN	245	266	193	129	4.34	3.98	2.68	1.74
±SD	±46	±49	±57	±31	±1.03	±0.68	±0.53	±0.26

* n=5, @ n=4,#n=3, $n=2, nd = nondetectable. PFC = pair fed control, ALD = ad libitum deficient.

Appendix 4. Urinary urea nitrogen (mg/day) and creatinine (mg/day) in 3 day fasted rats.

	UREA NITROGEN				CREATININE			
ANIMAL	DAY 0	DAY 1	DAY 2	DAY 3	DAY 0	DAY 1	DAY 2	DAY 3
WEEK 6 PFC								
25	17.0	9.5	10.8	9.6	4.86	8.22	6.92	7.18
26	15.4	13.2	13.8	12.9	5.42	8.19	7.77	7.50
27	13.0	8.0	7.8	7.0	4.62	7.77	7.17	7.04
28	14.7	10.1	10.4	9.1	4.36	7.52	6.04	6.55
29	15.1	8.8	7.8	8.5	4.46	7.34	6.49	6.76
30	17.1	9.9	11.4	8.9	4.78	7.73	7.70	6.52
MEAN*	15.4	9.9	10.3	9.4	4.75	7.80	7.02	6.93
±SD	±1.5	±1.8	±2.3	±2.2	0.38	0.35	0.68	0.38
WEEK 6 ALD								
61	24.9	9.9	9.0	5.2	5.69	5.97	7.24	6.49
62	18.8	9.2	10.0	5.2	6.61	7.28	7.66	6.38
63	16.0	7.6	8.6	6.1	6.37	8.52	7.17	7.11
64	12.0	6.1	12.9	9.0	4.99	5.48	8.22	6.03
65	22.7	8.9	10.0	5.5	4.92	6.36	9.54	6.43
66	17.1	12.7	13.7	10.5	6.32	5.83	8.75	5.07
MEAN	18.6	9.0	10.7	6.9	5.82	6.57	8.10	6.25
±SD	±4.7	±2.0	±2.1	±2.3	±0.73	±1.14	±0.92	±0.68
REPLETION PFC								
43	27.0	5.8	9.9	10.2	6.58	5.88	9.22	9.93
44	26.1	9.2	12.6	6.8	6.58	5.50	8.34	6.90
45	26.9	17.2	10.8	8.8	5.92	6.73	7.64	8.63
46	26.3	14.7	10.2	6.9	6.20	9.33	8.10	8.63
47	23.4	15.8	9.5	11.1	5.74	7.46	8.14	9.82
48	22.4	13.1	9.7	6.1	6.13	7.96	8.81	8.59
MEAN	25.4	15.2@	10.4	8.3	6.19	7.14	8.38	8.60
±SD	±2.0	±1.7	±1.1	±2.0	±0.34	±1.42	±0.56	±1.18
REPLETION ALD								
79	22.3	14.3	11.5	9.4	6.83	7.96	9.51	9.86
80	25.4	12.2	10.2	8.2	5.56	6.41	6.58	7.11
81	19.9	13.1	5.0	5.7	6.55	7.36	6.94	6.58
82	18.9	12.7	10.7	10.5	6.83	7.92	7.88	7.64
83	29.1	17.2	10.6	8.8	6.09	7.57	8.34	8.98
84	27.4	16.0	11.1	11.4	6.94	8.15	8.94	9.86
MEAN	23.8	14.2	10.2	9.0	6.47	7.56	8.03	8.34
±SD	±4.1	±1.9	±1.5	±2.0	0.54	0.63	1.13	1.42

*n=5, @ n=4,# n=3, $n=2. PFC = pair fed control, ALD = ad libitum deficient.

Appendix 5a. Individual urinary data for:deoxycytidine excretion (umoles/day); ^{3}H-deoxycytidine excretion; ratio of ^{3}H-deoxycytidine/deoxycytidine (pmol/umol); creatinine. Page 1 of 2.

	ANIMAL	dC (umol/day)	^{3}H-dC (pmol/day)	^{3}H-dC/dC (pmol/umol)	CREATININE (umol/day)
WEEK 2					
ALC	1	1.09	46.0	42.2	30.0
	2	1.11	50.2	45.2	26.9
	3	1.54	102.1	66.3	29.7
	4	2.21	110.4	50.0	33.7
	5	1.45	82.1	56.6	32.4
	6	1.40	56.4	40.3	29.0
MEAN		1.42	74.5	50.1	30.3[ab]
± SD		±0.41	±27.7	±9.9	±2.45
PFC	13	0.92	47.7	51.9	30.6
	14	1.48	79.7	53.8	35.0
	15	1.73	96.1	55.5	32.8
	16	0.76	1.3	1.8	35.5
	17	3.98	0.9	0.2	40.2
	18	1.39	62.3	44.8	34.9
MEAN		1.71	71.4[#]	51.5[#]	34.8[a]
± SD		±1.17	±21.0	±4.7	±3.2
ALD	49	0.60	53.0	88.3	40.1
	50	0.41	41.6	101.4	30.6
	51	0.68	53.8	57.0	36.0
	52	0.80	45.6	57.0	30.3
	53	0.51	26.6	52.1	34.9
	54	0.50	0.6	1.2	35.2
MEAN		0.58	44.1	75.6[*]	34.5[a]
± SD		±0.14	±11.0	±20.8	±3.7
MFD	85	0.35	33.4	95.4	28.4
	86	0.72	29.1	40.5	17.2
	87	0.40	0.4	1.0	28.6
	88	0.73	0.2	0.3	24.8
	89	0.43	40.4	94.0	29.5
	90	0.70	83.4	119.1	34.6
MEAN		0.56	46.6[#]	87.2[#]	27.2[b]
± SD		±0.18	±25.0	±33.2	±5.82

n=4, * n=5. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 5a. Individual urinary data for:deoxycytidine excretion (umoles/day); ^{3}H-deoxycytidine excretion; ratio of ^{3}H-deoxycytidine/deoxycytidine (pmol/umol); creatinine. Page 2 of 2.

	ANIMAL	dC (umol/day)	^{3}H-dC (pmol/day)	^{3}H-dC/dC (pmol/umol)	CREATININE (umol/day)
WEEK 6					
ALC	7	3.81	371	97.3	68.6
	8	3.01	334	110.9	68.3
	9	5.26	451	85.7	56.4
	10	3.18	300	94.4	58.0
	11	2.17	242	94.4	63.6
	12	2.42	226	93.5	79.9
MEAN		3.31	321	98.9	65.0
± SD		±1.12	±83.3	±10.3	±8.5
PFC	13	1.59	213	133.8	71.7
	14	0.14	19	135.7	66.4
	15	1.24	212	171.0	62.5
	16	1.31	3	2.3	60.5
	17	0.74	102	138.4	69.1
	18	0.65	90	138.8	68.9
MEAN		0.94	154#	143.5*	66.5
± SD		±0.53	±67	±15.4	±4.3
ALD	55	0.43	41.6	96.7	56.4
	56	0.16	25.4	158.4	57.6
	57	0.13	32.0	246.5	64.2
	58	0.34	37.3	109.8	58.0
	59	0.12	20.7	172.1	70.2
	60	0.22	28.1	127.6	65.9
MEAN		0.23	30.8	132.9*	62.0
± SD		±0.13	±7.7	±31.9	±5.5
MFD	91	0.20	19.2	95.8	44.3
	92	0.04	34.0	850.7	41.2
	93	0.11	35.7	324.6	56.4
	94	0.35	67.1	191.6	24.7
	95	0.61	69.4	113.7	52.3
	96	0.21	32.7	155.6	50.8
MEAN		0.26	43.0	139.2#	45.0
± SD		±0.20	±20.4	±43.0	±11.4

n=4, * n=5. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 5b. Individual deoxycytidine excretion (umol/day) from 6 week fasted, repleted fasted rats.

	ANIMAL	PREFAST	DAY 1	DAY 2	DAY 3
WEEK 6 FASTED					
PFC	25	0.86	0.67	0.37	0.40
	26	0.61	0.97	0.57	0.46
	27	0.82	1.02	0.31	0.31
	28	0.80	0.76	0.63	0.72
	29	1.86	2.25	0.82	0.66
	30	0.81	0.60	0.31	0.23
MEAN		0.96	1.06	0.50	0.46
± SD		±0.45	±0.65	±0.20	±0.19
ALD	61	0.23	0.17	0.10	0.10
	62	0.25	0.26	0.17	0.07
	63	0.25	0.14	0.13	0.12
	64	0.06	0.05	0.07	0.09
	65	0.38	0.52	0.16	0.04
	66	0.60	0.24	0.21	0.03
MEAN		0.26	0.23	0.12	0.08
± SD		±0.18	±0.16	±0.07	±0.04
REPLETION FASTED					
PFC	43	1.77	0.96	1.53	0.69
	44	1.55	1.27	0.94	0.16
	45	1.35	1.34	1.09	0.25
	46	2.82	1.89	1.46	0.42
	47	3.08	3.51	1.29	0,82
	48	2.47	2.65	1.47	nd
MEAN		2.17	1.94	1.30	0.39
± SD		±0.72	±0.97	±0.24	±0.32
	79	1.80	1.98	0.63	0.21
	80	0.95	1.50	0.74	0.36
	81	2.36	2.34	1.08	0.41
	82	3.94	6.29	2.95	1.26
	83	3.79	3.73	1.11	0.57
	84	1.29	1.70	0.58	0.14
MEAN		2.36	2.92	1.18	0.49
± SD		±1.24	±1.83	±0.87	±0.41

PFC = pair fed control, ALD = ad libitum deficient.

Appendix 6a. Individual data on urinary excretion of label from ip injected ^{3}H-deoxycytidine in 2 and 6 week vitamin B-6 deficient rats and their respective controls. Page 1 of 2.

ANIMALS		% ^{3}H IN URINE EXCRETED[1]: as dC	in FRACT 1[3]	in URINE	% INJECTED ^{3}H EXCRETED[2]: as dC	in FRACT 1
				WEEK 2		
ALC[4]	1	37	54	6.4	2.3	3.5
	2	34	44	7.8	2.7	3.4
	3	38	47	13.8	5.3	6.5
	4	38	46	13.2	5.1	6.1
	5	43	66	8.5	3.7	5.6
	6	40	52	7.2	2.8	3.8
MEAN		38	52	9.5	3.6	4.8
± SD		±3	±8	±3.2	±1.3	±1.4
PFC	13	40	54	6.3	2.6	3
	14	44	55	9.9	4.3	6
	15	43	51	12.8	5.5	7
	16	2	6	4.5	0.1	0.2
	17	6	30	0.8	0.1	0.2
	18	47	62	8.1	3.8	1.3
MEAN		44#	56#	8.3#	4.0#	4.3#
± SD		±3	±5	±3.1	±1.2	±2.6
ALD	49	38	52	9.2	3.5	4.8
	50	29	44	8.6	2.5	3.8
	51	53	70	3.0	1.6	2.1
	52	38	54	5.3	2.0	2.8
	53	20	31	7.5	1.5	2.3
	54	1	8	3.4	0.03	0.3
MEAN		35x	50x	6.7x	2.2x	3.2x
± SD		±12	±14	±2.6	±0.8	±1.1
MFD	85	24	40	0.3	2.5	1.6
	86	49	17	6.4	3.1	1.1
	87	0.08	0.02	2.0	0.03	0.4
	88	---	19	0.4	---	0.1
	89	37	19	7.9	3.0	1.5
	90	44	34	10.2	4.4	1.5
MEAN		39#	24#	8.2#	3.2#	1.4#
± SD		±11	±9	±1.9	±0.8	±0.2

1. % OF ^{3}H IN URINE, refers to the radioavitiity excreted in the urine in the first 24 hours after injection.
2. % OF INJECTED ^{3}H refers to the % of radioactive dose given to each rat.
3. Fraction 1 refers to the eluent from the affigel 601 column which contained deoxynucleosides and bases.
4. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum control, MFD = meal fed deficient.

Appendix 6A. Individual data on urinary excretion of label from ip injected ^{3}H-deoxycytidine in 2 and 6 wek vitamin B-6 deficient rats and their respective controls. Page 2 of 2.

ANIMALS		% ^{3}H IN URINE EXCRETED[1]: as dC	in FRACT 1	in URINE	% INJECTED ^{3}H EXCRETED[2]: as dC	in FRACT 1
				WEEK 6		
ALC	7	59	71	17.8	10.6	12.6
	8	46	55	16.8	7.7	9.2
	9	59	66	21.1	13.0	14.0
	10	55	64	15.5	8.5	10.0
	11	48	60	14.7	7.1	8.9
	12	36	45	16.1	5.7	7.3
MEAN		50	60	17.0	8.8	10.3
± SD		±9	±9	±2.3	±2.6	±2.5
PFC	19	55	72	13.0	7.1	9.4
	20	8	11	??8.0	0.6	0.9
	21	67	93	11.3	7.5	10.5
	22	4	21	2.6	0.1	0.5
	23	33	47	9.9	3.3	4.6
	24	32	47	9.5	3.1	4.4
MEAN		47[#]	65[#]	10.9[#]	5.2[#]	7.2[#]
± SD		±16	±22	±1.6	±2.4	±3.2
ALD	55	34	66	5.3	1.8	3.5
	56	28	62	3.6	1.0	2.2
	57	29	69	4.3	1.3	3.0
	58	36	66	4.1	1.5	2.7
	59	26	59	3.7	1.0	2.2
	60	18	62	4.6	1.3	2.8
MEAN		29	62	4.6	1.3	2.8
± SD		±6	±6	±1.0	±0.3	±0.5
MFD	91	18	53	5.2	1.0	2.0
	92	35	66	5.4	1.9	3.6
	93	31	66	6.0	1.9	4.0
	94	55	82	12.1	6.6	9.9
	95	42	51	7.5	3.2	3.8
	96	26	54	5.9	1.6	3.2
MEAN		30[x]	58[x]	5.6[x]	1.9[x]	3.3[x]
± SD		±9	±7	±0.9	±0.8	±0.8

1. % OF ^{3}H IN URINE, refers to the radioavitiity excreted in the urine in the first 24 hours after injection.
2. % OF INJECTED ^{3}H refers to the % of radioactive dose given to each rat.
3. Fraction 1 refers to the eluent from the affigel 601 column which contained deoxynucleosides and bases.
4. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum control, MFD = meal fed deficient.

Appendix 6b. Individual data on urinary excretion of label from ^{3}H-deoxycytidine in control and vitamin B-6 deficient rats after 2 or 6 weeks of deficiency. Page 1 of 8.

	METAB[1]	dC[2]	FRAC 1[3]	URINE[4]	DOSE[5]
			WEEK 2 ALC[6]		
RAT 1					
CPM/DAY	87,530	218,526	321,383	595,440	5,792,568
% URINE CTS	14.7	36.7	54.0	100	---
% DOSE	1.5	3.8	5.5	10.3	100
RAT 2					
CPM/DAY	59,587	238,347	307,387	692,870	4,674,704
% URINE CTS	8.6	34.4	44.0	100	---
% DOSE	1.3	5.1	6.6	14.8	100
RAT 3					
CPM/DAY	105,090	484,392	598,632	1,266,140	5,182,824
% URINE CTS	8.3	38.3	47.3	100	---
% DOSE	2.0	9.4	11.6	24.4	100
RAT 4					
CPM/DAY	95,367	524,520	628,620	1,361,390	5,995,816
% URINE CTS	7.0	38.5	46.2	100	---
% DOSE	1.6	8.7	10.6	22.7	100
RAT 5					
CPM/DAY	107,906	389,829	500,152	759,900	5,284,4482
% URINE CTS	14.2	51.3	65.8	100	---
% DOSE	2.0	7.4	9.5	14.4	100
RAT 6					
CPM/DAY	86,839	267,980	356,031	678,430	4,979,576
% URINE CTS	12.8	39.5	52.5	100	---
% DOSE	1.7	5.4	7.1	13.6	100
MEANS $\pm$ SD					
CPM/DAY	90,386	354,022	452,034	892,362	5,318,323
$\pm$SD	$\pm$17,420	$\pm$131,629	$\pm$142,967	$\pm$331,955	$\pm$496,469
% URINE CTS	10.9	39.8	51.6	---	---
$\pm$SD	$\pm$3.0	$\pm$5.9	$\pm$7.9	---	---
% DOSE	1.7$\pm$0.3	6.6$\pm$2.2	8.5$\pm$2.4	16.7$\pm$5.6	---

1. Metab = all HPLC fraction except dC which contain label.
2. dC = HPLC fraction coeluting with dC.
3. Frac 1 = Affigel fraction containing deoxynucleosides and bases.
4. Radioactive label excreted in urine in the first 24 hours after injection.
5. Dose = amount of radioactive label injected.
6. ALC = ad libitum control

Appendix 6b. Individual data on urinary excretion of label from ^{3}H-deoxycytidine in control and vitamin B-6 deficient rats after 2 or 6 weeks of deficiency. Page 2 of 8.

	METAB[1]	dC[2]	FRAC 1[3]	URINE[4]	DOSE[5]
			WEEK 2 PFC[6]		
RAT 13					
CPM/DAY	78,799	226,772	306,159	562,710	5,081,200
% URINE CTS	14.0	40.3	54.4	100	---
% DOSE	1.6	4.5	6.0	11.1	100
RAT 14					
CPM/DAY	98,951	378,444	481,175	867,990	4,877,952
% URINE CTS	11.4	43.6	55.3	100	---
% DOSE	2.0	7.8	9.8	17.8	100
RAT 15					
CPM/DAY	83,648	456,360	543,088	1,058,840	4,877,952
% URINE CTS	7.9	43.1	55.3	100	---
% DOSE	1.7	9.4	11.1	21.7	100
RAT 16					
CPM/DAY	14,686	6,351	22,078	396,910	4,776,328
% URINE CTS	3.7	1.6	5.6	100	---
% DOSE	0.3	0.1	0.5	8.3	100
RAT 17					
CPM/DAY	12,440	4,330	20,672	68,730	5,182,824
% URINE CTS	18.1	6.3	30.0	100	---
% DOSE	0.2	0.1	0.4	1.3	100
RAT 18					
CPM/DAY	91,914	295,888	393,333	629,550	4,573,086
% URINE CTS	14.6	47.0	62.5	100	---
% DOSE	2.0	6.5	8.6	13.8	100
MEANS + SD					
CPM/DAY	88,328#	339,366#	430,939#	779,772#	4,959,251#
±SD	±8,915	±99,637	±103,417	±227,555	±166,985
% URINE CTS	14.5#	7.0#	56.9#	---	---
±SD	±2.8	±2.1	±3.8	---	---
% DOSE	1.8±0.2#	7.0±2.1#	8.3±3.2#	16.1±4.6#	---

1. Metab = all HPLC fraction except dC which contain label.
2. dC = HPLC fraction coeluting with dC.
3. Frac 1 = Affigel fraction containing deoxynucleosides and bases.
4. Radioactive label excreted in urine in the first 24 hours after injection.
5. Dose = amount of radioactive label injected.
6. PFC = pair fed control

Appendix 6b. Individual data on urinary excretion of label from ^{3}H-deoxycytidine in control and vitamin B-6 deficient rats after 2 or 6 weeks of deficiency. Page 3 of 8.

	METAB[1]	dC[2]	FRAC 1[3]	URINE[4]	DOSE[5]
			WEEK 2 ALD[6]		
RAT 49					
CPM/DAY	92,410	251,488	343,818	660,074	5,487,696
% URINE CTS	14.0	38.1	52.1	100	---
% DOSE	1.7	4.6	6.3	12.0	100
RAT 50					
CPM/DAY	102,792	197,415	300,190	680,741	4,674,704
% URINE CTS	15.1	29.0	44.1	100	---
% DOSE	2.2	4.2	6.4	14.6	100
RAT 51					
CPM/DAY	82,004	255,659	337,446	482,375	5,386,072
% URINE CTS	17.0	53.0	70.0	100	---
% DOSE	1.5	4.7	6.3	9.0	100
RAT 52					
CPM/DAY	92,350	216,444	308,987	577,185	5,284,448
% URINE CTS	16.0	37.5	58.5	100	---
% DOSE	1.7	4.1	5.8	10.9	100
RAT 53					
CPM/DAY	75,972	126,191	201,869	643,834	4,877,952
% URINE CTS	11.8	19.6	31.4	100	---
% DOSE	1.6	2.6	4.1	13.2	100
RAT 54					
CPM/DAY	23,308	2,825	26,767	353,150	5,792,568
% URINE CTS	5.6	0.8	7.6	100	---
% DOSE	0.4	0.05	0.5	6.1	100
MEANS $\pm$ SD					
CPM/DAY	71,106*	209,439*	298,462*	608,842*	5,120,174*
$\pm$SD	$\pm$39,739	$\pm$52,508	$\pm$57,055	$\pm$80,645	$\pm$349,090
% URINE CTS	14.8	35.4*	51.2*	---	---
$\pm$SD	$\pm$2.0	$\pm$12.4	$\pm$14.6	---	---
% DOSE	1.7$\pm$0.3*	4.0$\pm$0.8*	5.8$\pm$1.0*	11.9$\pm$2.1*	---

1. Metab = all HPLC fraction except dC which contain label.
2. dC = HPLC fraction coeluting with dC.
3. Frac 1 = Affigel fraction containing deoxynucleosides and bases.
4. Radioactive label excreted in urine in the first 24 hours after injection.
5. Dose = amount of radioactive label injected.
6. ALD = ad libitum deficient

Appendix 6b. Individual data on urinary excretion of label from ^{3}H-deoxycytidine in control and vitamin B-6 deficient rats after 2 or 6 weeks of deficiency. Page 4 of 8.

