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DISTRIBUTION OF PANTOTHENIC ACID IN THE RAT

By

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ABSTRACT

DISTRIBUTION OF PANTOTHENIC ACID IN THE RAT

By

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Forty-eight male Sprague-Dawley weanling rats were used to determine the distribution of pantothenic acid (PA) in organs, blood and urine. The purpose was to determine the best indicator of PA status by correlating PA concentration of bodily fluid with those of organs. The rats were divided into 4 groups and fed PA deficient AIN-76 diets supplemented with: A)120 mg Ca-PA/kg diet, ad lib; B)12 mg/kg, ad lib; C)0 mg/kg, ad lib; D)12 mg/kg, pair-fed with group C for 8 weeks. Heart, spleen, kidney, whole blood, plasma and urine were collected at week 2, 5 and 8 for determination of total and free forms of PA. Free PA concentration in kidney and total PA concentration in heart, spleen and whole blood of group C were significantly lower ($p<0.05$) than those of group B at all weeks examined. Free PA concentration in plasma correlated the best with heart, spleen, kidney free PA concentration in group C and was identified to be the best indicator of PA status.

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INTRODUCTION

It has been well established that pantothenic acid is essential for humans and animals. Pantothenate, a B vitamin sometimes called vitamin B-5, is a precursor for coenzyme A (CoA) and phosphopantetheine of acyl carrier protein. CoA and phosphopantetheine are known to be biochemically active and bound forms of pantothenic acid and are required in many different metabolic reactions (Krehl, 1954; Lehninger, 1982).

Pantothenate is found in many foodstuffs. The most concentrated sources are egg, liver, meat, and legumes. An average American diet has been reported to provide 2 - 3 mg pantothenate/1000 kcal (Essenstat et al., 1986; Song et al., 1985; Srinivasan et al., 1981; Walsh et al., 1981). Because pantothenate is widely distributed and no overt clinical deficiency of the nutrient has been reported in humans, it has been generally assumed that intake is adequate for persons consuming a normal diet.

A Recommended Dietary Allowance has not been established for pantothenic acid. However, the Food and Nutrition Board of the National Academy of Sciences, National Research Council has recommended an Estimated Safe and Adequate Daily Dietary Intake of pantothenate for adult of 4 - 7 mg/day. A Recommended Dietary Allowance will be

set after sufficient experimental data accumulate and accurate methods of assessment are found. Studies with experimental animal demonstrate that pantothenic acid deficiencies can occur. Rats deficient in pantothenate were reported to be weak and anorexic, and grew poorly compared to normal rats. The need for pantothenate was more evident during growth (Henderson et al., 1941; Lotspeich, 1950; Reibel et al., 1982).

Irregular and unusual eating habits may also place a person at risk for pantothenic acid deficiency (Eissenstat et al., 1986). However, most of the symptoms such as irritability and anorexia noted in humans were reversible with the administration of pantothenic acid (Henderson et al., 1942).

Determining the concentration of pantothenic acid in organs of rats fed diets deficient in or supplemented with various amounts of pantothenic acid may give an insight to the pattern of depletion or storage in the whole organism. It was hypothesized in this study that 1) distribution of pantothenic acid among various organs differs for rats fed different amount of pantothenate 2) pantothenic acid level in urine and blood (plasma or whole blood) reflect pantothenic acid levels in diet and various organs at different stages of pantothenate status. Growth patterns and clinical symptoms also were expected to correspond to pantothenate intake and organ level.

In this study, pantothenic acid levels in urine, plasma and whole blood were measured and correlated to the pantothenate concentration in selected organs to identify the most valid and reliable indicator of pantothenate status in rats. Growth patterns and clinical symptoms were also examined throughout the study. Specific objectives of the study included:

1.To determine the distribution of pantothenate in heart, spleen, and kidney of rats fed deficient, adequate or excessive amount of pantothenic acid.

2.To determine pantothenate levels in urine and blood of rats fed deficient, adequate and excessive amount of pantothenate for different periods.