	METAB[1]	dC[2]	FRAC 1[3]	URINE[4]	DOSE[5]
			WEEK 2 MFD[6]		
RAT 85					
CPM/DAY	103,257	158,502	261,954	657,685	3,556,840
% URINE CTS	15.7	24.1	39.8	100	---
% DOSE	2.9	4.5	7.4	18.5	100
RAT 86					
CPM/DAY	50,086	138,440	188,445	281,382	2,438,976
% URINE CTS	17.8	49.2	67.0	100	---
% DOSE	2.1	5.7	7.7	11.5	100
RAT 87					
CPM/DAY	25,544	1,909	27,645	146,860	4,268,208
% URINE CTS	17.4	1.3	18.8	100	---
% DOSE	0.6	0.04	0.6	3.4	100
RAT 88					
CPM/DAY	3,398	923	7,296	20,974	3,861,712
% URINE CTS	16.2	4.4	34.8	100	---
% DOSE	0.1	0.02	0.2	0.5	100
RAT 89					
CPM/DAY	98,534	191,936	200,003	513,199	3,861,712
% URINE CTS	19.2	37.4	56.6	100	---
% DOSE	2.6	5.0	5.2	13.3	100
RAT 90					
CPM/DAY	131,089	395,998	527,501	910,341	4,979,576
% URINE CTS	14.4	43.5	57.9	100	---
% DOSE	2.6	8.0	10.6	18.3	100
MEANS + SD					
CPM/DAY	94,811#	212,219#	294,478#	515,652#	3,827,837#
±SD	±41,157	±118,590	±158,667	±280,251	±839,655
% URINE CTS	16.8#	38.6#	55.3#	---	---
+SD	+2.1	+10.8	+11.3	---	---
% DOSE	2.6±0.3#	5.8±1.5#	7.7±2.2#	15.4±3.5#	---

1. Metab = all HPLC fraction except dC which contain label.
2. dC = HPLC fraction coeluting with dC.
3. Frac 1 = Affigel fraction containing deoxynucleosides and bases.
4. Radioactive label excreted in urine in the first 24 hours after injection.
5. Dose = amount of radioactive label injected.
6. MFD = meal fed deficient

Appendix 6b. Individual data on urinary excretion of label from ^{3}H-deoxycytidine in control and vitamin B-6 deficient rats after 2 or 6 weeks of deficiency. Page 5 of 8.

	METAB[1]	dC[2]	FRAC 1[3]	URINE[4]	DOSE[5]
			WEEK 6 ALC[6]		
RAT 7					
CPM/DAY	332,021	1,760,898	2,092,068	2,964,475	9,451,032
% URINE CTS	11.2	59.4	70.6	100	---
% DOSE	3.5	18.6	22.1	31.4	100
RAT 8					
CPM/DAY	321,570	1,585,252	1,907,473	3,476,430	10,772,144
% URINE CTS	9.2	45.6	54.9	100	---
% DOSE	3.0	14.7	17.7	32.3	100
RAT 9					
CPM/DAY	261,334	2,141,488	2,402,055	3,629,640	9,247,784
% URINE CTS	7.2	59.0	66.2	100	---
% DOSE	2.8	23.2	26.0	39.2	100
RAT 10					
CPM/DAY	235,053	1,145,816	1,661,808	2,583,000	8,934,792
% URINE CTS	9.1	55.2	64.3	100	---
DOSE	2.6	15.9	18.6	28.9	100
RAT 11					
CPM/DAY	279,879	1,149,785	1,429,526	2,370,690	8,434,792
% URINE CTS	11.8	48.5	60.3	100	---
% DOSE	3.3	13.6	16.9	28.1	100
RAT 12					
CPM/DAY	289,879	1,074,969	1,365,771	3,019,575	9,775,904
% URINE CTS	9.6	35.6	45.2	100	---
% DOSE	3.0	11.0	14.0	31.0	100
MEANS + SD					
CPM/DAY	286,623	1,476,368	1,809,671	3,007,302	9,432,741
±SD	±36,434	±427.139	±401,041	±488,810	±797,099
% URINE CTS	9.7	50.6	60.2	---	---
+SD	+1.6	+9.2	+9.1	---	---
% DOSE	3.0±0.3	16.2±4.3	19.2±4.2	31.8±3.9	---

1. Metab = all HPLC fraction except dC which contain label.
2. dC = HPLC fraction coeluting with dC.
3. Frac 1 = Affigel fraction containing deoxynucleosides and bases.
4. Radioactive label excreted in urine in the first 24 hours after injection.
5. Dose = amount of radioactive label injected.
6. ALC = ad libitum control

Appendix 6b. Individual data on urinary excretion of label from ^{3}H-deoxycytidine in control and vitamin B-6 deficient rats after 2 or 6 weeks of deficiency. Page 6 of 8.

	METAB[1]	dC[2]	FRAC 1[3]	URINE[4]	DOSE[5]
			WEEK 6 PFC[6]		
RAT 19					
CPM/DAY	322,152	1,010,638	1,332,708	1,840,870	7,621,800
% URINE CTS	17.5	54.9	72.4	100	---
% DOSE	4.2	13.3	17.5	24.2	100
RAT 20					
CPM/DAY	3,385	90,257	124,412	1,128,210	7,418,552
% URINE CTS	3.0	8.0	11.0	100	---
% DOSE	0.05	1.2	1.7	15.2	100
RAT 21					
CPM/DAY	393,969	1,006,810	1,401,262	1,509,460	7,215,304
% URINE CTS	26.1	66.7	92.8	100	---
% DOSE	5.5	14.0	19.4	20.9	100
RAT 22					
CPM/DAY	57,037	14,173	71,361	345,680	7,316,928
% URINE CTS	16.5	4.1	20.6	100	---
% DOSE	0.8	0.2	1.0	4.7	100
RAT 23					
CPM/DAY	201,863	486,238	688,632	1,473,450	7,723,424
% URINE CTS	13.7	33.0	46.7	100	---
% DOSE	2.6	6.3	8.9	19.1	100
RAT 24					
CPM/DAY	189,125	428,506	618,279	1,322,550	7,316,928
% URINE CTS	14.3	32.4	46.7	100	---
% DOSE	2.6	5.9	8.4	18.1	100
MEANS $\pm$ SD					
CPM/DAY[#]	254,527	733,048	1,010,970	1,536,582	7,435,489
$\pm$SD	$\pm$94,784	$\pm$319,198	$\pm$414,850	$\pm$218,420	$\pm$197,231
% URINE CTS	17.9	46.8	64.6	---	---
+SD	+5.7[#]	+16.9[#]	+22.3[#]	---	---
% DOSE	3.7$\pm$1.4[#]	9.9$\pm$4.4[#]	13.6$\pm$5.7[#]	19.5$\pm$3.3[#]	---

1. Metab = all HPLC fraction except dC which contain label.
2. dC = HPLC fraction coeluting with dC.
3. Frac 1 = Affigel fraction containing deoxynucleosides and bases.
4. Radioactive label excreted in urine in the first 24 hours after injection.
5. Dose = amount of radioactive label injected.
6. PFC = pair fed control

Appendix 6b. Individual data on urinary excretion of label from ^{3}H-deoxycytidine in control and vitamin B-6 deficient rats after 2 or 6 weeks of deficiency. Page 7 of 8.

	METAB1	dC[2]	FRAC 1[3]	URINE[4]	DOSE[5]
			WEEK 6 ALD[6]		
RAT 55					
CPM/DAY	183,616	197,387	380,760	573,800	6,300,688
% URINE CTS	32.0	34.4	66.4	100	---
% DOSE	2.9	3.1	6.0	9.1	100
RAT 56					
CPM/DAY	143,969	120,403	264,520	428,480	6,605,560
% URINE CTS	33.6	28.1	61.7	100	---
% DOSE	2.2	1.8	4.0	10.5	100
RAT 57					
CPM/DAY	208,492	152,199	360,544	521,230	6,707,184
% URINE CTS	40.0	29.2	69.2	100	---
% DOSE	3.1	2.3	5.4	7.8	100
RAT 58					
CPM/DAY	147,060	177,264	324,307	495,150	6,707,184
% URINE CTS	29.7	35.8	65.5	100	---
% DOSE	2.2	2.6	4.8	7.4	100
RAT 59					
CPM/DAY	124,991	98,099	222,990	378,762	5,894,192
% URINE CTS	33.0	25.9	58.9	100	---
% DOSE	2.1	1.7	3.8	6.4	100
RAT 60					
CPM/DAY	242,243	133,270	375,490	736,300	6,605,560
% URINE CTS	32.9	18.1	51.0	100	---
% DOSE	3.7	2.0	5.7	11.1	100
MEANS $\pm$ SD					
CPM/DAY	175,062	149,130	321,435	522,287	6,503,936
$\pm$SD	$\pm$44,636	$\pm$40,584	$\pm$64,668	$\pm$125,373	$\pm$30,487
% URINE CTS	33.5	28.6	62.1	---	---
$\pm$SD	$\pm$3.5	$\pm$6.4	$\pm$6.5	---	---
% DOSE	2.7$\pm$0.6	2.4$\pm$0.5	5.0$\pm$0.9	8.7$\pm$1.8	---

1. Metab = all HPLC fraction except dC which contain label.
2. dC = HPLC fraction coeluting with dC.
3. Frac 1 = Affigel fraction containing deoxynucleosides and bases.
4. Radioactive label excreted in urine in the first 24 hours after injection.
5. Dose = amount of radioactive label injected.
6. ALD = ad libitum deficient

Appendix 6b. Individual data on urinary excretion of label from ^{3}H-deoxycytidine in control and vitamin B-6 deficient rats after 2 or 6 weeks of deficiency. Page 8 of 8.

	METAB[1]	dC[2]	FRAC 1[3]	URINE[4]	DOSE[5]
			WEEK 6 MFD[6]		
RAT 91					
CPM/DAY	80,163	91,033	171,128	452,900	4,877,952
% URINE CTS	17.7	20.1	37.8	100	---
% DOSE	1.6	1.9	3.5	9.3	100
RAT 92					
CPM/DAY	146,050	161,603	308,350	467,060	4,776,328
% URINE CTS	31.3	34.6	66.0	100	---
% DOSE	3.1	3.4	6.5	9.8	100
RAT 93					
CPM/DAY	193,250	169,576	362,704	550,570	5,081,200
% URINE CTS	35.1	30.8	65.9	100	---
% DOSE	3.8	3.3	7.1	10.8	100
RAT 94					
CPM/DAY	160,421	318,509	479,480	583,350	2,642,224
% URINE CTS	27.5	54.6	82.1	100	---
% DOSE	6.1	12.1	18.1	22.1	100
RAT 95					
CPM/DAY	68,717	329,531	398,154	780,880	5,284,448
% URINE CTS	8.8	42.2	51.0	100	---
% DOSE	1.3	6.2	7.5	14.8	100
RAT 96					
CPM/DAY	162,252	155,198	317,429	587,870	4,877,952
% URINE CTS	27.6	26.4	54.0	100	---
% DOSE	3.3	3.2	6.5	12.1	100
MEANS + SD					
CPM/DAY	130,086*	181,388*	311,553*	567,856*	4,979,576*
±SD	±53,707	±88,498	±86,429	±131,770	±203,428
% URINE CTS	24.1	34.8	54.9	---	---
+SD	+10.78	+12.2	+11.8*	---	---
% DOSE	2.6±1.1*	3.6±1.6*	6.2±1.6*	11.4±2.2*	---

1. Metab = all HPLC fraction except dC which contain label.
2. dC = HPLC fraction coeluting with dC.
3. Frac 1 = Affigel fraction containing deoxynucleosides and bases.
4. Radioactive label excreted in urine in the first 24 hours after injection.
5. Dose = amount of radioactive label injected.
6. MFD = meal fed deficient

Appendix 7a. Individual data on tissue PLP levels (nmol/g wet weight). Page 1 of 4.

ANIMAL	LIVER	SPLEEN	THYMUS	MUSCLE	HEART	INTEST INE	BONE MARROW	PLASMA
WEEK 2								
ALC 1	28.2	2.80	1.97	22.19	8.12	2.45	1.02	1518
2	27.7	2.41	2.06	22.35	3.54	1.72	1.13	937
3	27.5	2.05	1.82	20.97	7.60	2.58	0.64	1242
4	22.5	2.60	1.84	25.10	8.10	2.89	0.87	1253
5	27.3	2.68	2.05	22.03	7.12	2.17	1.09	1390
6	32.6	2.87	2.21	18.13	7.70	2.04	1.09	762
MEAN	27.6	2.57	1.99	21.78	7.03	2.31	0.97	1184
±SD	±3.2	±0.30	±0.15	±2.26	±1.75	±0.42	±0.19	±283
PFC 13	26.7	3.20	1.72	23.00	4.71	2.68	0.86	1219
14	30.4	2.74	1.96	20.42	8.19	1.69	0.83	843
15	27.6	3.43	2.12	19.69	8.73	2.26	0.86	1073
16	25.8	2.84	2.18	22.44	8.67	1.87	0.39	962
17	24.2	2.16	1.84	19.61	7.27	1.63	1.21	885
18	25.8	2.97	1.61	19.22	6.68	2.17	1.03	1862
MEAN	26.8	2.89	1.91	20.73	7.39	2.05	0.86	1141
±SD	±2.1	0.44	±0.22	±1.60	±1.51	±0.40	±0.27	±378
ALD 49	9.0	0.60	0.71	9.92	5.54	0.30	0.77	19.2
50	10.3	0.74	0.55	11.22	5.15	0.44	0.32	23.9
51	5.5	0.86	0.69	10.13	5.58	0.46	0.32	51.4
52	8.5	0.84	0.65	11.01	5.17	0.52	0.26	30.0
53	7.7	0.60	0.60	9.06	4.90	0.33	0.31	9.6
54	9.0	0.97	0.38	11.07	4.43	0.35	0.32	19.1
MEAN	10.0	0.77	0.60	10.40	5.13	0.40	0.38	25.5
±SD	±2.8	±0.15	±0.12	±0.85	±0.43	±0.09	±0.19	±14.3
MFD 85	13.9	0.78	0.79	12.04	4.26	0.47	0.30	47.6
86	10.8	0.96	0.83	10.61	5.35	0.29	0.17	85.6
87	15.5	0.70	0.77	7.30	4.37	0.48	0.28	48.0
88	13.0	1.05	0.75	12.62	4.27	0.44	0.36	61.1
89	17.7	0.98	0.71	11.81	4.38	0.65	0.29	63.8
90	19.7	0.96	0.73	8.29	5.27	0.35	0.31	79.2
MEAN	15.0	0.90	0.76	10.45	4.65	0.45	0.28	64.2
±SD	±3.3	±0.14	±0.04	±2.18	±0.51	±0.14	±0.06	±15.7

ALC =ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 7a. Individual data on tissue PLP levels (nmol/g wet weight). Page 2 of 4.

ANIMAL	LIVER	SPLEEN	THYMUS	MUSCLE	HEART	INTESTINE	BONE MARROW	PLASMA
WEEK 6								
ALC 7	27.2	2.73	--	25.99	8.74	1.54	1.54	1072
8	29.4	2.54	--	22.67	7.91	1.33	2.05	1371
9	26.3	2.35	--	24.56	8.66	1.39	2.27	1289
10	28.5	2.78	--	20.96	8.94	2.32	2.37	835
11	25.8	2.64	--	23.00	7.21	1.91	2.09	1278
12	27.5	2.69	--	21.51	7.80	1.69	1.92	1116
MEAN	27.5	2.62	--	23.11	8.21	1.70	2.04	1160
±SD	±1.3	±0.16	--	±1.89	±0.67	±0.37	±0.29	±195
PFC19	32.8	2.45	--	20.14	7.50	1.87	1.82	677
20	36.2	2.35	--	19.50	8.74	2.13	1.70	354
21	32.7	2.03	--	22.40	8.12	1.85	2.17	417
22	36.5	3.08	--	24.53	8.26	2.00	2.15	841
23	29.0	3.20	--	18.36	7.51	2.05	2.80	1064
24	32.7	3.12	--	24.10	7.08	1.68	2.26	700
MEAN	33.7	2.71	--	21.51	7.87	1.93	2.15	676
±SD	±2.7	±0.49	--	±2.55	±0.61	±0.16	±0.39	±264
ALD55	9.0	0.74	--	6.84	5.26	0.79	0.38	16.0
56	9.7	0.79	--	8.02	1.28	0.50	0.56	18.0
57	5.2	0.69	--	7.65	4.99	0.47	0.37	10.7
58	9.9	0.73	--	6.35	5.04	0.37	0.42	8.9
59	4.7	0.55	--	7.46	4.87	0.35	0.42	3.7
60	8.4	0.54	--	7.26	4.86	0.33	0.30	28.6
MEAN	7.8	0.68	--	7.26	4.38	0.47	0.41	13.4
±SD	±2.3	±0.10	--	±0.60	±1.53	±0.17	±0.09	±8.3
MFD91	11.7	0.52	--	4.10	4.65	0.43	0.19	12.1
92	13.3	0.65	--	8.28	4.76	0.44	0.20	17.0
93	12.1	0.51	--	7.68	5.16	0.10	0.36	35.7
94	11.1	0.67	--	6.72	3.86	0.07	0.47	21.2
95	12.8	0.59	--	7.44	5.77	0.44	0.25	22.0
96	8.1	0.64	--	6.49	4.30	0.49	0.50	22.4
MEAN	11.5	0.60	--	6.79	4.75	0.33	0.33	21.2
±SD	±1.9	±0.07	--	±1.47	±0.67	±0.19	±0.14	±8.3

ALC =ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 7a. Individual data an tissue PLP levels (nmol/g wet weight). Page 3 of 4.

ANIMAL	LIVER	SPLEEN	THYMUS	MUSCLE	HEART	INTESTINE	BONE MARROW	PLASMA
WEEK 6 FAST DAY 1								
PFC31	29.3	3.47	--	18.0	9.06	1.59	3.26	604
32	28.8	2.85	--	23.0	9.06	1.49	2.36	424
33	29.9	3.34	--	23.9	8.86	2.13	2.13	484
34	30.1	3.24	--	24.1	9.00	1.90	2.05	586
35	27.4	2.91	--	26.9	8.80	1.84	2.36	778
36	29.2	2.93	--	26.9	8.02	1.93	2.40	622
MEAN	29.1	3.12	--	23.8	8.80	1.81	2.43	583
±SD	±0.9	±0.20	--	±3.3	±0.40	±0.23	±0.43	±123
ALD67	11.9	0.65	--	7.4	5.52	0.34	--	25.7
68	13.6	0.94	--	8.8	6.05	0.79	0.91	40.2
69	18.1	0.94	--	7.2	5.91	0.76	0.88	31.1
70	12.8	0.79	--	5.5	5.88	0.58	0.78	33.6
71	12.8	0.81	--	6.0	7.69	0.47	0.86	45.0
72	11.0	0.82	--	6.0	4.76	0.49	0.60	21.9
MEAN	13.4	0.83	--	6.8	5.97	0.57	0.81	32.9
±D	±2.5	±0.11	--	±1.2	±0.96	±0.18	±0.12	±8.7
DAY 3								
PFC25	35.5	2.85	--	17.3	8.30	1.08	4.17	360
26	27.5	2.63	--	17.3	9.10	1.66	2.23	468
27	25.2	2.56	--	23.5	8.16	1.38	2.31	603
28	26.2	3.02	--	16.8	8.47	1.86	2.67	944
29	23.1	2.74	--	16.6	8.99	2.35	2.06	601
302	29.4	2.64	--	20.2	8.16	1.44	1.58	430
MEAN	27.8	2.74	--	18.6	8.60	1.63	2.50	568
±SD	±4.3	±0.17	--	±2.7	±0.42	±0.44	±0.89	±208
ALD61	13.6	0.81	--	6.3	3.82	0.67	0.73	40.5
62	15.0	0.94	--	7.0	5.53	0.84	0.86	62.1
63	15.9	1.11	--	7.8	5.71	0.97	1.41	48.0
64	13.4	0.84	--	8.4	5.52	0.45	0.54	35.4
65	14.8	0.93	--	6.7	5.02	0.59	0.70	35.8
66	12.6	0.79	--	5.9	5.19	0.59	0.76	45.7
MEAN	14.2	0.90	--	7.0	5.13	0.67	0.83	44.6
±SD	±1.2	0.12	--	±0.9	±0.69	±0.18	±0.30	±10.0

ALC =ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 7a. Individual data on tissue PLP levels (nmol/g wet weight). Page 4 of 4.

ANIMAL	LIVER	SPLEEN	THYMUS	MUSCLE	HEART	INTEST INE	BONE MARROW	PLASMA
REPLETION								
PFC37	30.5	2.60	2.05	25.1	7.49	1.84	2.13	830
38	35.7	2.20	2.25	22.6	7.64	2.24	2.19	980
39	29.2	2.66	3.32	20.2	6.91	1.76	2.62	902
40	32.4	2.65	2.23	24.0	7.67	2.19	2.30	758
41	34.6	2.54	2.53	24.4	7.60	2.52	2.80	705
42	36.2	3.03	2.86	24.5	8.58	1.83	2.24	1578
MEAN	33.1	2.61	2.28	23.6	7.65	2.06	2.38	959
±SD	±2.8	±0.27	±0.25	±1.7	±0.54	±0.30	±0.27	±319
ALD73	33.8	2.24	2.32	15.1	8.20	2.15	2.10	549
74	33.9	2.71	2.40	13.6	8.62	2.01	2.03	806
75	28.5	2.30	2.38	15.0	8.66	1.91	1.75	585
76	30.8	2.08	2.52	16.5	8.32	2.15	2.10	782
77	35.5	2.57	2.49	10.6	8.63	1.41	2.48	1209
78	28.7	2.40	2.61	17.2	7.75	1.66	2.16	1196
MAN	31.9	2.38	2.45	14.7	8.36	1.88	2.10	854
±SD	±2.9	±0.22	±0.11	±2.3	±0.35	±0.29	±0.23	±288
REPLETION DAY 3 FAST								
PFC43	24.3	2.55	2.40	27.4	8.67	1.86	2.01	884
44	28.2	0.87	1.95	21.6	6.62	1.63	2.06	595
45	26.0	2.54	2.42	20.8	8.28	2.04	2.16	713
46	26.1	3.14	2.57	24.2	8.31	1.62	2.38	982
47	24.4	2.87	2.31	26.7	7.65	1.75	1.64	899
48	28.5	2.25	2.00	21.7	7.18	1.70	1.68	691
MEAN	26.3	2.37	2.54	23.7	7.78	1.77	1.99	794
±SD	±1.8	±0.80	±0.48	±2.8	±0.78	±0.16	±0.28	±149
ALD79	25.7	2.28	1.94	10.7	7.08	1.51	1.63	1072
80	21.4	2.28	2.45	15.4	7.60	1.46	1.57	516
81	23.1	2.54	2.29	17.6	8.33	1.51	2.29	982
82	28.8	1.96	2.36	15.0	7.69	1.30	2.29	680
83	26.2	2.18	2.99	16.3	7.94	1.18	1.75	736
84	21.6	2.24	1.82	13.4	7.50	1.21	1.92	712
MEAN	24.5	2.25	2.31	14.8	7.69	1.36	1.91	783
±SD	±2.9	±0.19	±0.42	±2.4	±0.42	±0.15	±0.32	±206

ALC =ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 7b. Individual data on tissue PLP values (nmol/g protein). Page 1 of 4.