3.To correlate pantothenic acid levels in urine and blood with those in various organs at different stages of pantothenic acid deficiency.

4.To examine clinical symptoms and growth patterns of rats fed deficient, adequate and excessive amount of pantothenate.

REVIEW OF LITERATURE

DISCOVERY

Pantothenic acid was first identified by Roger Williams in 1933. Williams detected pantothenate as an acidic substance which was a required growth factor for yeast (Williams, 1943). Pantothenate was found in all plant and animal tissues as well as in microorganisms analyzed. Thus, Williams named it pantothenic acid ("pan-" meaning "everywhere").

Pantothenic acid was also called "filtrate factor" because it was not retained on the absorbent fuller earth. Lepkovsky et al. (1936) determined that this filtrate factor, distinct from thiamine, riboflavin and pyridoxine, was required by rats for normal growth.

Pantothenic acid was also known as "chick antidermatitis factor" which was discovered independently by Woolley et al. (1939) and Jukes (1939). The factor prevented and cured a characteristic dermatitis in chicks but not in rats. They described the disease as characterized by skin lesions that could be alleviated by administration of pantothenic acid. Thus, a need for the status of pantothenic acid as a vitamin for animals was established. To further understand this phenomenon, the

structure of the factor was studied. Woolley et al. (1939) showed that the structure was a hydroxy acid destroyed by alkali and had a beta-alanine united through its amino group to the carboxyl of the hydroxy acid (Figure 1).

It was not until a dozen years after Williams' discovery of pantothenic acid that the biochemical role of pantothenic acid was discovered by Lipmann and Kaplan (1948). They discovered a heat-stable co-factor necessary for promoting ATP-dependent enzymes which functions as an acyl acceptor for the pyruvate and alpha-ketoglutarate dehydrogenase complexes. This co-factor was later named coenzyme A (CoA) for acetylation. Upon purification and analysis of CoA, they discovered that it contained pantothenate in a bound form.

ANALYTICAL METHODS

Concerns regarding the requirement of pantothenic acid in man led to the development of several methods for measuring pantothenate. Today, there are two commonly used methods to measure pantothenic acid: microbiological assay and radioimmunoassay (RIA). Microbiological assay employs the use of a microorganism, most commonly Lactobacillus plantarum, which requires pantothenic acid for growth (Strong, 1941). Suspensions of samples are incubated with the microorganism and the acid produced by the organism is a measure of pantothenate in the sample by direct titration of entire culture. An RIA for pantothenate acid