	ANIMAL	LIVER	SPLEEN	THYMUS	MUSCLE	HEART	INTESTINE
WEEK 2							
ALC	1	176	26.7	39.8	179	128	74
	2	157	26.3	42.2	173	65	40
	3	163	23.7	37.7	175	97	59
	4	147	26.2	40.4	185	103	59
	5	154	27.5	44.9	179	112	58
	6	204	30.4	49.5	189	112	53
MEAN		166	26.8	42.4	180	103	57
±SD		±22	±2.1	±4.2	±6	±22	±11
PFC	13	143	35.6	35.7	209	156	33
	14	150	28.7	41.7	216	113	72
	15	140	34.5	45.1	191	60	92
	16	127	28.5	44.8	192	110	70
	17	122	25.6	34.4	149	73	62
	18	136	33.1	36.5	183	89	55
MEAN		136	31.0	39.7	190	100	64
±SD		±10	±4.0	±4.8	±24	±34	±20
ALD	49	56	6.4	14.1	66	61	10
	50	63	7.6	11.6	114	62	15
	51	27	10.2	14.5	106	47	22
	52	55	8.2	13.5	90	66	19
	53	47	6.3	11.8	84	64	14
	54	56	9.9	8.9	83	48	13
MEAN		51	8.1	12.4	90	58	16
±SD		±13	±1.7	±2.1	±17	±8	±4
MFD	85	62	8.2	16.4	78	52	17
	86	47	9.5	17.1	104	65	17
	87	72	7.0	14.7	60	46	13
	88	65	10.8	14.5	105	41	15
	89	77	10.8	14.3	93	58	39
	90	89	9.3	14.9	79	56	32
MEAN		69	9.3	15.3	87	53	22
±SD		±14	±1.5	±1.1	±17	±9	±11

ALC =ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 7b. Individual data on tissue PLP values (nmol/g protein). Page 2 of 4.

	ANIMAL	LIVER	SPLEEN	THYMUS	MUSCLE	HEART	INTESTINE
WEEK 6							
ALC	7	158	27.9	--	167	135	33
	8	168	22.4	--	203	110	32
	9	146	21.5	--	244	135	42
	10	162	29.0	--	137	129	47
	11	143	25.8	--	186	107	87
	12	161	24.9	--	154	115	63
MEAN		156	25.3	--	181	122	51
±SD		±10	±3.0	--	±38	±13	±21
PFC	19	165	26.1	--	139	109	55
	20	174	23.8	--	138	118	58
	21	150	19.1	--	197	122	52
	22	171	26.4	--	196	126	61
	23	135	29.1	--	112	116	52
	24	154	28.1	--	148	125	46
MEAN		158	25.4	--	155	119	54
±SD		±15	±3.6	--	±34	±6	±5
ALD	55	52	7.1	--	55	71	32
	56	54	7.4	--	73	41	12
	57	28	6.6	--	58	69	16
	58	47	6.2	--	53	70	24
	59	29	4.5	--	77	71	10
	60	44	5.0	--	67	62	13
MEAN		42	6.0	--	64	64	18
±SD		±11	±1.1	--	±10	±12	±8
MFD	91	55	5.1	--	35	66	10
	92	65	6.7	--	61	71	10
	93	61	5.2	--	51	72	10
	94	52	7.8	--	76	62	6
	95	58	5.6	--	54	82	14
	96	38	6.6	--	58	77	18
MEAN		55	6.2	--	56	72	12
±SD		±9	±1.1	--	±14	±7	±4

ALC =ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 7b. Individual data on tissue PLP values (nmol/g protein). Page 3 of 4.

	ANIMAL	LIVER	SPLEEN	THYMUS	MUSCLE	HEART	INTESTINE
WEEK 6 FAST DAY 1							
PFC	31	137	31.2	--	142	114	59
	32	135	26.2	--	177	108	70
	33	137	29.1	--	186	124	55
	34	148	34.0	--	173	123	83
	35	125	29.1	--	208	69	69
	36	129	28.0	--	202	82	54
MEAN		135	29.6	--	181	99	65
±SD		±8	±2.7	--	±23	±23	±11
ALD	67	57	5.1	--	53	77	17
	68	64	8.7	--	60	67	23
	69	82	8.2	--	49	45	26
	70	59	7.8	--	40	64	24
	71	71	8.6	--	39	91	18
	72	55	7.6	--	62	55	16
MEAN		65	7.7	--	50	66	21
±SD		±10	±1.3	--	±10	±16	±4
DAY 3							
PFC	25	160	28.1	--	169	99	55
	26	127	27.3	--	142	98	63
	27	120	24.7	--	171	90	80
	28	133	29.7	--	176	104	87
	29	111	29.6	--	121	108	78
	30	127	23.8	--	158	84	82
MEAN		130	27.2	--	156	97	74
±SD		±17	±2.5	--	±21	±9	±12
ALD	61	62	6.4	--	72	62	38
	62	66	8.2	--	48	68	38
	63	73	10.2	--	56	74	73
	64	66	8.0	--	71	45	22
	65	63	9.3	--	64	58	26
	66	58	6.8	--	44	63	21
MEAN		65	8.2	--	59	62	36
±SD		±5	±1.4	--	±12	±10	±20

ALC =ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 7b. Individual data on tissue PLP values (nmol/g protein). Page 4 of 4.

	ANIMAL	LIVER	SPLEEN	THYMUS	MUSCLE	HEART	INTESTINE
REPLETION							
PFC	37	172	28.1	38.2	198	115	48
	38	191	26.3	45.2	240	102	68
	39	153	28.0	60.9	192	89	72
	40	162	28.0	42.8	210	112	87
	41	177	24.7	49.9	236	111	64
	42	201	31.7	54.3	193	126	61
MEAN		176	27.8	48.6	211	109	67
±SD		±18	±2.3	±8.2	±22	±12	±13
ALD	73	197	22.6	44.9	142	116	54
	74	172	30.0	44.5	103	122	100
	75	176	29.8	44.9	129	129	67
	76	165	22.3	49.7	143	126	61
	77	194	22.9	47.9	88	114	61
	78	170	26.4	50.0	120	108	60
MEAN		179	25.7	47.0	121	119	67
±SD		±13	±3.6	±2.5	±22	±8	±17
REPLETION DAY 3 FAST							
PFC	43	110	27.6	42.5	199	131	71
	44	137	12.7	33.6	173	106	56
	45	132	30.9	42.5	228	129	90
	46	122	34.9	53.7	152	130	78
	47	115	32.6	40.3	201	129	84
	48	140	30.0	41.4	135	108	70
MEAN		126	28.0	35.6	181	122	75
±SD		±12	±8.0	±18	±34	±12	±12
ALD	79	137	22.6	38.6	85	113	82
	80	108	24.3	43.7	118	113	83
	81	118	29.6	41.2	148	126	67
	82	168	23.5	47.4	113	109	57
	83	139	26.2	59.7	121	127	52
	84	98	25.1	35.5	97	113	63
MEAN		128	25.2	44.5	113	117	67
±SD		±25	±2.5	±9.6	±22	±8	±13

ALC =ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 7c. Individual data on tissue PLP levels (nmol/mg DNA). Page 1 of 4

	ANIMAL	LIVER	THYMUS	MUSCLE	INTESTINE	BONE MARROW
WEEK 2						
ALC	1	37.9	3.16	108	1.81	110.9
	2	33.6	2.44	168	2.81	90.6
	3	40.6	4.40	151	5.36	79.6
	4	41.2	4.95	216	8.03	81.1
	5	42.2	3.88	193	1.49	106.4
	6	49.2	5.86	208	2.86	92.4
MEAN		40.8	4.12	174	3.73	93.5
±SD		±5.2	±1.23	±40	±2.51	±12.8
PFC	13	44.2	3.89	261	5.61	75.2
	14	61.3	4.14	272	2.82	30.7
	15	45.4	1.99	177	7.24	86.1
	16	42.6	4.88	255	6.56	59.3
	17	37.3	3.10	174	2.25	135.5
	18	41.2	3.52	180	6.86	65.4
MEAN		45.3	2.59	220	5.21	75.4
±SD		±8.3	±0.99	±47	±2.15	±32.0
ALD	49	15.6	0.82	83	0.49	67.8
	50	20.4	1.16	83	0.36	24.4
	51	10.7	0.86	119	0.63	27.6
	52	15.5	0.76	111	0.89	13.6
	53	13.5	0.88	97	0.86	27.0
	54	17.0	0.65	101	0.87	21.0
MEAN		15.5	0.86	99	0.68	30.2
±SD		±3.3	±0.17	±15	±0.23	±19.1
MFD	85	30.4	0.96	142	0.29	27.8
	86	17.2	--	109	0.23	216.2
	87	29.6	0.98	70	0.32	22.8
	88	26.9	0.91	158	0.20	30.5
	89	30.9	0.92	76	2.03	38.5
	90	36.0	0.85	76	0.68	25.9
MEAN		28.5	0.93	105	0.65	27.0
±SD		±6.3	±0.05	±38	±0.70	±7.5

ALC =ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 7c. Individual data on tissue PLP levels (nmol/mg DNA). Page 2 of 4.

	ANIMAL	LIVER	THYMUS	MUSCLE	INTESTINE	BONE MARROW
WEEK 6						
ALC	7	38.4	--	126	2.57	82.4
	8	33.9	--	171	3.91	99.2
	9	39.2	--	177	2.34	102.1
	10	41.5	--	181	6.86	98.8
	11	50.3	--	202	7.29	86.5
	12	44.1	--	247	5.73	85.7
MEAN		41.2	--	184	4.78	92.5
±SD		±5.6	--	±38	±2.15	±8.5
PFC	19	39.3	--	228	9.74	133.4
	20	52.0	--	260	6.87	81.9
	21	59.7	--	202	5.73	110.3
	22	50.7	--	279	5.85	152.1
	23	31.7	--	162	5.12	136.8
	24	37.6	--	225	5.94	122.4
MEAN		45.2	--	226	6.54	122.8
±SD		±10.6	--	±41	±1.66	±24.5
ALD	55	13.9	--	58	1.85	21.4
	56	14.9	--	59	1.32	33.5
	57	13.9	--	90	1.14	28.8
	58	13.5	--	64	1.15	29.7
	59	12.7	--	80	1.52	19.3
	60	12.2	--	66	0.90	16.4
MEAN		13.5	--	70	1.31	24.9
±SD		±1.0	--	±13	±0.33	±6.7
MFD	91	22.3	--	48	0.95	14.6
	92	25.1	--	85	1.48	18.5
	93	19.1	--	73	0.22	21.9
	94	15.1	--	84	0.27	31.1
	95	18.2	--	48	1.07	19.8
	96	11.3	--	60	1.66	19.1
MEAN		18.5	--	66	0.94	20.8
±SD		±4.9	--	±16	±0.60	±5.6

ALC =ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 7c. Individual data on tissue PLP levels (nmol/mg DNA). Page 3 of 4.

	ANIMAL	LIVER	THYMUS	MUSCLE	INTESTINE	BONE MARROW
WEEK 6 FAST						
DAY 1						
PFC	31	32.5	--	180	1.53	153.1
	32	27.9	--	176	1.50	160.7
	33	27.4	--	254	3.87	151.2
	34	35.6	--	402	6.17	166.4
	35	33.0	--	448	3.79	172.1
	36	34.6	--	292	3.91	182.8
MEAN		31.8	--	292	3.46	164.4
±SD		±3.4	--	±113	±1.75	±12.0
ALD	67	15.9	--	73	0.26	--
	68	15.0	--	126	1.20	53.2
	69	25.1	--	68	0.77	47.1
	70	19.2	--	53	0.45	52.6
	71	18.0	--	58	0.34	58.0
	72	11.3	--	70	0.32	44.1
MEAN		17.4	--	75	0.56	51.0
±SD		±4.6	--	±26	±0.36	±5.4
DAY 3						
PFC	25	39.4	--	154	0.75	279.5
	26	45.9	--	180	6.72	188.0
	27	35.3	--	247	3.84	192.4
	28	36.1	--	198	3.20	251.3
	29	51.6	--	163	2.35	173.9
	30	45.4	--	213	3.77	218.8
MEAN		42.3	--	192	3.44	217.3
±SD		±6.4	--	±34	±1.98	±41.0
ALD	61	18.3	--	69	1.61	91.2
	62	22.6	--	62	0.69	70.5
	63	21.3	--	75	0.80	100.5
	64	17.3	--	79	0.62	74.1
	65	17.0	--	70	0.60	72.1
	66	22.3	--	53		70.7
MEAN		19.8	--	68	0.86	79.9
±SD		±2.6	--	±9	±0.42	±12.8

ALC =ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 7c. Individual data on tissue PLP levels (nmol/mg DNA). Page 4 of 4.

	ANIMAL	LIVER	THYMUS	MUSCLE	INTESTINE	BONE MARROW
REPLETION						
PFC	37	34.0	--	189	2.94	145.8
	38	37.5	6.32	226	3.26	168.2
	39	42.1	7.90	177	7.67	180.2
	40	57.8	7.29	231	7.00	148.4
	41	49.2	10.81	212	6.57	179.8
	42	55.9	12.12	194	5.66	137.4
MEAN		46.1	8.93	205	5.52	160.0
±SD		±9.8	±1.49	±22	±1.98	±18.5
ALD	73	47.4	6.19	134	11.67	130.8
	74	38.8	8.25	142	6.58	144.5
	75	34.6	9.96	179	3.52	284.1
	76	42.0	9.40	119	8.51	128.8
	77	46.8	6.97	84	2.34	145.8
	78	33.0	9.29	158	3.61	129.7
MEAN		40.4	8.34	136	6.04	160.6
±SD		±6.1	±1.49	±33	±3.58	±61.0
REPLETION FAST DAY 3						
PFC	43	23.7	5.88	342	5.72	162.5
	44	38.9	7.09	251	2.20	144.2
	45	31.7	5.99	245	13.05	155.2
	46	35.6	9.73	250	4.78	179.3
	47	32.1	8.52	254	7.33	111.7
	48	32.7	7.52	268	10.30	84.6
MEAN		32.4	7.46	268	7.23	139.6
±SD		±5.1	±1.49	±37	±3.92	±35.1
ALD	79	58.9	8.26	127	2.06	133.3
	80	30.3	11.61	177	4.07	101.0
	81	28.6	10.65	169	4.09	149.4
	82	37.1	8.25	172	1.52	180.6
	83	39.8	12.72	129	3.52	118.5
	84	28.4	9.15	158	1.34	155.9
MEAN		37.2	10.11	156	2.77	139.8
±SD		±11.6	±1.85	±22	±1.27	±28.3

ALC =ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 7d. Individual data on tissue PLP levels (nmol/tissue). Page 1 of 4.

	ANIMAL	LIVER	SPLEEN	THYMUS	HEART
WEEK 2					
ALC	1	409	2.24	1.43	7.42
	2	470	1.84	1.50	2.96
	3	435	1.61	1.46	7.24
	4	305	2.30	1.50	7.83
	5	300	2.20	1.04	6.22
	6	400	2.24	1.96	6.85
MEAN		382	2.08	1.48	6.42
±SD		±77	±0.25	±0.29	±1.78
PFC	13	262	2.62	1.28	4.46
	14	262	2.57	1.62	7.74
	15	240	2.60	1.23	6.76
	16	269	2.11	1.29	7.33
	17	202	1.34	0.86	5.55
	18	203	1.91	0.93	5.06
MEAN		240	2.19	1.20	6.15
±SD		±30	±0.51	±0.28	±1.31
ALD	49	132	0.46	0.19	4.73
	50	130	0.46	0.29	4.68
	51	134	0.55	0.28	3.69
	52	118	0.73	0.23	4.34
	53	112	0.38	0.23	3.89
	54	127	0.95	0.23	4.22
MEAN		125	0.59	0.24	4.26
±SD		±9	±0.21	±0.04	±0.42
MFD	85	83	0.49	0.26	2.68
	86	50	0.31	0.11	1.33
	87	111	0.45	0.20	3.13
	88	83	0.52	0.19	2.81
	89	86	0.38	0.29	2.92
	90	165	0.59	0.34	3.90
MEAN		96	0.46	0.76	2.80
±SD		±39	±0.10	±0.04	±0.84

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 7d. Individual data on tissue PLP levels (nmol/tissue). Page 2 of 4.

	ANIMAL	LIVER	SPLEEN	THYMUS	HEART
WEEK 6					
ALC	7	370	2.46	--	9.01
	8	465	2.41	--	9.58
	9	422	2.46	--	9.85
	10	417	2.02	--	8.68
	11	315	2.00	--	6.25
	12	306	2.19	--	7.96
MEAN		382	2.26	--	8.56
±SD		±63	±0.22	--	±1.31
PFC	19	286	1.60	--	7.15
	20	292	1.76	--	7.62
	21	264	1.19	--	6.72
	22	302	2.53	--	7.16
	23	237	2.49	--	6.21
	24	258	1.84	--	5.46
MEAN		273	1.90	--	6.72
±SD		±24	±0.52	--	±0.78
ALD	55	119	0.34	--	5.24
	56	110	0.44	--	0.97
	57	60	0.47	--	3.99
	58	120	0.43	--	3.73
	59	66	0.42	--	2.87
	60	103	0.30	--	3.36
MEAN		96	0.40	--	3.36
±SD		±27	±0.07	--	±1.42
MFD	91	78	0.24	--	2.56
	92	85	0.26	--	2.49
	93	92	0.23	--	3.38
	94	40	0.55	--	1.72
	95	84	0.22	--	3.39
	96	42	0.30	--	2.39
MEAN		71	0.30	--	2.66
±SD		±22	±0.13	--	±0.64

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 7d. Individual data on tissue PLP levels (nmol/tissue). Page 3 of 4.

	ANIMAL	LIVER	SPLEEN	THYMUS	HEART
WEEK 6 FAST					
DAY 1					
PFC	31	219	2.79	--	8.15
	32	196	1.86	--	7.60
	33	210	2.13	--	6.88
	34	220	1.94	--	7.01
	35	196	1.60	--	7.53
	36	202	1.66	--	6.29
MEAN		207	2.00	--	7.24
±SD		±11	±0.43	--	±0.65
ALD	67	111	0.46	--	4.06
	68	112	0.54	--	4.26
	69	172	0.55	--	5.11
	70	98	0.52	--	4.13
	71	77	0.54	--	4.51
	72	86	0.53	--	3.27
MEAN		109	0.52	--	4.22
±SD		±34	±0.03	--	±0.60
DAY 3					
PFC	25	231	1.25	--	6.36
	26	184	1.39	--	7.15
	27	147	1.58	--	7.55
	28	164	1.26	--	6.11
	29	156	1.52	--	6.34
	30	199	1.72	--	6.32
MEAN		180	1.45	--	6.64
±SD		±31	±0.19	--	±0.57
ALD	61	92	0.76	--	2.67
	62	111	0.55	--	3.97
	63	110	0.87	--	4.17
	64	92	0.40	--	2.96
	65	87	0.39	--	3.38
	66	107	0.49	--	3.42
MEAN		100	0.58	--	3.43
±SD		±11	±0.20	--	±0.57

ALC = ad libitum control, PFC = pair fed control,
ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 7d. Individual data on tissue PLP levels (nmol/tissue). Page 4 of 4.

	ANIMAL	LIVER	SPLEEN	THYMUS	HEART
REPLETION					
PFC	37	386	1.96	1.20	7.49
	38	484	2.18	1.45	7.72
	39	244	2.77	0.87	5.60
	40	385	2.16	0.98	7.04
	41	445	2.14	1.82	7.96
	42	522	2.78	1.36	8.63
MEAN		411	2.33	1.28	7.41
±SD		±98	±0.35	±0.39	±1.03
ALD	73	544	1.99	1.32	7.38
	74	433	2.27	1.45	8.68
	75	406	1.99	1.70	8.04
	76	385	2.27	1.24	8.41
	77	520	2.73	1.70	8.68
	78	421	2.07	1.48	7.75
MEAN		452	2.22	1.48	8.16
±SD		±65	±0.28	±0.19	±0.53
REPLETION FAST DAY 3					
PFC	43	185	1.78	1.05	8.40
	44	217	0.50	0.83	6.18
	45	189	1.89	0.97	7.33
	46	190	2.16	1.22	7.20
	47	187	2.16	0.96	7.35
	48	220	1.40	0.98	7.89
MEAN		198	1.64	1.00	7.39
SD		±16	±0.63	±0.13	±0.75
ALD	79	218	1.41	1.02	8.17
	80	154	1.42	1.29	6.04
	81	167	1.27	1.49	7.64
	82	238	1.50	1.07	7.01
	83	219	1.75	2.30	7.51
	84	177	1.71	1.32	6.82
MEAN		196	1.51	1.42	7.20
±SD		±34	±0.19	±0.47	±0.74

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ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 8a. Individual data on liver serine transhydroxymethylase activity. Page 1 of 4

RAT	% STIM[2]	SERINE TRANSHYDROXYMETHYLASE ACTIVITY U/MG TISSUE[1] -PLP	+PLP	U/TISSUE -PLP	+PLP	U/G PROTEIN -PLP	+PLP	U/UG DNA -PLP	+PLP
				WEEK 2 ALC[3]					
1	81	34.6	182	503	2,643	216	1,137	3.20	16.8
2	80	25.3	128	277	1,400	142	818	2.80	14.2
3	73	57.1	210	901	3,320	338	1,241	5.32	19.5
4	76	49.7	206	676	2,810	315	1,306	6.70	27.8
5	76	43.4	184	477	2,030	245	1,042	6.10	26.0
6	76	45.2	189	549	2,320	282	1,181	5.57	23.3
MEAN	77	42.5	183	564	2,420	256	1,104	4.95	21.3
± SD	±3	±11.2	±29	±210	±665	±72	±209	±1.59	±5.3
				WEEK 2 PFC					
13	88	23.4	189.6	225	1,858	125	1,014	3.95	32.0
14	80	49.3	247.4	424	2,129	244	1,225	11.55	57.9
15	88	24.1	194.1	209	1,689	122	985	4.56	36.7
16	83	36.7	215.0	382	2,236	181	1,059	5.83	34.1
17	80	43.4	211.8	361	1,772	219	1,070	8.01	39.1
18	89	20.5	188.8	161	1,487	108	994	4.16	38.3
MEAN	85	32.9	207.7	290	1,860	166	1,058	6.34	39.7
± SD	±4	±12.0	±22.5	±110	±280	±57	±89	±2.96	±9.3
				WEEK 2 ALD					
49	88	19.8	171.2	289	2,497	123	1,063	2.35	20.3
50	86	27.4	191.1	345	2,407	169	1,180	4.32	30.1
51	82	25.5	137.9	222	1,198	126	683	5.72	30.9
52	86	22.0	161.4	304	2,220	143	1,048	2.91	21.4
53	79	21.9	106.5	274	1,330	132	642	3.07	14.9
54	80	30.9	154.5	439	2,200	192	960	4.14	20.7
MEAN	84	24.6	154.1	312	1,980	148	929	3.75	23.0
± SD	±4	±4.1	±29.7	±70	±560	±27	±219	±1.22	±6.2
				WEEK 2 MFD					
85	80	36.1	182.0	216	1,090	161	872	13.22	66.7
86	87	25.7	196.9	118	910	113	864	8.86	67.9
87	83	27.8	160.5	200	1,150	130	750	7.41	42.8
88	90	17.6	173.7	113	1,110	88	868	5.71	56.4
89	93	12.4	167.2	60	800	54	727	4.46	60.1
90	82	25.0	136.4	210	1,140	113	617	5.45	29.7
MEAN	86	24.1	169.4	150	1,030	110	773	7.52	53.9
± SD	±5	±8.2	±20.5	±60	±140	±36	±96	±3.20	±14.9

1. U=umoles formaldehyde/hour/mg tissue, tissue, g protein or ug DNA.
2. % STIM = (activity of STHM with PLP - activity of STHM without PLP)/(activity STHM without PLP) X 100.
3. ALC=ad libitum control, PFC=pair fed control, ALD=ad libitum deficient, MFD = meal fed deficient.

* n=5, # n=4, + not included in calculation of mean.

Appendix 8a. Individual data on liver serine transhydroxymethylase activity. Page 2 of 4.

		SERINE TRANSHYDROXYMETHYLASE ACTIVITY							
	%	U/MG TISSUE[1]		U/TISSUE		U/G PROTEIN		U/UG DNA	
RAT	STIM[2]	-PLP	+PLP	-PLP	+PLP	-PLP	+PLP	-PLP	+PLP
				WEEK 6 ALC[3]					
7	73	84.6	309	1,151	4,202	489	1,788	8.78	32.1
8	74	76.1	292	1,261	4,614	435	1,669	5.55	21.3
9	69	87.6	281	1,408	4,496	487	1,562	8.15	26.2
10	73	82.4	305	1,197	4,453	468	1,733	8.20	30.4
11	74	72.4	282	814	3,187	402	1,566	12.44	48.4
12	73	77.8	285	866	3,164	454	1,668	11.21	41.1
MEAN	80.15	80.2	292	1,110	4,020	456	1,664	9.06	33.2
± SD	±5.70	±5.7	±12	±230	±670	±33	±90	±2.45	±10.0
				WEEK 6 PFC					
19	73	59.1	220	513	1,914	297	1,106	8.12	30.2
20	66	65.1	191	526	1,547	313	916	11.56	33.8
21	68	46.2	144	373	1,166	212	663	10.45	32.7
22	68	57.3	180	473	1,494	269	847	9.63	30.3
23	76	56.2	236	459	1,935	261	1,097	7.51	31.5
24	82	48.8	269	386	2,125	230	1,269	7.10	39.2
MEAN	72	55.4	207	460	1,700	264	983	9.06	33.0
± SD	±6	±6.93	±44	±60	±360	±38	±217	±1.77	±3.3
				WEEK 6 ALD					
55	77	32.9	144	436	1,900	188	825	3.85	16.9
56	77	20.6	92	233	1,040	116	515	2.80	12.4
57	73	42.7	158	487	1,800	228	843	9.91	36.6
58	80	40.7	207	492	2.500	193	982	4.60	23.4
59	81	32.3	171	449	2,380	195	1,031	6.24	33.0
60	82	43.5	189	531	2,310	224	976	5.12	22.3
MEAN	78	35.4	160	440	1,990	191	862	±5.42	24.1
± SD	±3	±8.75	±40	±110	±540	±40	±189	±2.49	±9.2
				WEEK 6 MFD					
91	81	31.5	165	210	1,110	147	771	8.97	47.0
92	86	31.2	218	200	1,370	151	1,060	9.26	64.8
93	81	43.5	225	330	1,700	220	1,133	9.02	46.5
94	73	63.5	232	230	840	297	1,085	24.24	88.6
95	77	31.7	137	210	890	144	625	6.92	30.0
96	83	30.7	182	180	1,060	145	862	7.38	43.7
MEAN	80	38.7	199	230	1,060	184	923	10.96	53.4
± SD	±5	±13.1	±39	±50	±320	±63	±202	±6.57	±20.

1. U=umoles formaldehyde/hour/mg tissue, tissue, g protein or ug DNA.
2. % STIM = (activity of STHM with PLP - activity of STHM without PLP)/(activity STHM without PLP) X 100.
3. ALC=ad libitum control, PFC=pair fed control, ALD=ad libitum deficient, MFD = meal fed deficient.

* n=5, # n=4, + not included in calculation of mean.

Appendix 8a. Individual data on liver serine transhydroxymethylase activity. Page 3 of 4.

		SERINE TRANSHYDROXYMETHYLASE ACTIVITY					
	%	U/MG TISSUE[1]		U/TISSUE		U/G PROTEIN	
RAT	STIM	-PLP	+PLP	-PLP	+PLP	-PLP	+PLP
			WEEK 6 1 DAY FASTED PFC[3]				
31	84	35.4	218	265	1,640	166	1,025
32	80	53.0	265	360	1,800	249	1,246
33	69	58.5	190	410	1,330	268	874
34	71	49.5	172	361	1,260	243	842
35	76	62.3	261	440	1,850	284	1,190
36	77	47.2	208	324	1,440	209	922
MEAN	76	51.0	219	360	1,550	236	1,016
± SD	±6	±9.5	±38	±60	±250	±43	±169
			WEEK 6 1 DAY FASTED ALD				
67	84	39.0	238	363	2,210	186	1,135
68	64	70.6	225	579	1,840	333	1,061
69	82	24.4	139	232	1,320	111	634
70	82	38.3	211	295	1,620	175	962
71	76	35.4	148	212	890	197	821
72	73	50.4	185	393	1,440	252	923
MEAN	78	43.0	191	340	1,550	209	923
± SD	±6	±15.8	±41	±140	±450	±76	±179
			WEEK 6 3 DAY FASTED PFC				
25	68	44.0	139	290	901	198	624
26	65	62.4	180	418	1,208	289	835
27	62	39.6	104	230	601	189	496
28	75	55.7	219	351	1,380	281	1,106
29	77	34.1	149	231	1,016	165	722
30	73	41.7	153	280	1,040	180	659
MEAN	70	46.2	157	300	1,020	217	740
± SD	±6	±10.6	±39	±70	±270	±54	±211
			WEEK 6 3 DAY FASTED ALD				
61	74	44.6	169	303	1,150	202	764
62	69	56.5	185	418	1,360	250	818
63	77	44.5	196	307	1,350	203	894
64	85	40.1	259	273	1,760	198	1,277
65	74	36.9	140	218	830	158	600
66	73	47.5	177	404	1,500	218	813
MEAN	75	45.0	188	325	1,320	205	861
± SD	±5	±6.7	±40	±77	±32	±30	±226

1. U=umoles formaldehyde/hour/mg tissue, tissue, g protein or ug DNA.
2. % STIM = (activity of STHM with PLP - activity of STHM without PLP)/(activity STHM without PLP) X 100.
3. ALC=ad libitum control, PFC=pair fed control, ALD=ad libitum deficient, MFD=meal fed deficient.

* n=5, # n=4, + not included in calculation of mean.

Appendix 8a. Individual data on liver serine transhydroxymethylase activity. Page 4 of 4

		SERINE TRANSHYDROXYMETHYLASE ACTIVITY					
	%	U/MG TISSUE[1]		U/TISSUE		U/G PROTEIN	
RAT	STIM[2]	-PLP	+PLP	-PLP	+PLP	-PLP	+PLP
		REPLETED PFC[3]					
37	74	42.0	163	530	2,050	236	916
38	74	53.9	209	730	2,840	288	1,120
39	78	53.2	239	440	2,010	279	1,252
40	67	53.1	162	630	1,930	266	812
41	68	60.4	188	770	2,430	310	964
42	74	52.6	204	560	2,940	292	1,129
MEAN	72	52.5	194	610	2,370	278	1,132
± SD	±4	±5.94	±30	±130	±440	±25	±162
		REPLETED ALD					
73	61	68.4	177	1,090	2,850	398	1,028
74	69	65.4	213	830	2,720	332	1,080
75	64	83.2	233	1,190	3,330	514	1,440
76	74	54.8	210	680	2,620	293	1,122
77	79	43.6	212	640	3,120	238	1,158
78	74	53.4	209	780	3,070	316	1,236
MEAN	70	61.5	210	860	2,950	348	1,177
± SD	±672	±13.9	±18	±220	±270	±96	±147
		REPLETED 3 DAY FASTED					
43	72	50.3	182	380	1,380	229	827
44	75	47.1	191	360	1,470	229	928
45	78	35.3	161	260	1,180	179	819
46	62	52.3	136	380	990	243	633
47	65	58.0	164	450	1,260	275	778
48	69	49.8	163.2	380	1,260	244	800
MEAN	70	48.8	166	370	1,260	233	798
± SD	±6	±7.6	±19.0	±60	±170	±31	±96
		REPLETED 3 DAY FASTED ALD					
79	73	55.4	207	470	1,760	295	1,102
80	57	34.9	80	250	580	173	404
81	69	37.9	123	270	880	194	630
82	68	34.7	110	290	910	203	641
83	76	38.0	159	320	1,330	201	839
84	66	40.9	122	340	1,000	187	557
MEAN	68	40.3	133	320	1,070	209	696
± SD	±7	±7.73	±44	±80	±41	±44	±244

1. U=umoles formaldehyde/hour/mg tissue, tissue, g protein or ug DNA.
2. % STIM=(activity of STHM with PLP - activity of STHM without PLP)/(activity STHM without PLP) X 100.
3. ALC=ad libitum control, PFC=pair fed control, ALD=ad libitum deficient, MFD=meal fed deficient.

* n=5, # n=4, + not included in calculation of mean.

Appendix 8b. Individual data on spleen serine transhydroxymethylase activity. Page 1 of 4

		SERINE TRANSHYDROXYMETHYLASE ACTIVITY					
		U/G TISSUE[1]		U/TISSUE		U/G PROTEIN	
ANIMAL	% STIM[2]	- PLP	+ PLP	- PLP	+ PLP	- PLP	+ PLP
			WEEK 2 ALC[3]				
1	87	3.77	28.94	3.02	23.15	36.0	275.9
2	82	5.52	30.57	4.21	23.29	60.2	333.4
3	89	2.77	24.56	2.22	19.65	32.0	284.0
4	84	4.07	25.01	3.60	22.13	41.1	252.5
5	80	6.80	34.84	5.58	28.57	69.7	357.3
6	79	5.97	27.82	4.66	21.70	63.3	295.1
MEAN	84	4.82	28.62	3.88	23.08	50.4	299.7
± SD	±4	±1.52	±3.81	±1.20	±2.99	±15.0	±38.8
			WEEK 2 PFC				
13	78	6.91	31.06	5.65	25.41	77.0	346.0
14	82	4.83	27.00	4.53	25.33	50.6	282.8
15	69	8.45	27.25	6.40	20.63	84.9	273.7
16	72	6.89	24.46	5.12	18.17	69.1	245.4
17	73	6.68	25.05	4.13	14.89	79.1	296.4
18	75	6.64	26.24	4.28	16.90	74.1	292.8
MEAN	75	6.73	26.84	5.02	20.22	72.5	289.5
± SD	±5	±1.15	±2.33	±0.88	±4.40	±11.9	±33.1
			WEEK 2 ALD				
49	95	1.40	25.96	1.07	19.86	14.8	275.4
50	90	2.91	28.87	1.79	17.78	30.0	297.7
51	91	2.76	29.81	1.75	18.93	32.7	353.7
52	91	2.54	27.14	2.21	23.67	24.8	265.4
53	88	2.71	22.56	1.73	14.42	28.5	237.2
54	91	2.37	26.49	2.11	23.63	24.1	269.7
MEAN	91	2.45	26.81	1.78	19.72	25.8	283.2
± SD	±2	±0.55	±2.54	±0.40	±3.56	±6.3	39.6
			Week 2 MFD				
85	89	3.22	30.19	2.01	18.84	33.9	317.2
86	90	3.11	30.63	1.02	10.02	30.6	301.5
87	86	3.18	22.54	2.04	14.49	31.8	225.5
88	83	5.20	30.90	2.60	15.45	53.5	317.9
89	80	6.34	32.34	2.43	12.39	69.6	354.8
90	89	2.95	27.84	1.81	17.04	29.5	270.8
MEAN	86	4.00	29.06	1.99	14.71	41.5	298.0
± SD	±4	±1.42	±3.51	±0.56	±3.18	±16.4	±44.7

1. U=umoles formaldehyde/hour/mg tissue, tissue, g protein or ug DNA.
2. % STIM=(activity of STHM with PLP - activity of STHM without PLP)/(activity STHM without PLP) X 100.
3. ALC=ad libitum control, PFC=pair fed control, ALD=ad libitum deficient, MFD=meal fed deficient.

* n=5, # n=4, + not included in calculation of mean.

Appendix 8b. Individual data on spleen serine transhydroxymethylase activity. Page 2 of 4.

		SERINE TRANSHYDROXYMETHYLASE ACTIVITY					
		U/MG TISSUE[1]		U/TISSUE		U\G PROTEIN	
ANIMAL	% STIM[2]	- PLP	+ PLP	- PLP	+ PLP	- PLP	+ PLP
				WEEK 6 ALC[3]			
7	71	8.79	30.55	7.91	27.50	89.9	312.4
8	81	6.42	34.06	6.08	32.25	56.6	300.5
9	80	5.18	26.49	5.43	27.76	47.5	242.9
10	79	6.10	28.54	4.43	20.75	63.6	297.6
11	75	6.97	28.02	5.29	21.27	68.2	274.2
12	76	6.68	27.40	5.44	22.33	61.8	253.3
MEAN	77	6.69	29.18	5.76	25.31	64.6	280.2
± SD	±4	±1.20	±2.75	±1.18	±4.58	±14.3	±27.9
				WEEK 6 PFC			
19	79	7.40	35.54	4.82	23.17	78.9	379.1
20	82	7.02	38.77	5.26	29.08	71.1	392.9
21	77	8.59	37.17	5.05	21.86	81.0	350.3
22	69	7.50	23.84	6.16	19.60	64.4	204.7
23	78	8.81	40.32	6.85	31.33	80.0	366.2
24	69	9.18	29.91	5.43	17.68	82.6	269.1
MEAN	76	8.08	34.29	5.60	23.79	76.3	327.0
± SD	±5	0.89	±6.24	±0.77	±5.37	±7.1	±74.1
				WEEK 6 ALD			
55	92	2.06	24.92	0.94	11.31	19.7	238.1
56	96	1.12	27.83	0.62	15.42	10.5	260.8
57	86	3.13	22.89	0.22	15.75	30.0	219.1
58	99	0.39	29.53	0.23	17.45	3.3	249.9
59	98	0.53	22.94	0.40	17.4	4.4	189.5
60	93	1.35	19.39	0.75	10.82	12.5	179.9
MEAN	94	1.43	24.58	0.53	14.71	13.3	222.9
± SD	±4	±1.03	±3.68	±0.29	±2.95	±10.0	±32.8
				WEEK 6 MFD			
91	90	1.98	20.61	0.90	9.32	19.3	201.2
92	91	1.95	20.71	0.77	8.16	20.2	214.5
93	84	3.02	19.15	1.34	8.52	30.7	194.5
94	100	0	10.70	0	8.81	0	124.0
95	88	2.86	23.51	1.08	8.91	27.4	224.8
96	74	2.79	16.70	1.30	7.77	28.6	171.2
MEAN	88	2.10	18.56	0.90	8.58	21.0	188.4
± SD	±9	±1.13	±4.45	±0.49	±0.56	±11.3	±36.5

1. U=umoles formaldehyde/hour/mg tissue, tissue, g protein or ug DNA.
2. % STIM = (activity of STHM with PLP - activity of STHM without PLP)/(activity STHM without PLP) X 100.
3. ALC=ad libitum control, PFC=pair fed control, ALD=ad libitum deficient, MFD=meal fed deficient.

* n=5, # n=4, + not included in calculation of mean.

Appendix 8b. Individual data on spleen serine transhydroxymethylase activity. Page 3 of 4.

		SERINE TRANSHYDROXYMETHYLASE ACTIVITY					
		U/MG TISSUE[1]		U/TISSUE		U/G PROTEIN	
ANIMAL	% STIM[2]	- PLP	+ PLP	- PLP	+ PLP	- PLP	+ PLP
			WEEK 6 1 DAY FASTED PFC[3]				
31	69	8.58	27.81	6.06	19.63	77.1	249.9
32	68	6.68	21.16	3.84	12.17	61.4	195.6
33	71	7.11	24.55	4.15	14.31	61.9	213.8
34	78	6.10	27.45	4.01	18.06	64.1	320.5
35	81	5.82	30.50	3.89	20.40	58.1	304.7
36	81	5.67	29.17	3.67	18.90	54.1	278.3
MEAN	75	6.66	26.77	4.27	17.24	62.8	260.3
± SD	±6	±1.09	±3.39	±0.89	±3.27	±7.8	±50.0
			WEEK 6 1 DAY FASTED ALD				
67	98	0.46	25.58	0.43	24.15	3.6+	202.0
68	85	2.84	19.54	1.66	11.45	26.2	180.5
69	90	2.93	30.31	2.29	23.73	25.7	265.8
70	89	2.64	24.64	1.26	11.78	26.4	243.5
71	88	2.50	20.81	1.04	8.64	26.4	219.7
72	86	2.38	17.35	3.00	10.67	22.1	161.0
MEAN	89	2.29+	23.04	1.61	15.07	25.4*	214.1
± SD	±5	±0.92	±4.72	±0.92	6.96	±1.8	±43.4
			WEEK 6 3 DAY FASTED PFC				
25	79	5.88	27.57	3.10	14.56	58.0	272.0
26	70	6.52	21.87	4.25	14.26	67.6	226.9
27	82	4.46	24.54	2.85	15.66	43.0	236.8
28	59	8.01	19.72	4.81	11.83	78.7	193.8
29	71	9.30	31.60	5.11	17.35	100.5	341.1
30	77	6.42	28.51	3.63	16.14	57.8	256.5
MEAN	73	6.77	25.64	3.96	14.97	67.6	254.5
± SD	±8	±1.69	±4.43	±0.92	±1.90	±20.0	±50.1
			WEEK 6 3 DAY FASTED ALD				
61	86	2.94	21.38	1.29	9.41	24.8	180.0
62	86	3.87	27.20	2.04	14.36	33.9	238.6
63	86	4.07	28.34	2.51	17.46	37.6	261.7
64	92	1.80+	22.29	0.75	9.27	17.1	212.1
65	85	4.31	29.15	2.40	16.21	43.1	291.8
66	90	2.33	24.51	1.52	16.01	19.9	209.6
MEAN	88	3.22*	25.48	1.75	13.79	29.4	232.3
± SD	±3	±1.02	±3.24	±0.65	±3.58	±10.4	±40.3

1. U=umoles formaldehyde/hour/mg tissue, tissue, g protein or ug DNA.
2. % STIM = (activity of STHM with PLP - activity of STHM without PLP)/(activity STHM without PLP) X 100.
3. ALC=ad libitum control, PFC=pair fed control, ALD=ad libitum deficient, MFD = meal fed deficient.

* n=5, # n=4, + not included in calculation of mean.

Appendix 8b. Individual data on spleen serine transhydroxymethylase activity. Page 4 of 4

		SERINE TRANSHYDROXYMETHYLASE ACTIVITY					
		U/MG TISSUE[1]		U/TISSUE		U/G PROTEIN	
ANIMAL	% STIM[2]	- PLP	+ PLP	- PLP	+ PLP	- PLP	+ PLP
			REPLETED PFC[3]				
37	77	6.76	29.0	5.10	21.8	72.9	312.7
38	81	4.52	23.7	4.48	23.5	54.1	283.5
39	73	7.52	27.4	7.84	28.6	79.2	289.1
40	68	7.86	24.5	6.41	20.0	83.0	258.9
41	76	7.39	30.4	6.23	25.6	72.0	296.5
42	71	5.87	20.3	5.38	18.6	61.4	211.8
MEAN	74	6.65	25.9	5.91	23.0	70.4	275.4
± SD	±5	±1.26	±3.8	±1.19	±3.7	±10.9	±35.8
			REPLETED ALD				
73	89	2.68	24.7	2.38	22.0	27.0	249.0
74	72	6.26	22.5	5.25	18.9	69.4	249.6
75	81	3.92	20.1	3.39	17.4	50.9	261.4
76	77	5.35	23.8	5.83	25.9	57.3	254.6
77	82	3.17	27.3	3.36	29.0	28.2	243.0
78	84	4.39	27.1	3.79	23.4	48.3	297.9
MEAN	81	4.30	24.2	4.00	22.8	46.8	259.2
± SD	±6	±1.34	±2.74	±1.29	±4.3	±16.6	19.9
			REPLETED 3 DAY FASTED PFC				
43	50+	4.68	9.27	3.28	6.49	66.4	131.5
44	100+	0+	4.50+	0+	2.59+	0+	65.6+
45	86	2.22	16.07	1.62	11.75	27.0	195.7
46	73	3.97	14.81	2.73	10.19	44.1	164.5
47	78	4.55	20.93	3.43	15.78	51.7	237.9
48	86	1.83	13.04	1.14	8.12	24.4	173.8
MEAN	79#	3.45#	14.8#	2.03#	#9.15	42.7#	180.7#
± SD	±2	±1.34	±4.3	±1.35	±4.54	±17.5	±39.5
			REPLETED 3 DAY FASTED ALD				
79	87	2.50	19.37	1.55	12.01	24.7	191.7
80	82	4.88	26.60	3.03	16.52	52.1	283.7
81	76	2.48	10.24	1.24	5.12	28.9	119.3
82	79	4.14	19.95	3.17	15.28	49.7	239.4
83	81	3.62	19.31	2.91	15,53	43.4	231.7
84	67	7.95	24.10	6.07	18.41	88.9	269.5
MEAN	79	4.26	19.93	3.00	13.81	48.0	222.6
± SD	±7	±2.03	±5.59	±1.71	±4.74	22.9	±59.9

1. U=umoles formaldehyde/hour/mg tissue, tissue, g protein or ug DNA.
2. % STIM = (activity of STHM with PLP - activity of STHM without PLP)/(activity STHM without PLP) X 100.
3. ALC=ad libitum control, PFC=pair fed control, ALD=ad libitum deficient, MFD = meal fed deficient.