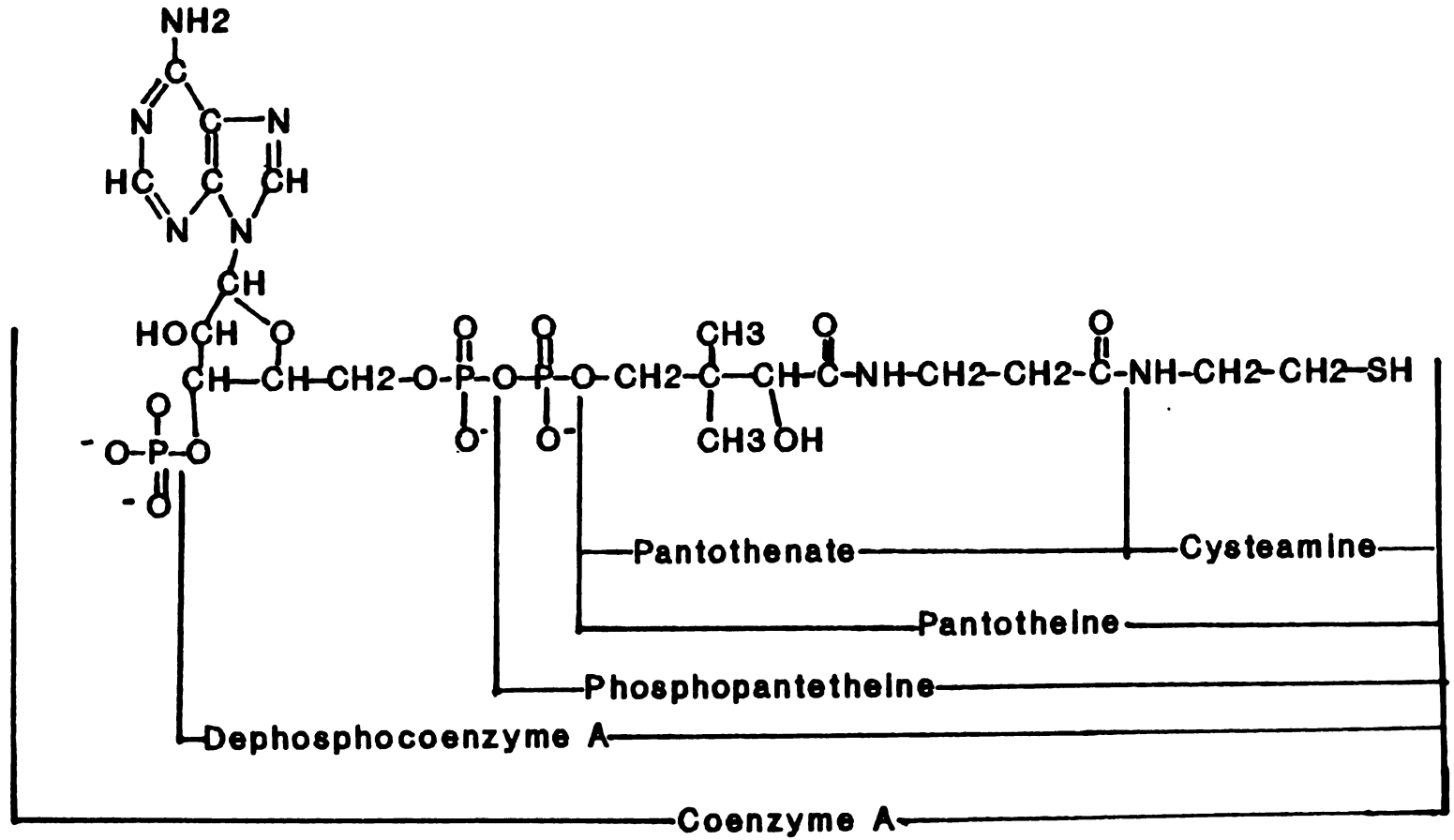


Figure 1. Stucture of CoA and its derivatives.

was developed by Wyse et al. (1979). RIA is based on the binding specificity of an antibody specific for pantothenic acid. RIA employs the use of expensive radiolabelled chemicals; however, more samples can be assayed within a given time when compared to microbiological methods. RIA measures the ability of the unlabelled antigen (pantothenate) to inhibit binding of the radioactive antigen by antibody (rabbit antiserum). The process is a simple competition in which antigen occupies a portion of the antibody binding sites reducing the free antigen available to the labelled antigen. Thus the pantothenic acid concentration of an unknown sample is determined by comparing the decreased labelled pantothenate binding it produces with a standard curve. However both methods can only measure free pantothenate. With enzymatic treatment, bound pantothenate can be liberated and therefore be measured for total amount of the free pantothenic acid content. Thus, bound pantothenic acid can be determine mathematically by simple subtraction of free pantothenate content from total pantothenate content.

PANTOTHENATE REQUIREMENT

Rats deficient in pantothenate exhibit reduced growth rate, loss of body weight, roughening and achromotrichia of the coat, muscle weakness, impaired adrenal function, inhibition of antibody formation, neural damage and

eventual death (Reibel et al., 1982). Hatano (1962) reported that weight loss and fur changes appeared only when urinary pantothenate excretion reached 1 ug/day compared to the control group's excretion of 95 - 118 ug/day. It took 18 days for weanling rats maintained on a deficient diet to reach this low level (of 1 ug/day) in urine.

Anorexia was observed by McCarthy et al. (1966) within the first week in mink kits fed a pantothenic acid deficient diet. Weight loss in these animals was noted consistently after the first week. The average weight loss was 40 - 60g/week and by week 8, fifty percent of the animals had died. The final body weight of the surviving animals at week 8 was half their original body weight. Other signs reported by McCarthy et al. included emaciation, dehydration, vomiting after water or food ingestion, and loose mucoid-like feces which changed to melena 6 - 9 days prior to death.

In human studies, pantothenate deficiency was established with use of an antagonist, omega-methyl-pantothenic acid along with a pantothenate deficient diet (Lubin et al., 1956; Hodges et al., 1958). Omega-methyl-pantothenic acid hastened the development of pantothenate deficiency and increased the severity of the abnormalities. Hodges et al. (1958) worked with three groups of human subjects: one group was the control and received 20 mg of Ca-pantothenate a day, the second group received diets

devoid of pantothenate, and the last group received diets devoid of pantothenate plus 750 mg - 1000 mg per day of omega-methyl-pantothenate. Subjects remained on the diet for nine weeks. In the third group, individuals exhibited personality changes, complained of fatigue, sleep disturbances and neurological disorders such as numbness, paresthesis and muscle cramps. Whereas, the other two groups reported no symptoms. Similarly, Lubin et al. (1956) reported in a study in which human subjects received a diet devoid of pantothenic acid plus 500 mg of omega-methyl-pantothenic acid per day for 4 weeks experienced depression, paresthesis, cardiovascular instability, neuralmotor disorder, muscle weakness, abdominal pains and frequent infections.

Pantothenic acid has a growth promoting effect independent of its role through CoA (Reibel et al., 1982; Henderson et al., 1942; Lotspeich, 1950). Reibel et al. reported that male Sprague-Dawley rats weighing 250 - 300 grams maintained on a pantothenic acid deficient diet did not grow as well as the control at week four of the experiment. These deficient adult rats, however, had maintained CoA concentration at normal levels although free pantothenate levels were depressed in heart, kidney, gastrocnemius, liver, and testes at the fourth week of their experiment.

Lotspeich (1950) also demonstrated that rats fed a

pantothenic acid deficient diet produced a "plateaued" effect on weight curves in adult female Sprague-Dawley rats when subjected to growth hormone injection as early as the second week on a deficient diet. Under normal conditions, a deficiency of pantothenate is reported to be difficult to produce in adult rats. A dramatic weight gain resulted after an intraperitoneal injection of 1.0 mg of Ca-pantothenate per day for four days into the rats (Lotspeich, 1950).

The role of pantothenic acid to growth was further tested by Krehl (1954). He noted that as rats matured and reached adulthood, the requirement for pantothenate was greatly reduced. He demonstrated this by placing young hypophysectomized rats on pantothenic acid deficient diet. Doing so, the rats were deprived of growth hormone as well as other trophic hormones. These animals did not show any sign of deficiency. In fact they remained "immature, non-growing rats". Thus, when the impetus to grow was removed, the requirement for the vitamin decreased. This could also explain why it is difficult to produce a deficient state in adult animals.

Henderson and co-workers (1942) were able to raise four generations of rats using ration 789 (containing all known vitamins including 20 mg Ca-pantothenate/kg diet) without difficulty. These rats reached maturity without optimum growth but were able to reproduce. Therefore, Henderson et al. (1942) concluded that the requirement for

pantothenic acid may be lower than the 20 mg Ca-pantothenate/kg diet supplemented to 789 ration. In addition, Henderson et al. (1942) suggested that the requirement for pantothenic acid may be lower when the diet included all the other essential vitamins.