* n=5, # n=4, + not included in calculation of mean.

Appendix 8c. Individual data on thymus serine transhydroxymethylase activity. Page 1 of 2

		SERINE TRANSHYDROXYMETHYLASE ACTIVITY					
	%	U/MG TISSUE[1]		U/TISSSUE		U/UG PROTEIN	
RAT	STIM[2]	-PLP	+PLP	-PLP	+PLP	-PLP	+PLP
				WEEK 2 ALC[3]			
1	72	8.28	29.34	5.99	21.24	167	592
2	72	9.14	32.93	6.66	24.01	187	675
3	87	5.56	42.45	4.45	33.96	115	880
4	83	7.35	42.61	5.98	34.68	162	936
5	80	8.31	42.12	4.20	21.27	182	923
6	72	10.76	38.64	9.54	34.27	241	866
MEAN	78	8.23	38.64	6.14	28.24	176	812
± SD	±7	±1.74	±5.64	±1.92	±6.72	±41	±143
				WEEK 2 PFC			
13	83	6.94	40.14	5.16	29.82	144	832
14	73	10.52	39.62	8.68	32.69	224	843
15	75	10.26	41.80	5.94	24.20	218	889
16	73	10.09	37.87	5.95	22.34	207	778
17	80	9.26	45.52	4.32	21.21	173	851
18	84	6.46	40.15	3.73	23.17	147	911
MEAN	78	8.92	40.85	5.63	25.57	186	851
± SD	±5	±1.78	±2.61	±1.74	±4.60	±36	±46
				WEEK 2 ALD			
49	96	1.29	34.42	0.35	9.40	26	681
50	95	1.34	25.22	0.72	13.47	28	533
51	90	3.08	29.99	1.27	12.36	65	628
52	94	1.75	30.22	0.61	10.61	36	628
53	95	1.25	24.62	0.48	9.40	24	483
54	98	0.29	19.00	0.17	11.40	7	447
MEAN	95	1.50	27.25	0.60	11.11	31	567
± SD	±3	±0.91	±5.42	0.38	±1.63	±19	±93
				WEEK 2 MFD			
85	94	1.77	31.31	0.58	10.21	37	652
86	96	1.37	35.71	0.18	4.82	28	737
87	100	0	31.14	0	7.97	0	0
88	93	2.40	32.62	0.61	8.25	46	629
89	95	1.50	31.00	0.61	12.68	30	627
90	89	3.69	32.37	1.70	14.92	75	662
MEAN	94	1.79	32.36	0.61	9.81	43	661
± SD	±6	±1.22	±1.78	±0.59	±3.61	±19	±45

1. U=umoles formaldehyde/hour/ mg tissue, tissue, g protein or ug DNA.
2. % STIM = (activity of STHM with PLP - activity of STHM without PLP)/(activity STHM without PLP) X 100.
3. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.
* n=5, # n=4, + not included in calculation of mean.

Appendix 8c. Individual data on thymus serine transhydroxymethylase activity. Page 2 of 2.

RAT	% STIM[2]	SERINE TRANSHYDROXYMETHYLASE ACTIVITY U/MG TISSUE[1] -PLP	+PLP	U/TISSUE -PLP	+PLP	U/G PROTEIN -PLP	+PLP
				REPLETED PFC[3]			
37	88	6.36	52.83	3.72	30.91	118	984
38	83	7.13	42.75	4.60	27.57	143	859
39	76	9.38	39.37	2.45	10.28	172	723
40	83	8.49	51.12	3.74	22.49	163	982
41	77	13.03	56.72	9.38	40.84	257	1,118
42	73	14.83	55.51	7.03	26.31	282	1,054
MEAN	80	9.87	49.72	5.15	26.40	189	953
± SD	±6	±3.36	±7.07	±2.57	±10.05	±65	±142
				REPLETED ALD			
73	75	13.61	54.27	7.73	30.83	263	1,051
74	79	13.12	61.20	7.35	37.09	243	1,135
75	77	14.58	62.32	10.44	44.62	275	1,176
76	70	16.44	55.09	8.10	27.16	309	1,086
77	72	16.38	58.77	11.20	40.20	315	1,131
78	70	14.87	49.66	8.42	28.11	285	951
MEAN	74	14.87	56.89	8.87	34.67	282	1,088
± SD	±4	±1.42	±4.77	±1.57	±7.07	±27	±79
				REPLETED 3 DAY FASTED PFC			
43	77	11.22	48.63	4.93	21.35	199	862
44	78	10.38	47.17	4.44	20.19	179	812
45	80	9.00	45.54	3.60	18.22	158	800
46	78	9.26	42.32	4.39	20.06	193	884
47	79	10.51	49.35	4.38	20.58	183	860
48	78	7.99	36.79	3.91	18.03	165	762
MEAN	78	9.73	44.96	4.28	19.74	180	830
± SD	±1	±1.19	±4.72	±0.46	±1.33	±16	±46
				REPLETED 3 DAY FASTED ALD			
79	83	8.18	47.51	4.29	24.90	163	945
80	81	8.90	45.68	4.70	24.12	159	814
81	78	12.11	55.43	7.87	36.03	218	997
82	73	12.49	46.75	5.67	21.22	251	938
83	74	11.43	43.66	8.79	33.53	228	872
84	79	9.23	43.90	6.67	31.74	180	856
MEAN	78	10.39	47.16	6.33	28.59	200	904
± SD	±4	±1.84	±4.33	±1.67	±5.96	±38	±68

1. U=umoles formaldehyde/hour/ mg tissue, tissue, g protein or ug DNA.
2. % STIM = (activity of STHM with PLP - activity of STHM without PLP)/(activity STHM without PLP) X 100.
3. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.
* n=5, # n=4, + not included in calculation of mean.

Appendix 8d. Individual data on bone marrow serine transhydroxymethylase activity. Page 1 of 4.

ANIMAL	% STIM[2]	SERINE TRANSHYDROXYMETHYLASE ACTIVITY U/MG TISSUE[1] - PLP	+ PLP	PER UG DNA - PLP	+ PLP
			WEEK 2 ALC[3]		
1	61	4.07	10.26	0.44	1.11
2	59	5.61	13.33	0.45	1.07
3	63	3.54	9.65	0.44	1.20
4	62	5.61	14.77	0.52	1.37
5	54	5.63	12.19	0.55	1.19
6	54	5.88	12.70	0.50	1.08
MEAN	59	5.06	12.15	0.48	1.17
± SD	±4	±0.99	±1.92	±0.06	±0.11
			WEEK 2 PFC		
13	61	5.03	13.04	0.44	1.14
14	68	2.79	8.75	0.44	1.38
15	73	3.40	12.40	0.34	1.24
16	67	2.33	7.43	0.35	0.90
17	61	3.58	9.21	0.40	1.03
18	54	7.56	16.71	0.48	1.06
MEAN	64	4.12	11.26	0.40	1.12
± SD	±7	±1.92	±3.44	±0.07	±0.17
			WEEK 2 ALD		
49	96	0.45	10.10	0.04	0.89
50	100	0	8.61	0	0.65
51	84	2.46	15.59	0.21	1.33
52	98	0.19	10.28	0.01	0.53
53	93	0.81	11.41	0.07	0.98
54	96	0.30	6.99	0.02	0.46
MEAN	94	0.70	10.50	0.06	0.81
± SD	±6	±0.90	±2.92	±0.08	±0.33
			WEEK 2 MFD		
85	100	ND	9.64	ND	0.88
86	---	0.93	2.26	0.09	0.22
87	---	---	10.41	---	0.86
88	85	1.29	8.58	0.11	0.73
89	86	0.91	6.32	0.12	0.83
90	98	0.24	9.54	0.02	0.81
MEAN	92#	0.67	7.79	0.06	0.72
± SD	±8	±0.33	±3.06	±0.06	±0.25

1. U=umoles formaldehyde/hour/mg tissue, tissue, g protein or ug DNA.
2. % STIM = (activity of STHM with PLP - activity of STHM without PLP)/(activity STHM without PLP) X 100.
3. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

* n=5, # n=4, + not included in calculation of mean.

Appendix 8d. Individual data on bone marrow serine transhydroxymethylase activity. Page 2 of 4.

		SERINE TRANSHYDROXYMETHYLASE ACTIVITY			
		U/MG TISSUE[1]		U/UG DNA	
ANIMAL	% STIM[2]	- PLP	+ PLP	- PLP	+ PLP
			WEEK 6 ALC[3]		
7	80	2.24	11.03	0.12	0.59
8	88	1.24	10.76	0.06	0.52
9	91	1.11	11.77	0.05	0.53
10	78	4.79	21.32	0.20	0.89
11	80	3.15	15.73	0.13	0.65
12	78	2.01	8.95	0.09	0.40
MEAN	82	2.42	13.26	0.11	0.58
± SD	±6	±1.38	±4.54	±0.05	±0.16
			WEEK 6 PFC		
19	70	1.91	6.41	0.14	0.47
20	85	1.24	8.29	0.06	0.40
21	---	---	---	---	---
22	70	2.05	6.91	0.16	0.54
23	61	7.15	18.40	0.35	0.90
24	64	6.46	17.72	0.35	0.96
MEAN	70*	3.76*	11.55*	0.21*	0.65*
± SD	±9	±2.81	±5.99	±0.13	±0.26
			WEEK 6 ALD		
55	100	ND	9.12	ND	0.52
56	100	ND	9.81	ND	0.58
57	100	ND	10.80	ND	0.84
58	100	ND	---	ND	---
59	100	ND	9.65	ND	0.44
60	100	ND	5.45	ND	0.30
MEAN	100	ND	8.97	ND	0.54*
± SD	±0	±	±2.06	±	±0.20
			WEEK 6 MFD		
91	100	ND	1.42	ND	0.11
92	83	0.53	3.18	0.05	0.30
93	89	0.66	5.77	0.04	0.35
94	94	1.06	16.93	0.07	1.12
95	91	0.38	4.20	0.03	0.33
96	95	0.78	15.11	0.03	0.58
MEAN	92	0.57	7.77	0.04	0.46
± SD	±6	±0.36	±6.57	±0.02	±0.35

1. U=umoles formaldehyde/hour/mg tissue, tissue, g protein or ug DNA.
2. % STIM = (activity of STHM with PLP - activity of STHM without PLP)/(activity STHM without PLP) X 100.
3. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

* n=5, # n=4, + not included in calculation of mean.

Appendix 8d. Bone marrow serine transhydroxymethylase activity. Page 3 of 4.

ANIMAL	% STIM[2]	SERINE TRANSHYDROXYMETHYLASE ACTIVITY U/MG TISSUE[1] - PLP	+ PLP	U/UG DNA - PLP	+ PLP
			WEEK 6 1 DAY FASTED PFC[3]		
31	61	13.43	34.20	0.63	1.60
32	57	20.24	24.07	0.70	1.64
33	66	9.02	26.41	0.64	1.87
34	62	10.00	26.43	0.80	2.11
35	52	12.04	24.83	0.88	1.81
36	43	11.68	20.58	0.88	1.54
MEAN	55	11.07	25.16	0.76	1.76
± SD	±8	±1.61	±4.98	±0.11	±0.21
			WEEK 6 1 DAY FASTED ALD		
67	---	---	---	---	---
68	72	6.89	24.86	0.40	1.46
69	71	8.02	27.64	0.43	1.49
70	73	5.10	18.89	0.35	1.28
71	77	5.48	23.43	0.37	1.58
72	81	3.13	16.27	0.23	1.20
MEAN	75*	5.72*	22.20*	0.36*	1.39*
± SD	±4	±1.86	±4.59	±0.08	±0.17
			WEEK 6 3 DAY FASTED PFC		
25	---	---	---	---	---
26	62	3.59	9.39	0.30	0.79
27	67	3.85	11.95	0.32	0.97
28	51	3.82	11.95	0.36	0.74
29	74	2.96	11.62	0.25	0.98
30	---	---	---	---	---
MEAN	64#	3.55#	10.20#	0.31#	0.87#
± SD	±10	±0.41	±1.94	±0.05	±0.12
			WEEK 6 3 DAY FASTED ALD		
61	87	0.24	1.84	0.03	0.23
62	83	1.83	10.84	0.15	0.89
63	83	1.46	8.47	0.10	0.58
64	81	1.31	6.84	0.08	0.94
65	80	1.26	6.42	0.13	0.66
66	89	0.97	8.86	0.09	0.82
MEAN	84	1.18	7.21	0.11	0.69
± SD	±3	±0.54	±3.07	±.05	±0.26

1. U=umoles formaldehyde/hour/mg tissue, tissue, g protein or ug DNA.
2. % STIM = (activity of STHM with PLP - activity of STHM without PLP)/(activity STHM without PLP) X 100.
3. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

* n=5, # n=4, + not included in calculation of mean.

Appendix 8d. Individual data on bone marrow serine transhydroxymethylase activity. Page 4 of 4

ANIMAL	% STIM[2]	SERINE TRANSHYDROXYMETHYLASE ACTIVITY U/MG TISSUE[1] - PLP	U/MG TISSUE[1] + PLP	U/UG DNA - PLP	U/UG DNA + PLP
				REPLETED PFC[3]	
37	70	2.77	9.04	0.19	0.62
38	66	5.33	15.60	0.41	1.20
39	52	4.94	10.31	0.34	0.71
40	64	4.18	11.61	0.27	0.75
41	71	3.74	12.95	0.24	0.83
42	60	4.56	11.40	0.28	0.70
MEAN	64	4.25	11.82	0.29	0.80
± SD	±7	±0.92	±2.27	±0.08	±0.21
			REPLETED ALD		
73	75	3.52	14.10	0.22	0.88
74	76	2.39	10.39	0.17	0.74
75	67	4.62	13.86	0.75	2.25
76	70	3.43	11.59	0.21	0.71
77	---	---	---	---	0.20+
78	76	2.83	12.00	0.17	0.72
MEAN	73*	3.36*	12.39*	0.30	1.06*
± SD	±4	±0.84	±1.57	±0.25	±0.67
			REPLETED 3 DAY FASTED PFC		
43	56	4.67	12.14	0.38	0.85
44	51	6.57	13.42	0.46	0.94
45	51	6.26	12.93	0.45	0.93
46	50	5.45	10.91	0.41	0.82
47	54	6.04	12.10	0.41	0.89
48	54	5.15	9.11	0.26	0.46
MEAN	51	5.69	11.94	0.40	0.82
± SD	±4	±0.72	±1.65	±0.07	±0.18
			REPLETED 3 DAY FASTED ALD		
79	65	4.28	12.00	0.35	0.98
80	56	3.72	8.52	0.24	0.55
81	61	5.74	14.54	0.37	0.95
82	69	3.04	10.00	0.24	0.79
83	51	6.34	12.82	0.43	0.87
84	65	3.46	10.00	0.28	0.81
MEAN	61	4.43	11.31	0.32	0.82
± SD	±7	±1.32	±2.21	±0.08	±0.15

1. U=umoles formaldehyde/hour/mg tissue, tissue, g protein or ug DNA.
2. % STIM = (activity of STHM with PLP - activity of STHM without PLP)/(activity STHM without PLP) X 100.
3. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

* n=5, # n=4, + not included in calculation of mean.

Appendix 8e. Individual data on intestine serine transhydroxymethylase activity. Page 1 of 4

ANIMAL	% STIM[2]	SERINE TRANSHYDROXYMETHYLASE ACTIVITY U/MG TISSUE[1] - PLP	U/MG TISSUE[1] + PLP	U/G PROTEIN - PLP	U/G PROTEIN + PLP
		WEEK 2 ALC[3]			
1	---	4.11	---	0.223	---
2	79	0.90	4.31	0.034	0.163
3	85	1.01	6.53	0.029	0.189
4	75	1.63	6.53	0.052	0.208
5	38	1.96	3.15	0.067	0.241
6	64	0.54	1.49	0.032	0.088
MEAN	68*	1.21	4.40*	0.043*	0.178*
± SD	±20	±0.58	±2.19	±0.016	±0.058
		WEEK 2 PFC			
13	96	0.25+	6.96	0.009	0.246
14	9+	2.05	2.25	0.157	0.173
15	74	1.58	6.00	0.078	0.298
16	62	1.66	4.38	0.095	0.251
17	33	3.06	4.55	0.203	0.301
18	63	2.69	7.35	0.102	0.280
MEAN	66*	1.88*	5.25	0.107	0.258
± SD	±23	±0.99	±1.90	±0.067	±0.048
		WEEK 2 ALD			
49	96	0.12	2.85	0.005	0.117
50	45	0.88	1.61	0.045	0.073
51	93	0.08	1.18	0.007	0.094
52	---	---	---	---	---
53	100	ND	1.43	ND	0.074
54	100	ND	1.88	ND	0.094
MEAN	87*	0.22*	1.90*	0.011*	0.099*
± SD	±24	±0.37	±0.64	±0.019	±0.025
		WEEK 2 MFD			
85	100	0	1.17	0	0.042
86	91	0.18	2.04	0.013	0.150
87	88	0.42	3.49	0.015	0.128
88	95	0.50	9.18	0.015	0.277
89	79	0.34	1.64	0.025	0.121
90	27+	0.43	0.59	0.035	0.048
MEAN	91	0.31	3.02	0.017	0.128
± SD	±8*	±0.19	±3.17	±0.012	±0.085

1. U=umoles formaldehyde/hour/mg tissue, tissue, g protein or ug DNA.
2. % STIM = (activity of STHM with PLP - activity of STHM without PLP)/(activity STHM without PLP) X 100.
3. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

* n=5, # n=4, + not included in calculation of mean.

Appendix 8e. Individual data on intestine serine transhydroxymethylase activity. Page 2 of 4.

ANIMAL	% STIM[2]	SERINE TRANSHYDROXYMETHYLASE ACTIVITY U/MG TISSUE[1] - PLP	+ PLP	U/G PROTEIN - PLP	+ PLP
		WEEK 6 ALC[3]			
7	78	3.35	15.17	72.0	326.2
8	74	2.88	10.90	70.2	265.7
9	42	3.97	6.90	119.7	208.0
10	68	4.62	14.28	92.9	287.2
11	49	3.84	7.48	174.3	229.5
12	57	2.35	5.47	87.3	203.3
MEAN	61	3.50	10.03	102.7	271.7
± SD	±14	±0.81	±4.06	±39.4	±57.6
		WEEK 6 PFC			
19	29	2.71	3.80	80.2	112.4
20	14	3.31	3.86	89.8	104.8
21	28	3.30	4.57	92.9	128.6
22	31	2.08	3.01	63.3	91.4
23	47	6.10	11.58	154.8	292.0
24	69	4.52	14.35	124.0	393.7
MEAN	36	3.67	6.86	100.6	187.1
± SD	±19	±1.44	±4 83	±32.8	±125.4
		WEEK 6 ALD			
55	45	1.65	3.01	67.4	122.9
56	50	2.54	5.12	63.4	127.8
57	16	1.78	2.13	61.0	73.0
58	22	1.77	2.26	74.3	94.9
59	28	2.28	3.15	67.7	93.5
60	21	1.75	2.22	67.8	86.0
MEAN	30	1.96	2.98	66.2	99.7
± SD	±14	±0.36	±1.13	±4.6	±21.4
		WEEK 6 MFD			
91	59	2.35	5.67	55.1	132.9
92	78	2.43	11.10	57.0	260.2
93	0	1.86	0.99	194.6[+]	103.6
94	0	1.83	1.52	168.5[+]	140.0
95	33	2.41	3.60	78.5	117.3
96	0	2.36	2.29	84.8	82.3
MEAN	28	2.21	4.20	68.8[#]	148.2
± SD	±34	±0.28	±3.78	±15.0	±77.6

1. U=umoles formaldehyde/hour/mg tissue, tissue, g protein or ug DNA.
2. % STIM = (activity of STHM with PLP - activity of STHM without PLP)/(activity STHM without PLP) X 100.
3. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

* n=5, # n=4, + not included in calculation of mean.

Appendix 8e. Individual data on intestine serine transhydroxymethylase activity. Page 3 of 4.

ANIMAL	% STIM[2]	SERINE TRANSHYDROXYMETHYLASE ACTIVITY U/MG TISSUE[1] - PLP	+ PLP	U/G PROTEIN - PLP	+ PLP
		WEEK 6 1 DAY FASTED PFC[3]			
31	44	3.09	5.47	114.4	202.5
32	60	0.76	1.90	35.5	88.7
33	49	0.88	1.72	22.9	44.8
34	36	0.96	1.50	41.9	65.4
35	19	1.54	1.89	57.5	70.6
36	73	1.50	5.59	41.6	155.0
MEAN	47	1.46	3.01	52.3	104.5
± SD	±19	±0.87	±1.96	±32.4	±61.1
		WEEK 6 1 DAY FASTED ALD			
67	33	1.41	2.09	71.6	106.1
68	66	0.43	1.28	12.4	37.0
69	36	1.38	2.17	47.9	75.4
70	58	0.89	2.14	90.2	90.2
71	57	1.62	3.81	60.3	141.9
72	65	1.20	3.46	38.9	112.3
MEAN	53	1.15	2.49	53.6	93.8
± SD	±14	±0.48	±0.95	±27.0	±35.7
		WEEK 6 3 DAY FASTED PFC			
25	0	1.25	0.71	64.2	36.4
26	84	0.49	3.05	18.7	116.1
27	0	0.50	0.37	29.0	21.5
28	69	0.30	0.97	14.0	45.4
29	100	0	0.80	0	26.6
30	24	0.42	0.55	23.9	31.3
MEAN	46	0.49	1.08	25.0	46.2
± SD	±44	±0.41	±0.99	±21.6	±35.2
		WEEK 6 3 DAY FASTED ALD			
61	81	0.19	0.98	10.7	55.1
62	100	0	1.38	0	63.2
63	0	0.32	0.22	24.2	16.6
64	70	0.55	1.82	26.8	90.0
65	76	0.43	1.78	18.8	78.0
66	94	0.24	4.01	8.6	143.4
MEAN	70	0.29	1.68	14.8	74.4
± SD	±36	±0.19	±1.43	±10.2	±42.1

1. U=umoles formaldehyde/hour/mg tissue, tissue, g protein or ug DNA.
2. % STIM = (activity of STHM with PLP - activity of STHM without PLP)/(activity STHM without PLP) X 100.
3. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

* n=5, # n=4, + not included in calculation of mean.

Appendix 8e. Individual data on intestine serine transhydroxymethylase activity. Page 4 of 4

ANIMAL	% STIM[2]	SERINE TRANSHYDROXYMETHYLASE ACTIVITY U/MG TISSUE[1] - PLP	U/MG TISSUE[1] + PLP	U/G PROTEIN - PLP	U/G PROTEIN + PLP
				REPLETED PFC[3]	
37	58	2.70	6.39	70.7	165.7
38	55	1.94	4.28	58.8	129.7
39	0	3.21	3.64	131.6	149.2
40	48	1.18	2.29	47.0	91.2
41	70	2.29	7.56	57.9	191.3
42	65	2.33	6.59	77.8	220.0
MEAN	49	2.28	5.12	73.8	157.8
± SD	±25	±0.67	±2.03	±30.2	±45.5
				REPLETED ALD	
73	72	3.54	12.71	88.5	317.9
74	17	2.22	2.69	110.6	134.0
75	54	3.91	8.52	138.3	301.4
76	55	2.40	5.37	68.4	153.1
77	28	1.72	2.39	74.5	103.5
78	66	2.78	8.16	99.7	292.8
MEAN	49	2.76	6.64	96.7	217.1
± SD	±22	±0.83	±3.95	±25.7	±96.9
		REPLETED 3 DAY FASTED PFC			
43	38	1.76	2.86	66.8	108.5
44	56	2.63	6.03	90.8	208.3
45	0	1.87	1.79	82.8	79.3
46	0	1.72	1.83	82.7	88.0
47	23	1.61	2.08	77.0	99.5
48	0	1.18	1.17	48.3	47.9
MEAN	20	1.80	2.63	74.7	105.2
± SD	±24	±0.47	±1.75	±15.2	±54.6
		REPLETED 3 DAY FASTED ALD			
79	15	1.71	2.02	92.8	109.6
80	27	1.31	1.80	74.4	102.3
81	49	0.49	0.96	21.7	42.5
82	54	2.36	5.10	104.1	225.1
83	44	1.31	2.33	67.9	102.8
84	46	2.50	4.61	131.3	242.1
MEAN	39	1.61	2.80	82.0	137.4
± SD	±15	±0.75	±1.66	±37.2	±78.6

1. U=umoles formaldehyde/hour/mg tissue, tissue, g protein or ug DNA.
2. % STIM = (activity of STHM with PLP - activity of STHM without PLP) /(activity STHM without PLP) X 100.
3. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

* n=5, # n=4, + not included in calculation of mean.

Appendix 9a. Individual data on % of ^{3}H from injected ^{3}H-deoxcytidine retained by the liver. Page 1 of 2.

RAT	% DOSE[1] /G TISSUE	% DOSE /MG DNA	% DOSE /TISSUE	% DOSE /G PROTEIN	% HOMO CTS IN SUPER[2]
			WEEK 2 ALC[3]		
1	0.52	0.70	7.53	3.24	79
2	0.60	0.73	9.33	3.39	77
3	0.53	0.78	8.39	3.14	81
4	0.43	0.79	5.87	2.74	77
5	0.55	0.85	6.06	3.12	71
6	0.51	0.77	6.23	3.17	84
MEAN	0.52	0.77	7.24	3.13	78
± SD	±0.06	±0.05	±1.41	±0.22	±4
			WEEK 2 PFC		
13	0.55	0.91	5.42	2.96	78
14	0.47	0.97	4.69	2.56	84
15	0.45	0.73	3.86	2.25	77
16	0.57	0.93	5.89	2.78	80
17	0.51	0.78	4.22	2.55	80
18	0.61	0.98	4.70	3.24	80
MEAN	0.52	0.88	4.80	2.72	80
± SD	±0.06	±0.10	±0.75	±0.35	±2
			WEEK 2 ALD		
49	0.54	0.93	7.88	3.36	79
50	0.60	1.20	7.65	3.75	72
51	0.62	1.31	5.40	3.07	87
52	0.58	1.06	8.05	3.78	81
53	0.54	0.94	6.70	3.24	91
54	0.41	0.78	5.85	2.56	84
MEAN	0.55	1.02	6.92	3.29	82
± SD	±0.08	±0.17	±1.12	±0.45	±7
			WEEK 2 MFD		
85	0.75	1.63	4.45	3.33	79
86	0.57	1.44	6.67	6.32	65
87	0.73	1.40	5.24	3.42	89
88	0.69	1.43	4.41	3.46	89
89	0.73	1.28	3.55	3.17	78
90	0.60	1.10	5.04	2.73	82
MEAN	0.68	1.38	4.89	3.73	80
± SD	±0.08	±0.18	±1.05	±1.30	±9

1. % of dose (^{3}H-deoxycytidine) which was recovered in the homogenates.
2. % of dose (^{3}H-deoxycytidine) in tissue homogenates which remained in the supernatant after centrifuging.
3. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 9a. Individual data on % of ^{3}H from injected ^{3}H-deoxcytidine retained by the liver. Page 2 of 2.

CTS RAT	%DOSE[1] /G TISSUE	%DOSE /MG DNA	%DOSE /TISSUE	%DOSE /G PROTEIN	%HOMO CTS IN SUPER[2]	%SUPER CTS /dC FRAC[3]
			WEEK 6 ALC[4]			
7	0.38	0.54	5.2	2.2	75	95
8	0.36	0.41	5.6	2.0	83	97
9	0.38	0.57	6.1	2.1	80	97
10	0.43	0.63	6.3	2.4	79	105
11	0.44	0.86	5.0	2.4	89	91
12	0.38	0.61	4.2	2.2	79	103
MEAN	0.40	0.60	5.4	2.2	81	98
± SD	±0.03	±0.15	±0.8	±0.2	±5	±5
			WEEK 6 PFC			
19	0.51	0.61	4.4	2.6	70	98
20	0.51	0.74	3.0	2.5	68	100
21	0.55	1.00	4.4	2.7	73	102
22	0.57	0.80	4.8	2.7	75	109
23	0.49	0.53	4.0	2.3	69	103
24	0.57	0.65	4.5	2.7	70	102
MEAN	0.53	0.72	4.2	2.6	71	102
± SD	±0.03	±0.17	±0.6	±0.2	±3	±4
			WEEK 6 ALD			
55	0.62	0.96	8.2	3.6	77	97
56	0.58	0.88	6.6	3.2	77	101
57	0.53	1.39	6.0	2.8	70	107
58	0.60	0.82	7.2	2.8	75	98
59	0.62	1.67	8.6	3.5	74	110
60	0.65	0.94	8.0	3.4	70	106
MEAN	0.60	1.11	7.4	3.2	74	103
± SD	±0.04	±0.34	±1.0	±0.3	±3	±5
			WEEK 6 MFD			
91	0.77	1.46	5.1	3.6	72	105
92	0.84	1.58	5.3	4.1	70	103
93	0.59	0.93	4.5	3.0	85	100
94	1.43	1.96	5.1	6.7	68	108
95	0.72	1.02	4.7	3.3	71	108
96	0.74	1.04	4.3	3.5	72	105
MEAN	0.85	1.33	4.8	4.0	73	105
± SD	±0.30	±0.40	±0.4	±1.4	±6	±3

1. % of dose (^{3}H-deoxycytidine) which was recovered in the homogenates.
2. % of dose (^{3}H-deoxycytidine) in tissue homogenates which remained in the supernatant after centrifuging.
3. % of dose (^{3}H-deoxycytidine) in supernatant which was recovered in the deoxycytidine fraction from the HPLC.
4. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 9b. Individual data on % of ^{3}H from injected ^{3}H-deoxcytidine which was retained by the spleen. Page 1 of 2.

ANIMAL	% DOSE[1] /G TISSUE	% DOSE /MG DNA	% DOSE /TISSUE	% DOSE /G PROTEIN	% HOMO CTS IN SUPER[2]
			WEEK 2 ALC[3]		
1	0.57	0.41	0.46	5.46	66
2	0.60	0.33	0.46	6.60	76
3	0.56	0.36	0.44	6.42	79
4	0.48	0.41	0.43	4.90	68
5	0.54	0.47	0.47	5.50	75
6	0.55	0.44	0.43	5.88	85
MEAN	0.55	0.40	0.45	5.79	75
± SD	±0.04	±0.05	±0.02	±0.64	±7
			WEEK 2 PFC		
13	0.58	0.73	0.47	6.45	74
14	0.59	0.40	0.56	6.21	71
15	0.57	0.65	0.43	5.76	78
16	0.57	1.04	0.42	5.72	79
17	0.49	0.37	0.30	5.80	81
18	0.67	0.53	0.43	7.51	72
MEAN	0.58	0.62	0.44	6.24	76
± SD	±0.06	±0.25	±0.08	±0.69	±4
			WEEK 2 ALD		
49	0.48	0.24	0.37	5.13	77
50	0.61	0.39	0.38	6.33	70
51	0.59	0.16	0.38	7.04	75
52	0.66	0.58	0.57	6.43	68
53	0.53	0.45	0.34	5.62	72
54	0.39	0.52	0.35	3.96	81
MEAN	0.54	0.39	0.40	5.75	74
± SD	±0.10	±0.16	±0.09	±1.10	±5
			WEEK 2 MFD		
85	0.74	0.84	0.46	7.77	74
86	1.05	0.31	0.35	10.37	69
87	0.83	1.18	0.53	8.29	74
88	0.67	0.54	0.34	6.91	66
89	0.68	0.15	0.26	6.61	68
90	0.60	0.53	0.37	5.86	71
MEAN	0.76	0.59	0.38	7.64	70
± SD	±0.16	±0.37	±0.10	±1.59	±3

1. % of dose (^{3}H-deoxycytidine) which was recovered in the homogenates.
2. % of dose (^{3}H-deoxycytidine) in tissue homogenates which remained in the supernatant after centrifuging.
3. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 9b. Individual data on % of ^{3}H from injected ^{3}H-deoxcytidine which was retained by the spleen. Page 2 of 2.

ANIMAL	% DOSE[1] /G TISSUE	% DOSE /MG DNA	% DOSE /TISSUE	% DOSE /G PROTEIN	% HOMO CTS IN SUPER[2]
			WEEK 6 ALC[3]		
7	0.53	0.45	0.48	5.44	58
8	0.57	0.66	0.54	5.02	42
9	0.66	0.73	0.69	6.05	44
10	0.57	0.50	0.41	5.94	54
11	0.63	0.60	0.48	6.20	55
12	0.47	0.49	0.38	4.31	55
MEAN	0.57	0.57	0.50	5.49	51
± SD	±0.07	±0.11	±0.11	±0.73	
			WEEK 6 PFC		
19	0.49	0.45	0.32	5.22	68
20	0.52	0.53	0.39	5.23	60
21	0.53	0.64	0.31	4.96	63
22	0.73	0.89	0.60	6.29	57
23	0.49	0.40	0.38	4.42	66
24	0.44	0.30	0.26	3.94	64
MEAN	0.53	0.54	0.38	5.01	63
± SD	±0.10	±0.21	±0.12	±0.80	±4
			WEEK 6 ALD		
55	0.61	0.30	0.28	5.79	65
56	0.57	0.44	0.32	5.39	64
57	0.63	0.20	0.43	6.01	66
58	0.56	0.48	0.33	4.75	67
59	0.81	0.81	0.62	6.73	63
60	0.47	0.33	0.26	4.35	59
MEAN	0.61	0.43	0.37	5.50	64
± SD	±0.11	±0.21	±0.13	±0.87	±3
			WEEK 6 MFD		
91	0.57	0.38	0.26	5.57	67
92	0.77	0.52	0.30	7.97	76
93	0.68	0.77	0.30	6.89	68
94	1.35	1.33	1.11	15.60	68
95	0.61	0.47	0.23	5.83	65
96	0.76	0.49	0.35	7.75	65
MEAN	0.79	0.66	0.42	8.27	68
± SD	±0.29	±0.35	±0.34	±3.72	±4

1. % of dose (^{3}H-deoxycytidine) which was recovered in the homogenates.
2. % of dose (^{3}H-deoxycytidine) in tissue homogenates which remained in the supernatant after centrifuging.
3. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 9c. Individual data on % of ^{3}H from injected ^{3}H-deoxcytidine retained by the thymus.

ANIMAL	% DOSE[1] /G TISSUE	% DOSE /MG DNA	% DOSE /TISSUE	% DOSE /G PROTEIN	% HOMO CTS[2] IN SUPER
			WEEK 2 ALC[3]		
1	0.54	0.87	0.39	10.95	70
2	0.51	0.60	0.37	10.40	69
3	0.55	1.32	0.44	11.36	71
4	0.26	0.70	0.21	5.64	60
5	0.57	1.07	0.29	12.41	76
6	0.54	1.44	0.48	12.19	69
MEAN	0.50	1.00	0.36	10.51	69
± SD	±0.12	±0.34	±0.10	±2.45	±5
			WEEK 2 PFC		
13	0.58	1.31	0.43	12.01	73
14	0.53	1.13	0.44	11.37	69
15	0.52	0.49	0.30	11.10	67
16	0.51	1.15	0.30	10.58	66
17	0.43	0.73	0.20	8.12	73
18	0.65	1.42	0.37	14.72	70
MEAN	0.54	1.04	0.34	11.22	70
± SD	±0.07	±0.36	±0.09	±1.95	±3
			WEEK 2 ALD		
49	0.39	0.46	0.11	7.71	70
50	0.62	1.30	0.33	13.09	68
51	0.69	0.86	0.29	14.51	60
52	0.56	0.65	0.19	11.54	69
53	0.57	0.84	0.22	11.29	61
54	0.39	0.66	0.23	8.49	68
MEAN	0.54	0.80	0.23	11.10	66
± SD	±0.12	±0.29	±0.08	±2.61	±4
			WEEK 2 MFD		
85	0.82	0.99	0.27	17.03	68
86	---	---	---	---	---
87	0.44	0.56	0.11	8.32	66
88	0.49	0.60	0.12	9.49	73
89	0.77	1.03	0.32	15.58	63
90	0.76	0.88	0.35	15.54	64
MEAN	0.66*	0.81*	0.23*	13.19*	67*
± SD	±0.18	±0.22	±0.11	±3.98	±4

1. % of dose (^{3}H-deoxycytidine) which was recovered in the homogenates.
2. % of dose (^{3}H-deoxycytidine) in tissue homogenates which remained in the supernatant after centrifuging.
3. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient, * n=5.

Appendix 9d. Individual data on the % of ^{3}H from injected ^{3}H-deoxycytidine retained by the bone marrow.

ANIMAL	% DOSE /MG DNA	% HOMO CTS IN SUPER	ANIMAL	% DOSE /MG DNA	% HOMO CTS IN SUPER[2]
	WEEK 2 ALC		WEEK 6 ALC[4]		
1	0.073	TRACE	7	0.53	TRACE
2	0.08	TRACE	8	0.73	TRACE
3	0.06	TRACE	9	0.59	TRACE
4	0.06	TRACE	10	0.64	TRACE
5	0.06	TRACE	11	0.57	TRACE
6	0.06	TRACE	12	0.47	TRACE
MEAN	0.07	TRACE	MEAN	0.59	TRACE
± SD	±0.01	TRACE	± SD	±0.09	TRACE
	WEEK 2 PFC		WEEK 6 PFC		
13	0.05	TRACE	19	0.73	TRACE
14	0.05	TRACE	20	0.76	TRACE
15	0.06	TRACE	21	0.98	TRACE
16	0	TRACE	22	0.07	TRACE
17	0	TRACE	23	0.53	TRACE
18	0.04	TRACE	24	0.84	TRACE
MEAN	0.03#	TRACE	MEAN	0.77*	TRACE
± SD	±0.03	TRACE	± SD	±0.16	TRACE
	WEEK 2 ALD		WEEK 6 ALD		
49	0.14	TRACE	55	0.91	TRACE
50	0.01	TRACE	56	0.91	TRACE
51	0.09	TRACE	57	1.13	TRACE
52	0.03	TRACE	58	1.26	TRACE
53	0.04	TRACE	59	1.35	TRACE
54	0.01	TRACE	60	1.29	TRACE
MEAN	0.05	TRACE	MEAN	1.14	TRACE
± SD	±0.05	TRACE	± SD	±0.19	TRACE
	WEEK 2 MFD		WEEK 6 MFD		
85	0.11	TRACE	91	0.80	TRACE
86	0.17	TRACE	92	1.94	TRACE
87	0.01	TRACE	93	0.94	TRACE
88	0	TRACE	94	0.98	TRACE
89	0.09	TRACE	95	0.73	TRACE
90	0.07	TRACE	96	1.03	TRACE
MEAN	0.08	TRACE	MEAN	1.07	TRACE
± SD	±0.07	TRACE	± SD	±0.44	TRACE

1. % of dose (^{3}H-deoxycytidine) which was recovered in the homogenates.
2. % of dose (^{3}H-deoxycytidine) in tissue homogenates which remained in the supernatant after centrifuging.
3. % of dose (^{3}H-deoxycytidine) in supernatant which was recovered in the deoxycytidine fraction from the HPLC.
4. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 9e. Individual data on the % of ^{3}H from injected ^{3}H-deoxycytidine retained by the intestine. Page 1 of 2.

ANIMAL	% DOSE[1] /G TISSUE	% DOSE /MG DNA	% DOSE /G PROTEIN	% HOMO CTS[2] IN SUPER
		WEEK 2 ALC[3]		
1	0.24	0.17	12.8	60
2	0.41	0.66	15.4	75
3	0.35	0.74	10.3	71
4	0.32	0.90	10.4	72
5	0.38	0.26	29.3	59
6	0.36	0.51	21.3	65
MEAN	0.34	0.54	16.6	67
± SD	±0.06	±0.28	±7.44	±7
		WEEK 2 PFC		
13	0.38	0.79	13.4	77
14	0.26	0.43	14.6	67
15	0.21	0.68	10.6	67
16	0.30	1.04	16.9	79
17	0.25	0.34	16.4	72
18	0.37	1.15	14.0	70
MEAN	0.30	0.74	14.3	72
± SD	±0.07	±0.32	±2.3	±5
		WEEK 2 ALD		
49	0.32	0.52	13.0	67
50	0.39	0.32	20.0	53
51	0.40	0.55	33.4	64
52	0.32	0.91	18.2	63
53	0.35	0.87	18.0	64
54	0.35	0.62	17.5	71
MEAN	0.36	0.63	20.0	64
± SD	±0.03	±0.22	±6.93	±6
		WEEK 2 MFD		
85	0.45	0.47	16.0	68
86	0.72	0.56	25.5	49
87	0.52	0.34	19.0	63
88	0.44	0.20	13.2	71
89	0.22	0.69	16.2	61
90	0.21	0.41	16.5	66
MEAN	0.43	0.44	17.7	63
± SD	±0.19	±0.17	±4.24	±8

1. % of dose (^{3}H-deoxycytidine) which was recovered in the homogenates.
2. % of dose (^{3}H-deoxycytidine) in tissue homogenates which remained in the supernatant after centrifuging.
3. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 9e. Individual data on the % of ^{3}H from injected ^{3}H-deoxycytidine retained by the intestine. Page 2 of 2.

ANIMAL	% DOSE[1] /G TISSUE	% DOSE /MG DNA	% DOSE /G PROTEIN	% HOMO CTS IN SUPER[2]
		WEEK 6 ALC[4]		
7	0.26	0.43	5.60	75
8	0.27	0.79	6.57	69
9	0.23	0.39	6.90	67
10	0.28	0.84	5.73	71
11	0.31	1.19	14.12	68
12	0.24	0.80	8.79	65
MEAN	0.26	0.74	7.95	69
± SD	±0.03	±0.30	0.3.23	±3
		WEEK 6 PFC		
19	0.24	1.27	7.22	78
20	0.36	1.16	9.76	63
21	0.43	1.35	12.24	62
22	0.54	1.58	16.41	63
23	0.27	0.67	6.77	63
24	0.24	0.86	6.69	55
MEAN	0.35	1.15	9.85	63
± SD	±0.12	±0.33	±3.88	64 ±8
		WEEK 6 ALD		
55	0.39	0.92	16.10	63
56	0.30	0.79	7.48	70
57	0.32	0.78	11.07	58
58	0.32	0.99	13.33	65
59	0.24	1.03	7.02	80
60	0.46	1.24	12.42	66
MEAN	0.34	0.96	11.24	67
± SD	±0.08	±0.17	±3.50	±7
		WEEK 6 MFD		
91	0.43	0.96	10.11	64
92	0.53	1.77	11.55	65
93	0.28	0.62	29.35	59
94	0.51	1.99	47.35	66
95	0.43	1.05	14.04	60
96	0.33	1.12	11.96	66
MEAN	0.41	1.25	20.73	63
± SD	±0.10	±0.52	±14.84	±3

1. % of dose (^{3}H-deoxycytidine) which was recovered in the homogenates.
2. % of dose (^{3}H-deoxycytidine) in tissue homogenates which remained in the supernatant after centrifuging.
3. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 10. Effect of a vitamin B-6 deficiency on the incorporation of labeled deoxycytidine into DNA of selected tissues. Page 1 of 2

	CPM/UG OLIGOPOLYNUCLEOTIDES			
ANIMAL	SPLEEN	THYMUS	BONE MARROW	INTESTINE
		WEEK 2 ALC		
1	1.79	2.55	19.31	0.56
2	3.27	4.13	21.30	0.40
3	3.30	2.79	20.26	0.33
4	1.43	8.42	19.49	0.15
5	0.69	3.91	22.92	1.82
6	2.13	1.26	19.98	0.67
MEAN	2.10	3.84	20.54	0.42*
± SD	±1.03	±2.47	±1.36	±0.20
		WEEK 2 PFC		
13	1.14	1.40	---	0.44
14	1.21	1.06	---	0.20
15	0.84	3.05	---	0.24
16	0	0	---	0
17	0	0	---	0.14
18	1.70	1.64	---	0.96
MEAN	1.22#	1.81	---	0.40*
± SD	±0.36	±0.86#	---	±0.34
		WEEK 2 ALD		
49	0.87	5.43	29.62	0.98
50	0.89	3.44	24.07	1.84
51	0.86	6.03	29.27	1.55
52	1.60	0	22.45	0.73
53	0.24	4.76	17.50	0.63
54	0	0	0	0
MEAN	0.89*	4.92#	24.58*	1.15*
± SD	±0.48	±1.11	±5.06	±0.53
		WEEK 2 MFD		
85	0.63	0	21.20	0.54
86	---	---	25.21	2.47
87	0.57	0	0	0.09
88	0	5.79	0	0.06
89	0.40	6.00	21.81	0.50
90	0.74	1.38	24.86	1.18
MEAN	0.58#	2.48*	23.75#	1.17
± SD	±0.14	±2.84	±2.22	±0.92

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 10. Effect of a vitamin B-6 deficiency on the incorporation of labeled deoxycytidine into DNA of selected tissues. Page 2 of 2

	CPM/UG OLIGOPOLYNUCLEOTIDES			
ANIMAL	SPLEEN	THYMUS	BONE MARROW	INTESTINE
		WEEK 6 ALC		
7	3.16	---	40.78	0.76
8	2.05	---	44.51	0.65
9	5.08	---	37.15	1.48
10	3.25	---	35.26	1.12
11	3.37	---	38.66	0.62
12	3.43	---	45.17	0.48
MEAN	3.39	---	40.26	0.85
± SD	±0.97		±3.99	±0.38
		WEEK 6 PFC		
19	1.73	---	32.62	0.28
20	2.01	---	36.84	0.56
21	1.72	---	30.14	0.42
22	0.29	---	---	0.10
23	1.17	---	33.59	0.60
24	1.94	---	32.90	0.51
MEAN	1.71*	---	33.22	0.41
± SD	±0.33		±2.40	±0.19
		WEEK 6 ALD		
55	2.07	---	25.35	0.53
56	3.24	---	65.85	0.76
57	3.30	---	32.69	0.59
58	1.74	---	36.97	0.60
59	2.37	---	45.51	0.75
60	0.36	---	37.35	0.39
MEAN	2.54*	---	35.57*	0.60
± SD	±0.70		±7.36	±0.14
		WEEK 6 MFD		
91	2.30	---	24.55	0.31
92	2.53	---	32.51	0.51
93	2.20	---	25.91	0.66
94	1.32	---	8.66	0.13
95	2.59	---	28.66	---
96	2.41	---	29.08	0.48
MEAN	2.22	---	28.14*	0.42*
± SD	±0.47		±3.09	±0.20

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 11. Individual data on tissue protein levels (mg/g wet weight) (page 1 of 4).