Pantothenate was also reported to prevent graying in pigmented rats. In another study by Henderson et al. (1942) black rats, 21 days old, fed a pantothenic acid deficient diet became gray in 4 - 6 weeks. A daily oral supplemention of 40 ug per day was sufficient to prevent or cure achromotrichia in most of the rats. Others report higher levels, 80 - 500 ug, of Ca-pantothenate to prevent achromotrichia (Unna, 1940; Ansbacher et al., 1941). A ruffling and rusty discoloration of albino rats were reported as early as second week when 13 rats were fed a deficient diet (Hatano, 1962). Prevention of pigment loss in deficient rats was also possible if the rats were adrenalectomized (Ralli et al., 1944). Hair growth and melanin deposition were suppressed in the adrenalectomized rats when rats were injected with deoxycorticosterone acetate. Dumm et al. (1952) support this concept. Hypophysectomy produced an atrophy of the adrenal cortex and was associated with an increase in pigmentation or decrease in graying for black rats.

Pantothenate alone, however, may not be sufficient in curing graying. Lunde and Kringstad (1939) reported that pantothenic acid was distinct from the "anti-gray hair

factor" since pantothenate alone was not effective against graying. Oleson et al. (1939) agreed with this theory and suggested that a "second filtrate factor" was necessary for normal hair color but did not specify what the second factor was.

PANTOTHENIC ACID DEFICIENCY: EFFECTS ON HISTOLOGY OF TISSUES

Hatano (1962) investigated the effects of pantothenate deficiency on the histology of various organs with male Winstar rats (weighing an average of 45 g) starting at the age of 28 days. The liver of the rats on a deficient diet (<8 ng pantothenate/kg diet) for 32 days had areas of swollen cells which was not due to fatty infiltration. The glycogen content in liver was also reported to be decreased, though values were not reported by Hantano. These histological findings in liver of deficient rats were similar to those seen in riboflavin and pyridoxine deficiency. Thus, it will be inaccurate to identify pantothenate deficiency with these histological changes alone. Hatano did not examine if the changes were reversible when deficiency was not severe. The lungs of "most all" (n=4) deficient rats in Hatano's study were characterized by inflammation similar to prolonged bronchopneumonia, pulmonary alveolitis, and bronchitis. The adrenal glands showed irregular cellular arrangement of the zona fasciculate in the deficient group. The adrenal cortex appeared to be hyperactive. Contrarily, Deane and

McKibbin (1946) reported that deficient rats had adrenal depleted of ketosteroid material and also appeared enlarged and solid black due to hemorrhage. In addition, the adrenal cortex was characterized by loss of lipid. The signs of adrenal damage were magnified with the administration of adrenocorticotrophic hormone (ACTH) in the deficient animals (Winters et al., 1952). Cortisone appears to have a protective influence on the adrenal. With cortisone treatment, the adrenal of the deficient rats were indistinguishable from those of the normal control rats.

Hatano (1962) also reported that there was a primary decrease in spermatogenesis in the pantothenic acid deficient rats. The deficiency group exhibited atrophic seminiferous tubules and reduced number of seminal epithelia. Hatano demonstrated that spermatogenesis can be repaired, within 2 weeks, in the pantothenate deficient rats by adding pantothenate in the diet (feeding a control diet of 40 mg pantothenate/kg diet). Other tissues/organs affected by a pantothenate deficiency were also reported by others. McCarthy et al. (1966) observed stomach ulceration and hemorrhage in minks on a deficient diet for eight weeks.

PANTOTHENIC ACID DEFICIENCY: PANTOTHENATE CONTENT IN TISSUES, ORGANS AND BLOOD

The distribution of pantothenate has also been examined by Hatano (1962) to understand pantothenate metabolism. He determined the total and free pantothenic acid content

in liver, kidney, lung, spleen, brain, heart, testes, thymus, and whole blood of young (28 day old) male rats by microbiological assay method using Lactobacillus arabinosus (Figure 2). Total pantothenate was determined by breaking down the bound forms to the free form by enzymatic treatment with Lipmann's original enzymatic solution and then assayed for total free pantothenate. Lipmann's original enzymatic solution consisted of pigeon liver extract, 2% intestinal phosphatase solution and 1 M Tris hydroxymethylaminomethane (pH 8.3) mixed in a proportion of 2:1:1.