	ANIMAL	LIVER	SPLEEN	THYMUS	MUSCLE	HEART	INTESTINE
				WEEK 2			
ALC	1	160	105	50	124	63	18
	2	178	92	49	129	55	26
	3	169	86	48	120	78	34
	4	158	99	46	136	84	31
	5	177	.98	46	123	63	13
	6	160	94	45	96	69	17
MEAN		167	96	47	121	69	23
±SD		±9	±6	±2	±14	±11	±8
PFC	13	187	90	48	110	30	28
	14	201	95	47	95	73	13
	15	197	100	47	103	144	20
	16	203	100	49	117	79	17
	17	198	85	53	132	100	15
	18	190	90	44	105		26
MEAN		196	93	48	110	83	20
±SD		±7	±6	±3	±13	±38	±6
ALD	49	161	94	50	150	91	25
	50	162	97	47	98	83	19
	51	202	84	48	96	119	12
	52	154	102	48	121	79	18
	53	166	95	51	108	77	19
	54	161	98	43	133	92	20
MEAN		168	95	48	118	90	19
±SD		±18	±6	±3	±21	±15	±4
MFD	85	224	95	48	153	82	28
	86	228	102	48	102	83	14
	87	214	100	53	122	95	27
	88	200	97	52	120	105	33
	89	230	91	49	127	75	14
	90	221	103	49	105	94	12
MEAN		219	98	50	121	89	21
±SD		±11	±4	±2	±18	±11	±9

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 11. Individual data on tissue protein levels (mg/g wet weight) (page 2 of 4).

	ANIMAL	LIVER	SPLEEN	THYMUS	MUSCLE	HEART	INTESTINE
				WEEK 6			
ALC	7	173	98	--	156	65	46
	8	175	113	--	112	72	41
	9	180	109	--	101	64	33
	10	177	96	--	151	69	50
	11	180	102	--	124	67	22
	12	171	108	--	140	68	27
MEAN		176	104	--	130	68	37
±SD		±4	±7	--	±22	±3	±11
PFC	19	199	94	--	145	69	34
	20	208	99	--	141	74	37
	21	202	106	--	114	66	36
	22	213	116	--	125	66	33
	23	215	110	--	107	64	40
	24	212	111	--	163	57	36
MEAN		208	106	--	133	66	36
±SD		±6	±8	--	±21	±6	±2
ALD	55	175	105	--	125	74	24
	56	178	107	--	110	31	40
	57	187	104	--	133	72	29
	58	211	118	--	119	72	24
	59	166	121	--	96	69	31
	60	104	108	--	108	77	26
MEAN		185	110	--	115	65	30
±SD		±16	±7	--	±13	±18	±6
MFD	91	214	102	--	118	70	43
	92	206	97	--	136	67	46
	93	198	98	--	150	72	10
	94	214	86	--	88	62	11
	95	220	105	--	139	71	31
	96	211	98	--	112	56	28
MEAN		211	98	--	124	66	28
±SD		±8	±6	--	±23	±6	±15

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 11. Individual data on tissue protein levels (mg/g wet weight) (page 3 of 4).

	ANIMAL	LIVER	SPLEEN	THYMUS	MUSCLE	HEART	INTESTINE
			WEEK 6 1 DAY FAST				
PFC	31	213	111	---	127	80	27
	32	213	109	---	129	84	21
	33	218	115	---	129	72	38
	34	204	95	---	156	73	23
	35	219	100	---	130	128	27
	36	226	105	---	133	98	36
MEAN		216	106	---	134	89	29
±SD		±7	±8	---	±11	±21	±7
ALD	67	210	127	---	141	72	20
	68	212	108	---	146	90	35
	69	220	114	---	147	132	29
	70	219	101	---	137	92	24
	71	180	95	---	156	84	27
	72	200	108	---	96	86	31
MEAN		207	109	---	137	93	27
±SD		±15	±11	---	±21	±20	±5
			WEEK 6 3 DAY FAST				
PFC	25	222	101	---	102	84	19
	26	216	96	---	121	93	26
	27	209	104	---	138	91	17
	28	198	102	---	95	82	21
	29	207	92	---	137	83	30
	30	232	111	---	127	98	18
MEAN		214	101	---	120	88	22
±SD		±12	±6	---	±18	±6	±5
ALD	61	221	119	---	88	61	18
	62	226	114	---	148	81	22
	63	219	108	---	139	77	13
	64	203	105	---	117	124	21
	65	234	100	---	105	87	23
	66	218	117	---	137	82	28
MEAN		220	110	---	122	78	21
±		±10	±SD	---	±23	±10	±5

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 11. Individual data on tissue protein levels (mg/g wet weight) (page 4 of 4).

	ANIMAL	LIVER	SPLEEN	THYMUS	MUSCLE	HEART	INTESTINE
				REPLETION			
PFC	37	178	92	54	127	65	39
	38	187	84	50	94	75	33
	39	191	95	54	105	78	24
	40	200	95	52	114	69	25
	41	195	103	51	104	69	40
	42	180	96	53	127	68	30
MEAN		188	94	52	112	71	30
±SD		±8	±6	±2	±13	±5	±5
ALD	73	172	99	52	105	71	40
	74	197	90	54	132	71	20
	75	162	77	53	116	67	28
	76	187	93	51	115	66	35
	77	183	112	52	121	76	23
	78	169	91	52	143	72	28
MEAN		178	94	52	122	70	29
±SD		±13	±11	±1	±13	±3	±7
				REPLETION 3 DAY FAST			
PFC	43	220	71	56	138	66	26
	44	206	69	58	125	63	29
	45	197	82	57	91	64	23
	46	215	90	48	160	64	21
	47	211	88	57	133	59	21
	48	204	75	48	160	67	24
MEAN		209	79	54	135	64	24
±		±8	±9	±5	±26	±3	±3
ALD	79	188	101	50	126	62	18
	80	199	94	56	131	67	18
	81	195	86	56	119	66	23
	82	171	83	50	133	71	23
	83	189	83	50	134	62	15
	84	219	89	51	139	66	19
MEAN		194	89	52	130	66	19
±SD		±16	±7	±3	±7	±3	±3

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 12. Individual data on tissue DNA (ug/g wet weight).
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	ANIMAL	LIVER	SPLEEN	THYMUS	INTESTINE	MUSCLE	BONE MARROW
				WEEK 2			
ALC	1	744	139	624	1355	160	92
	2	825	186	845	612	116	125
	3	678	154	414	481	122	80
	4	546	117	372	360	99	108
	5	647	113	520	1461	118	102
	6	662	127	377	713	127	118
MEAN		684	139	543	820	124	104
±.SD		±94	±27	±176	±330	±20	±16
PFC	13	604	79	442	478	111	114
	14	496	147	473	600	117	63
	15	608	88	1066	312	115	100
	16	606	55	447	285	96	67
	17	648	133	594	724	134	89
	18	627	133	458	319	122	156
MEAN		598	105	580	453	116	99
±SD		±53	±36	±245	±180	±13	±35
ALD	49	577	200	855	614	100	114
	50	503	158	575	1226	98	132
	51	514	365	803	726	86	117
	52	549	113	856	585	131	194
	53	572	119	684	385	113	116
	54	527	75	588	404	122	152
MEAN		540	172	710	657	108	138
±SD		±31	±104	±156	±308	±17	±31
MFD	85	457	88	823	956	104	110
	86	625	342		1282	99	103
	87	523	70	782	1523	82	121
	88	483	124	820	2149	88	118
	89	574	465	752	320	76	76
	90	549	113	861	515	102	118
MEAN		535	200	808	1124	92	108
±SD		±61	±163	±42	±675	±12	±17

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 12. Individual data on tissue DNA (ug/g wet weight).
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	ANIMAL	LIVER	SPLEEN	THYMUS	INTESTINE	MUSCLE	BONE MARROW
				WEEK 6			
ALC	7	709	118	302	600	206	187
	8	867	86	268	340	133	207
	9	672	91	273	593	139	222
	10	686	114	349	338	116	240
	11	513	105	344	262	114	242
	12	624	96	507	295	87	224
MEAN		678	102	340	405	132	220
±SD		±116	±13	±88	±151	±40	±21
PFC	19	834	108	306	192	88	136
	20	695	97	229	310	75	207
	21	548	174	280	323	111	197
	22	719	82	253	342	88	128
	23	916	121	252	400	113	204
	24	871	148	250	283	107	185
MEAN		764	122	262	308	97	176
±SD		±136	±34	±27	±69	±15	±35
ALD	55	649	205	----	427	119	149
	56	652	130	----	387	136	119
	57	379	319	----	412	85	120
	58	733	116	----	322	99	106
	59	372	100	----	230	93	219
	60	694	142	----	367	110	182
MEAN		580	169	----	358	107	169
±SD		±161	±82	----	±72	±19	±32
MFD	91	526	150	----	451	85	129
	92	530	140	----	297	97	106
	93	632	89	----	455	105	165
	94	734	102	----	259	80	151
	95	705	130	----	412	155	127
	96	714	156	----	296	109	261
MEAN		640	128	----	362	105	103
±SD		±93	±27	----	±87	±27	±25

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 12. Individual data on tissue DNA (ug/g wet weight).
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	ANIMAL	LIVER	SPLEEN	THYMUS	INTESTINE	MUSCLE	BONE MARROW
				WEEK 6 FAST DAY 1			
PFC	31	902	----	240	1036	100	212
	32	1033	----	291	772	131	147
	33	1089	----	263	551	93	141
	34	847	----	188	308	63	123
	35	831	----	247	486	60	137
	36	844	----	282	494	92	131
MEAN		924	----	252	608	90	149
±SD		±110	----	±37	±257	±26	±33
ALD	67	746	----	----	1310	101	---
	68	907	----	----	658	70	171
	69	722	----	----	990	106	186
	70	668	----	----	1295	104	147
	71	707	----	----	1398	104	148
	72	970	----	----	1532	86	135
MEAN		787	----	----	1197	95	158
±SD		±122	----	----	±319	±14	±20
				WEEK 6 FAST DAY 3			
PFC	25	901	----	240	1446	112	149
	26	600	----	234	247	96	119
	27	714	----	289	360	95	120
	28	728	----	260	580	85	106
	29	447	----	215	1000	102	119
	30	648	----	237	382	94	72
MEAN		718*	----	246	669	97	114
±SD		±114	----	±26	±464	±9	±25
ALD	61	747	----	----	417	91	80
	62	661	----	----	1215	112	122
	63	750	----	----	1208	104	140
	64	777	----	----	728	106	73
	65	871	----	----	976	95	97
	66	564	----	----	?	111	108
MEAN		728	----	----	909*	103	103
±SD		±105	----	----	±340	±9	±25

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 12. Individual data on tissue DNA (ug/g wet weight).
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	ANIMAL	LIVER	SPLEEN	THYMUS	INTESTINE	MUSCLE	BONE MARROW
				REPLETION			
PFC	37	898	----	----	626	133	146
	38	951	----	345	688	100	130
	39	694	----	420	229	114	145
	40	561	----	306	313	104	155
	41	702	----	234	383	115	156
	42	648	----	236	323	126	163
MEAN		742	----	308	427	115	149
±SD		±151	----	±78	±186	±13	±12
ALD	73	714	----	375	184	113	160
	74	874	----	291	306	96	140
	75	722	----	239	542	84	62
	76	849	----	268	253	139	163
	77	888	----	357	604	126	170
	78	716	----	281	461	109	167
MEAN		794	----	302	392	111	144
±SD		±85	----	±53	±169	±20	±42
				REPLETION FAST DAY 3			
PFC	43	1026	----	408	326	80	123
	44	726	----	275	742	86	142
	45	822	----	404	156	85	139
	46	735	----	264	339	97	133
	47	758	----	271	239	105	147
	48	871	----	266	165	81	198
MEAN		823	----	315	328	89	147
±SD		±114	----	±71	±217	±10	±26
ALD	79	436	----	235	733	84	122
	80	707	----	211	359	87	155
	81	808	----	215	368	104	153
	82	776	----	286	854	87	127
	83	658	----	235	336	126	147
	84	761	----	199	902	85	123
MEAN		691	----	230	592	96	138
±SD		±136	----	±31	±266	±17	±15

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 13. Individual data on tissue weights (g/tissue) and final body weight (g). Page 1 of 4.

	ANIMAL	LIVER	SPLEEN	THYMUS	HEART	BONE MARROW	BODY WEIGHT
				WEEK 2			
ALC	1	14.53	0.800	0.724	0.924	0.186	235
	2	10.95	0.762	0.729	0.835	0.138	205
	3	15.83	0.800	0.800	0.953	0.179	220
	4	13.58	0.885	0.814	0.967	0.167	260
	5	10.99	0.820	0.505	0.873	0.166	230
	6	12.25	0.780	0.887	0.890	0.148	218
MEAN		13.02	0.808	0.743	0.905		228
±SD		±1.97	±0.042	±0.131	±0.050		±19
PFC	13	9.80	0.818	0.743	0.947	0.166	215
	14	5.61	0.938	0.825	0.945	0.208	210
	15	8.70	0.757	0.579	0.774	0.188	205
	16	10.40	0.743	0.590	0.845	0.126	205
	17	8.36	0.619	0.466	0.763	0.275	200
	18	7.87	0.644	0.577	0.757	0.198	195
MEAN		8.96	0.753	0.063	0.838		205
±SD		±0.95	±0.117	±0.130	±0.089		±7
ALD	49	14.60	0.765	0.273	0.854	0.222	235
	50	12.60	0.616	0.534	0.851	0.071	211
	51	8.69	0.635	0.412	0.661	0.222	201
	52	13.83	0.672	0.351	0.839	0.196	221
	53	12.48	0.639	0.382	0.794	0.196	205
	54	14.18	0.892	0.600	0.853	0.192	237
MEAN		12.73	0.736	0.425	0.829		218
±SD		±2.12	±0.125	±0.121	±0.088		±15
MFD	85	5.97	0.625	0.326	0.628	0.177	150
	86	4.63	0.327	0.135	0.248	0.109	100
	87	7.16	0.643	0.256	0.717	0.215	172
	88	6.37	0.500	0.253	0.659	0.114	150
	89	4.85	0.383	0.409	0.667	0.084	157
	90	8.36	0.612	0.461	0.740	0.141	200
MEAN		6.22	0.515	0.307	0.610		155
±SD		±1.41	±0.135	±0.118	±0.182		±33

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 13. Individual data on tissue weights (g/tissue) and final body weight (g). Page 2 of 4.

	ANIMAL	LIVER	SPLEEN	THYMUS	HEART	BONE MARROW	BODY WEIGHT
				WEEK 6			
ALC	7	13.59	0.900	0.784	1.031	0.184	362
	8	15.81	0.947	0.692	1.211	0.173	413
	9	16.01	1.048	0.539	1.137	0.221	365
	10	14.64	0.727	0.600	0.971	0.116	348
	11	11.35	0.759	0.528	0.866	0.162	328
	12	11.12	0.815	0.569	1.020	0.135	369
MEAN		13.75	0.866	0.619	0.039		364
±SD		±2.14	±0.122	±0.100	±0.122		±28
PFC	19	8.73	0.652	0.554	0.953	0.201	297
	20	7.09	0.750	0.422	0.872	0.140	291
	21	8.07	0.588	0.430	0.828	0.263	279
	22	8.28	0.822	0.520	0.867	0.175	287
	23	8.17	0.777	0.451	0.827	0.138	298
	24	7.89	0.591	0.407	0.782	0.143	283
MEAN		8.20	0.697	0.464	0.855		289
±SD		±0.29	±0.100	±0.059	±0.058		±8
ALD	55	13.16	0.454	0.016	0.996	0.130	254
	56	11.30	0.554	0.006	0.756	0.136	258
	57	11.38	0.688	0.100	0.800	0.221	259
	58	12.08	0.591	0.100	0.739	0.141	270
	59	13.92	0.762	0.048	0.589	0.072	218
	60	12.24	0.558	0.136	0.692	0.121	259
MEAN		12.35	0.601	0.096	0.762		254
±SD		±1.03	±0.109	±0.036	±0.135		±18
MFD	91	6.67	0.452	0.072	0.551	0.027	195
	92	6.34	0.392	0.084	0.523	0.151	179
	93	7.62	0.445	0.078	0.656	0.119	203
	94	3.57	0.823	0.042	0.445	0.242	100
	95	26.50	0.379	0.067	0.587	0.132	208
	96	5.82	0.465	0.089	0.555	0.089	191
MEAN		6.09	0.493	0.072	0.553		179
±SD		±1.37	±0.165	±0.017	±0.070		±40

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 13. Individual data on tissue weights (g/tissue), final body weight (g). Page 3 of 4.

	ANIMAL	LIVER	SPLEEN	THYMUS	HEART	BONE MARROW	BODY WEIGHT
			WEEK 6 FAST				
			DAY 1				
PFC	31	7.48	0.706	0.473	0.900	0.106	276
	32	6.79	0.575	0.319	0.839	0.176	269
	33	7.02	0.583	0.377	0.776	0.173	285
	34	7.30	0.658	0.433	0.779	0.208	274
	35	7.14	0.669	0.489	0.856	0.197	275
	36	6.92	0.648	0.332	0.784	0.137	269
MEAN		7.10	0.640	0.404	0.822		275
±SD		±0.25	±0.051	±0.072	±0.051		±6
ALD	67	9.32	0.944	0.100	0.736	--	241
	68	8.23	0.583	0.111	0.704	0.136	262
	69	9.47	0.783	0.210	0.865	0.184	292
	70	7.67	0.478	0.089	0.703	0.195	242
	71	6.01	0.415	0.100	0.586	0.158	200
	72	7.82	0.615	0.090	0.688	0.139	259
MEAN		8.09	0.637	0.117	0.714		249
±SD		±1.27	±0.197	0.046	±0.090		±30
			DAY 3				
PFC	25	6.50	0.528	0.485	0.900	0.071	240
	26	6.69	0.652	0.420	0.839	0.165	271
	27	5.83	0.638	0.555	0.776	0.163	280
	28	6..26	0.600	0.436	0.779	0.147	238
	29	6.75	0.549	0.319	0.856	0.172	231
	30	6.76	0.566	0.533	0.784	0.136	264
MEAN		6.47	0.589	0.458	0.822		254
±SD		±0.36	±0.050	±0.086	±0.051		±20
ALD	61	6.77	0.440	0.183	0.700	0.193	227
	62	7.44	0.528	0.207	0.718	0.197	262
	63	6.91	0.616	0.245	0.730	0.137?	252
	64	6.82	0.416	0.072	0.537	0.360	189
	65	5.88	0.556	0.159	0.673	0.226	221
	66	8.50	0.653	0.098	0.658	0.246	214
MEAN		7.05	0.535	0.161	0.669		228
±SD		±0.87	±0.094	±0.066	±0.070		±26

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 13. Individual data on tissue weights (g/tissue), final body weight (g). Page 4 of 4.

	ANIMAL	LIVER	SPLEEN	THYMUS	HEART	BONE MARROW	BODY WEIGHT
				REPLETION			
PFC	37	12.63	0.754	0.585	1.000	0.236	316
	38	13.56	0.992	0.645	1.010	0.223	380
	39	8.35	1.042	0.261	0.810	0.176	280
	40	11.88	0.815	0.440	0.918	0.221	348
	41	12.87	0.843	0.720	1.048	0.214	362
	42	14.42	0.916	0.474	1.006	0.199	345
MEAN		12.29	0.894	0.521	0.965		338
±SD		±2.11	±0.110	±0.165	±0.087		±36
ALD	73	16.07	0.889	0.568	0.900	0.241	323
	74	12.77	0.838	0.606	1.007	0.204	332
	75	14.27	0.865	0.716	0.928	0.308	350
	76	12.49	1.090	0.493	1.011	0.228	324
	77	14.66	1.061	0.684	1.006	0.207	375
	78	14.67	0.864	0.566	1.000	0.221	346
MEAN		14.16	0.934	0.606	0.975		342
±SD		±1.33	±0.111	±0.082	±0.048		±20
			REPLETION FAST DAY 3				
PFC	43	7.61	0.700	0.439	0.969	0.206	335
	44	7.68	0.575	0.428	0.933	0.189	320
	45	7.26	0.731	0.400	0.885	0.236	312
	46	7.25	0.688	0.474	0.867	0.188	315
	47	7.68	0.754	0.417	0.961	0.250	314
	48	7.72	0.623	0.490	1.099	0.191	323
MEAN		7.53	0.678	0.441	0.852		320
±SD		±0.22	±0.068	±0.034	±0.083		±8
ALD	79	8.48	0.620	0.524	1.154	0.304	340
	80	7.20	0.621	0.528	0.795	0.244	256
	81	7.24	0.500	0.650	0.917	0.196	280
	82	8.27	0.766	0.454	0.911	0.221	285
	83	8.36	0.804	0.769	0.946	0.190	336
	84	8.22	0.764	0.723	0.909	0.277	340
MEAN		7.96	0.679	0.608	0.939		306
±SD		±0.58	±0.118	±0.125	±0.118		±37

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 14. Individual data on serine concentration[1] in selected tissues.Page 1 of 2

ANIMAL	LIVER umol/g	SPLEEN umol/g	SERINE THYMUS umol/g	INTESTINE umol/g	BONE MARROW umol/ug DNA
			WEEK 2 ALC[2]		
1	73.4	50.8	41.1	53.9	1.69
2	79.6	51.4	58.6	79.5	1.52
3	66.9	50.3	45.8	62.0	1.68
4	76.2	39.4	33.2	63.7	1.44
5	69.4	38.3	38.7	42.7	1.71
6	76.2	37.7	35.2	63.8	1.25
MEAN	73.6	44.6	42.1	61.0	1.55
± SD	±4.7	±6.8	±9.2	±12.2	±0.20
			WEEK 2 PFC		
13	75.2	35.6	41.4	44.9	1.47
14	77.4	41.8	42.2	50.0	1.89
15	95.8	38.6	54.3	37.7	1.75
16	78.9	36.6	32.8	43.3	1.31
17	78.4	38.9	37.0	47.6	1.66
18	72.8	33.5	33.6	30.2	1.21
MEAN	79.8	37.5	40.2	42.3	1.55
± SD	±8.1	±2.4	±7.9	±7.3	±0.30
			WEEK 2 ALD		
49	47.4	45.6	56.6	61.7	2.56
50	59.9	35.3	36.1	57.6	1.92
51	68.4	43.6	35.7	36.6	3.38
52	51.0	41.8	29.2	63.1	2.23
53	60.1	38.5	31.5	49.1	1.59
54	51.8	34.5	30.0	50.9	1.51
MEAN	56.4	39.9	36.5	53.1	2.20
± SD	±7.76	±4.5	±10.3	±9.9	±0.70
			WEEK 2 MFD		
85	62.7	32.0	33.7	37.2	10.38
86	73.5	37.3	---	29.4	5.53
87	66.4	31.3	52.1	32.9	3.96
88	58.9	34.6	39.1	29.6	3.18
89	46.4	39.0	33.9	28.0	10.04
90	72.7	41.7	32.0	29.2	4.74
MEAN	63.4	36.0	38.1	31.0	6.31
±SD	±10.1	±4.1	±8.2	±3.4	±3.12

1. Frisell and Higginbotham-Wilcox, 19??.
2. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient

Appendix 14. Individual data on serine concentration in selected tissues. Page 2 of 2

ANIMAL	SERINE (umoles/g) LIVER umol/g	SPLEEN umol/g	THYMUS umol/g	INTESTINE umol/g	BONE MARROW umol/ug DNA
			WEEK 6 ALC		
7	83.0	50.0	---	47.0	2.72
8	65.4	40.2	---	42.0	2.04
9	67.1	42.5	---	57.3	1.54
10	64.0	39.6	---	46.6	3.13
11	82.2	37.8	---	49.7	3.52
12	69.0	38.2	---	41.7	4.50
MEAN	71.8	41.4	---	47.4	2.91
± SD	±8.6	±4.5	---	±5.8	±1.06
			WEEK 6 PFC		
19	58.1	37.4	---	26.0	3.41
20	59.1	38.4	---	31.7	2.28
21	56.1	39.1	---	38.4	5.12
22	55.9	40.4	---	35.5	2.99
23	52.7	36.4	---	35.8	0.56
24	58.9	39.9	---	30.8	1.58
MEAN	56.8	38.6	---	33.1	2.57
± SD	±2.43	±1.5	---	±4.4	±1.57
			WEEK 6 ALD		
55	66.7	46.2	---	60.2	2.55
56	67.6	38.1	---	67.9	3.37
57	99.5	43.2	---	34.8	6.59
58	69.0	36.9	---	41.2	8.90
59	73.6	36.4	---	31.4	3.27
60	58.0	53.2	---	45.8	2.31
MEAN	72.4	42.3	---	46.9	4.50
± SD	±14.2	±6.6	---	±14.4	±2.65
		WEEK 6 MFD			
91	51.9	43.2	---	41.8	4.22
92	46.0	38.8	---	35.5	6.44
93	58.2	35.4	---	24.7	2.35
94	43.7	37.6	---	29.8	2.73
95	44.7	36.0	---	40.9	6.77
96	51.6	39.1	---	28.0	3.49
MEAN	49.3	38.3	---	33.5	4.33
±SD	±5.6	±2.8	---	±7.1	±1.88

1. Frisell and Higginbotham-Wilcox, 19??.
2. ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient

Appendix 15. Mean food efficiency ratio (change in g body weight/g food consumed/day)

	WEEK 0-1	WEEK 1-2	WEEK 2-3	WEEK 4-5	WEEK 5-6
WEEK 2					
ALC	0.45±0.03	0.32±0.04	--------	--------	--------
PFC	0.38±0.06	0.37±0.06	--------	--------	--------
ALD	0.40±0.05	0.34±0.03	--------	--------	--------
MFD	0.42±0.13	0.11±0.07	--------	--------	--------
WEEK 6 NONFASTED					
ALC	0.41±0.03	---	0.30±0.07	0.20±0.02	0.18±0.03
PFC	0.37±0.05	---	0.28±0.06	0.12±0.04	0.12±0.07
ALD	0.40±0.03	---	0.14±0.05	0.10±0.07	0.03±0.08
MFD	0.43±0.06	---	0.20±0.11	0.13±0.11	0.09±0.08

	WEEK 5-6	WEEK 6-7	WEEK 7-8
REPLETION			
PFC	0.10±0.06	---	0.24±0.02
ALD	0.05±0.09	---	0.18±0.07

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient

Appendix 16a. Mean tissue PLP levels (nM/g wet weight)

	SPLEEN	THYMUS	BONE MARROW	INTEST-INE	MUSCLE	HEART
	nM/g	nM/g	nM/g	nM/g	nM/g	nM/g
WEEK 2						
ALC	2.6±0.3	2.0±0.2	0.97±0.19	2.3±0.4	22±2	7.0±1.8
PFC	2.9±0.4	1.9±0.2	0.86±0.27	2.0±0.4	21±2	7.4±1.5
ALD	0.8±0.2	0.6±0.1	0.38±0.19	0.4±0.1	10±1	5.1±0.4
MFD	0.9±0.1	0.8±0.0	0.28±0.06	0.4±0.1	10±2	4.6±0.5
WEEK 6						
ALC	2.6±0.2	------	2.04±0.29	1.7±0.4	23±0	8.2±0.7
PFC	2.7±0.5	------	2.15±0.39	1.9±0.2	22±3	7.9±0.6
ALD	0.7±0.1	------	0.41±0.09	0.5±0.2	7±1	5.0±0.2
MFD	0.6±0.1	------	0.33±0.14	0.3±0.2	7±1	4.8±0.7
WEEK 6 FAST						
DAY 1						
PFC	3.1±0.2	------	2.43±0.43	1.8±0.2	24±3	9.0±0.1
ALD	0.8±0.1	------	0.81±0.12	0.6±0.2	7±1	6.0±1.0
DAY 3						
PFC	2.7±0.2	------	2.50±0.89	1.6±0.4	19±3	8.6±0.4
ALD	0.9±0.1	------	0.83±0.30	0.7±0.2	7±1	5.1±0.7
REPLETION						
PFC	2.6±0.3	2.3±0.2	2.38±0.27	2.1±0.3	24±2	7.8±0.6
ALD	2.4±0.2	2.4±0.1	2.10±0.23	1.9±0.3	15±2	8.4±0.4
REPLETION FAST						
DAY 3						
PFC	2.4±0.8	2.5±0.1	1.99±0.28	1.8±0.2	24±3	7.8±0.8
ALD	2.2±0.2	2.3±0.4	1.91±0.32	1.4±0.2	15±2	7.7±0.4

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient

Appendix 16b. Mean tissue PLP levels (nM/g protein).

	SPLEEN	THYMUS	HEART	INTESTINE	MUSCLE
WEEK 2					
ALC	27±2	42±4	103±22	57±11	180±6
PFC	31±4	40±5	100±34	64±20	190±24
ALD	8±2	12±2	58±8	16±4	90±17
MFD	9±2	15±1	53±9	22±11	87±17
WEEK 6					
ALC	25±3	------	122±13	51±21	182±38
PFC	25±4	------	119±6	54±5	155±34
ALD	6±1	------	64±12	18±8	64±10
MFD	6±1	------	72±7	12±4	56±14
WEEK 6 FAST					
DAY 1					
PFC	30±3	------	99±23	65±11	181±23
ALD	8±1	------	66±16	21±4	50±10
DAY 3					
PFC	27±2	------	97±9	74±12	156±21
ALD	8±1	------	62±10	36±20	59±12
REPLETION					
PFC	28±2	49±8	109±12	67±13	211±22
ALD	26±4	47±2	119±8	67±17	121±22
REPLETION FAST					
DAY 3					
PFC	28±2	36±18	122±12	75±12	181±34
ALD	25±2	44±10	117±8	67±13	114±22

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient

Appendix 16c. Mean tissue PLP levels (nM/ug DNA).

	THYMUS	BONE MARROW	INTESTINE	MUSCLE
WEEK 2				
ALC	4.1±1.2	94±13	3.7±2.5	174±40
PFC	3.6±1.0	75±32	5.2±2.2	220±47
ALD	0.9±0.2	30±19	0.7±0.2	99±15
MFD	0.9±0.0	27±8	0.4±0.2	105±38
WEEK 6				
ALC	--------	92±8	4.8±2.2	184±40
PFC	--------	123±24	6.5±1.7	226±41
ALD	--------	25±7	1.3±0.3	70±13
MFD	--------	21±6	0.9±0.6	66±16
WEEK 6 FAST				
DAY 1				
PFC	-------	164±12	3.46±1.75	292±113
ALD	-------	51±5	0.56±0.36	75±34
DAY 3				
PFC	-------	217±41	3.44±1.98	192±34
ALD	-------	80±13	0.86±0.42*	68±9
REPLETION				
PFC	8.9±2.4	160±18	5.52±1.98	205±22
ALD	8.3±1.5	136±8	6.04±3.58	136±33
REPLETION FAST				
DAY 3				
PFC	7.5±1.5	140±35	7.23±3.92	268±37
ALD	10.1±1.8	140±28	2.77±1.27	156±22

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient

Appendix 16d. Mean tissue PLP (nM/tissue) and tissue weights (g).

	PLP (nM/tissue)			TISSUE WEIGHTS (mg)		
	SPLEEN	THYMUS	HEART	SPLEEN	THYMUS	HEART
WEEK 2						
ALC	2.08±0.25	1.48±0.29	6.42±1.78	808±42	743±131	905±50
PFC	2.19±0.51	1.20±0.28	6.15±1.31	753±117	630±130	838±89
ALD	0.59±0.21	0.24±0.04	4.26±0.42	736±125	425±121	829±88
MFD	0.46±0.10	0.23±0.27	2.80±0.84	515±135	307±118	610±182
WEEK 6						
ALC	2.26±0.22	--------	8.56±1.31	866±122	619±130	1039±122
PFC	1.90±0.52	--------	6.72±0.78	697±100	464±59	855±58
ALD	0.40±0.07	--------	3.36±1.42	601±109	-------	762±135
MFD	0.30±0.13	--------	2.66±0.64	493±165	-------	553±70
WEEK 6 FAST						
DAY 1						
PFC	2.00±0.43	--------	7.24±0.65	640±51	404±72	882±51
ALD	0.52±0.03	--------	4.22±0.60	637±197	------	669±70
DAY 3						
PFC	1.45±0.19	--------	6.64±0.57	589±50	458±86	669±70
ALD	0.58±0.20	--------	3.43±0.57	535±94	------	553±70
REPLETION						
PFC	2.33±0.35	1.28±0.34	7.41±1.03	894±110	521±165	965±87
ALD	2.22±0.28	1.48±0.19	8.16±0.53	934±111	606±82	975±48
REPLETION 3 DAY FAST						
PFC	1.64±0.63	1.00±0.30	7.39±0.75	678±68	441±34	952±83
ALD	1.51±0.15	1.42±0.47	7.20±0.74	679±68	608±125	939±118

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient

Appendix 16e. Mean liver and plasma PLP levels (nM per).

	LIVER nM / g	LIVER nM / ug DNA	LIVER nM / g protein	LIVER nM / liver	PLASMA nM / ml
WEEK 2					
ALC	28±3	41±5	166±22	382±77	1184±283
PFC	27±2	45±8	136±10	239±30	1141±378
ALD	10±3	15±3	51±13	125±9	26±14
MFD	15±3	28±6	69±14	96±39	64±16
WEEK 6					
ALC	27±1	41±6	156±10	382±63	1160±195
PFC	33±3	45±11	158±15	273±24	676±264
ALD	8±2	14±1	42±11	96±27	13±8
MFD	12±2	19±5	55±9	71±22	21±8
WEEK 6 FAST					
DAY 1					
PFC	29±1	30±3	135±8	207±11	583±123
ALD	13±2	16±4	65±10	109±34	33±9
DAY 3					
PFC	28±4	39±6	130±17	180±31	567±208
ALD	14±1	18±2	65±5	100±11	45±10
REPLETION					
PFC	33±3	46±10	176±8	411±98	959±319
ALD	32±3	40±6	179±13	452±65	854±288
REPLETION FAST					
DAY 3					
PFC	26±2	32±5	126±12	198±16	794±149
ALD	24±3	37±12	128±25	196±34	783±206

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient

Appendix 17a. Mean tissue protein levels (mg/g wet weight).

	SPLEEN	THYMUS	HEART	MUSCLE	INTESTINE
WEEK 2					
ALC	96±6	47±2	69±11	121±14	23±8
PFC	93±6	48±3	83±37	110±13	20±6
ALD	95±6	48±3	90±15	118±21	19±4
MFD	98±4	50±2	89±11	121±18	21±9
WEEK 6					
ALC	104±7	----	68±3	130±22	37±11
PFC	106±8	----	66±6	133±21	36±2
ALD	110±7	----	65±18	115±13	30±6
MFD	98±6	----	66±6	124±22	28±15
WEEK 6 FAST					
DAY 1					
PFC	106±8	----	89±21	134±11	29±7
ALD	108±11	----	93±20	137±21	27±5
DAY 3					
PFC	101±6	----	88±6	120±18	22±5
ALD	110±7	----	85±21	122±23	21±5
REPLETION					
PFC	94±6	52±2	71±5	112±14	30±5
ALD	94±12	52±1	70±3	122±26	29±7
REPLETION FAST					
DAY 3					
PFC	79±9	30±6	64±3	135±26	24±3
ALD	89±7	32±5	66±3	130±7	19±3

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 17b. Mean tissue protein levels (mg/tissue).

	SPLEEN	THYMUS	HEART
WEEK 2			
ALC	77.4±7.6	34.9±6.0	56.6±14.4
PFC	70.4±13.4	30.0±5.9	68.1±27.1
ALD	70.5±15.1	20.1±4.6	73.6±9.7
MFD	55.6±13.2	15.3±5.7	54.8±19.0
WEEK 6			
ALC	94.0±21.8	-----	70.3±9.6
PFC	74.1±14.0	-----	56.6±7.9
ALD	66.8±15.2	-----	49.9±16.2
MFD	46.9±12.5	-----	36.8±7.1
WEEK 6 FAST			
DAY 1			
PFC	67.6±5.9	-----	74.7±20.0
ALD	71.0±29.3	-----	69.2±10.2
DAY 3			
PFC	59.5±6.0	-----	67.3±23.7
ALD	59.1±11.4	-----	56.0±7.6
REPLETION			
PFC	83.8±9.9	29.6±5.6	67.9±5.2
ALD	88.3±19.3	31.7±4.7	60.9±6.7
DAY 3 FAST			
PFC	54.0±10.4	23.8±0.9	68.6±5.2
ALD	60.5±9.3	31.7±6.5	61.7±6.3

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient

Appendix 18. Mean tissue DNA (ug DNA/g wet weight).

	THYMUS	BONE MARROW	INTESTINE	MUSCLE	LIVER
WEEK 2					
ALC	543±176	104±16	820±330	124±20	684±94
PFC	580±245	99±35	453±180	116±13	598±53
ALD	710±156	138±31	657±308	108±17	540±31
MFD	808±42	108±17	1124±675	92±12	535±61
WEEK 6					
ALC	340±88	220±21	405±151	132±40	679±116
PFC	262±27	176±35	308±69	97±15	764±0
ALD	-------	169±32	358±72	107±19	580±161
MFD	-------	136±23*	362±87	105±27	640±94
WEEK 6 FAST					
DAY 1					
PFC	252±37	149±33	608±257	90±26	924±110
ALD	-------	158±20	1197±319	95±14	787±122
DAY 3					
PFC	246±26	123±25*	669±464	97±9	718±114
ALD	-------	103±25	909±340	103±9	728±105
REPLETION					
PFC	308±78	149±12	427±186	115±13	742±151
ALD	302±53	160±12*	392±169	111±20	794±85
REPLETION FAST					
DAY 3					
PFC	315±71	137±9*	328±217	89±10	823±114
ALD	230±31	138±15	592±266	96±17	691±136

Values reported as mean ± standard deviation for 6 rats unless otherwise indicated. (ie * n=5).
ALC = ad libitum control, PFC = pair fed control
ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 19. Mean serine levels

GROUP	LIVER	SPLEEN	THYMUS	BONE MARROW	INTESTINE
		(umoles/g wet weight)			
WEEK 2					
ALC	73.6±4.7	44.6±6.8	42.1±9.2	---	61.0±12.2
PFC	79.8±8.2	37.5±2.9	40.2±7.9	---	42.3±7.3
ALD	56.4±7.8	39.9±4.5	36.5±10.3	---	53.1±9.9
MFD	63.4±10.1	36.0±4.1	38.1±8.2	---	31.0±3.41
WEEK6					
ALC	71.8±8.6	41.4±4.5	---	---	47.4±5.8
PFC	56.8±2.4	38.6±1.5	---	---	33.1±4.4
ALD	72.4±14.2	42.3±6.6	---	---	46.9±14.4
MFD	49.3±5.6	38.3±2.8	---	---	33.5±7.1

	g pro	g pro	(umoles) per g pro	ug DNA	g pro
WEEK 2					
ALC	442±39	324±32	892±168	1.55±0.18	1,420±520
PFC	407±40	398±152	840±178	1.55±0.26	920±130
ALD	336±27	287±122	760±188	2.20±0.70	1,630±530
MFD	282±44	276±148	757±136	4.33±1.88	1,570±870
WEEK6					
ALC	408±49	---	---	2.91±1.06	---
PFC	275±13	---	---	2.60±1.58	---
ALD	390±75	---	---	4.50±2.65	---
MFD	235±34	---	---	4.33±1.88	---

ALC = ad libutum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient.

Appendix 20. Mean liver protein, glycogen, DNA and weight.

	PROTEIN mg/g	GLYCOGEN mg/g	DNA ug/g	PROTEIN g/liver	GLYCOGEN g/liver	DNA mg/liver	WEIGHT g/liver
WEEK 2							
ALC	167	31.9	684	2.14	400	8.87	12.72
	±9	±6.9	±94	±0.31	±153	±1.61	±2.05
PFC	196	5.3	598	1.82	48.5	5.36	8.96
	±7	±2.2	±53	±0.24	±25.3	±0.72	±0.95
ALD	168	38.7	540	2.10	508	6.90	12.73
	±18	±10.9	±31	±0.21	±192	±1.37	±2.15
MFD	219	3.1	535	1.36	22.0	3.30	6.22
	±11	±2.3	±61	±0.29	±20.1	±0.73	±1.41
WEEK 6							
ALC	176	32.0	679	2.42	452	9.48	13.75
	±4	±6.7	±116	±0.40	±155	±2.82	±2.14
PFC	208	0.2	764	1.70	1.5	6.27	8.20
	±136	±6	±0.0	±1.17	±0.05	±0.3	±0.29
ALD	185	50.4	580	2.28	630	7.12	12.35
	±16	±9.1	±161	±0.19	±162	±1.93	±1.03
MFD	211	0.3	640	1.28	1.5	3.84	6.07
	±8	±0.1	±94	±0.27	±0.5	±0.83	±1.37
WEEK 6 FAST							
DAY 1							
PFC	216	0.2	924	1.58	1.6	6.54	7.10
	±7	±0.1	±110	±0.14	±0.6	±0.73	±0.25
ALD	206	33.4	787	1.68	266	6.37	8.09
	±15	±10.2	±122	±0.35	±83	±1.36	±1.27
DAY 3							
PFC	214	0.2	718	1.40	1.2	4.33	6.47
	±12	±0.1	±114	±0.15	±0.4	±0.92	±0.36
ALD	220	0.5	728	1.55	3.6	5.06	7.05
	±10	±0.4	±105	±0.19	±2.9	±0.18	±0.87
REPLETION							
PFC	188	32.9	742	2.31	397	9.19	12.29
	±8	±8.0	±151	±0.37	±195	±2.71	±2.11
ALD	178	38.6	794	2.51	557	11.08	14.16
	±13	±0.2	±85	±0.18	±192	±0.80	±1.33
REPLETION FAST							
DAY 3							
PFC	209	0.2±	823	1.54	1.3	5.84	7.73
	±8	±0.0	±114	±0.09	±0.2	±0.49	±0.26
ALD	194	0.2	691	1.54	1.8	5.47	7.96
	±16	±0.1	±136	±0.15	±0.6	±0.99	±0.58

ALC = ad libitum control, PFC = pair fed control, ALD = ad libitum deficient, MFD = meal fed deficient