In Hatano's study (1962) 28 day old rats (4 rats per group) were fed either a pantothenate deficient diet or a control diet containing 40 mg pantothenate/kg diet for 32 days. Pantothenate deficient basal diet used in Hatano's study was a purified diet containing 67.8% sucrose, 17.0% casein and less than 8 ng pantothenate per kg of diet. It was noted in both groups that the highest total pantothenate concentration, in ug/g tissue, was found in the liver. Kidney, lung, and adrenal contained the next highest concentration of the vitamin. The rats fed the pantothenate deficient diet for 4.5 weeks showed a 94% reduction (37.5 ng/ml) from the control value of 615 ng/ml of pantothenate in the blood. Other reduction of pantothenate was also noted in lung, adrenal, testes, heart, and liver. Urinary pantothenate values dropped markedly from 95 - 118 ug pantothenate per day to 1 ug per day within 18 days fed

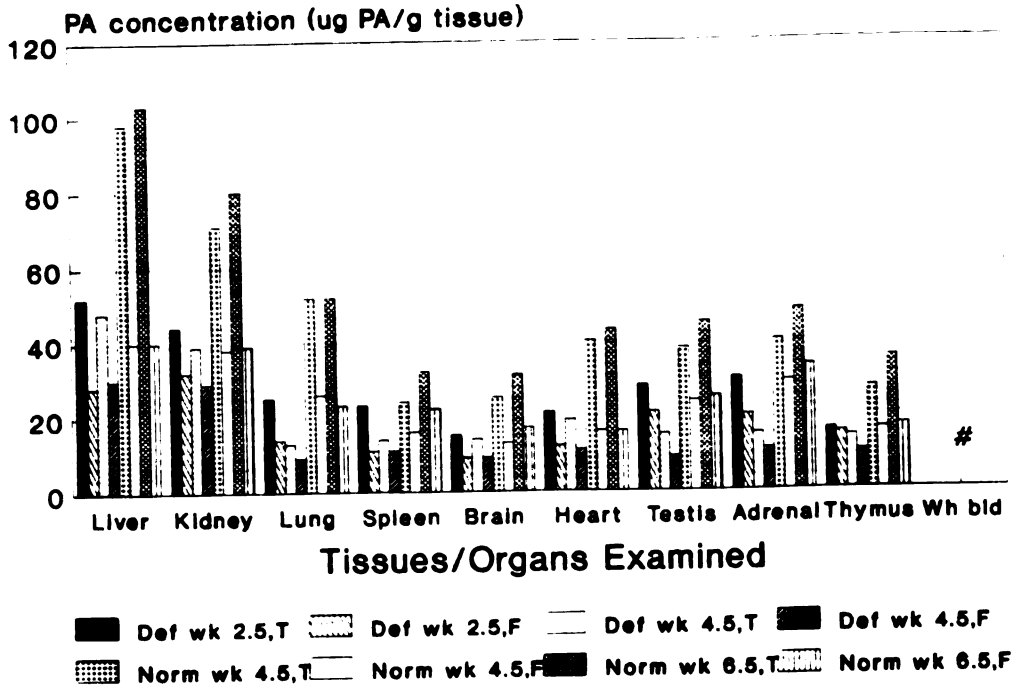


Figure 2. Reported results of pantothenate concentration in rat tissues and organs.
Data from Hatano (1962). Total (T) and free (F) pantothenate concentration of rats fed deficient pantothenate diet (def gp) at 2.5 and 4.5 weeks and normal diet (+40mg Ca-Pantothenate/kg diet) for 4.5 weeks and 6.5 weeks. Values represent mean (std dev. not reported) for 4 rats per group. # Whole blood (wh bld) values reported for norm rats at wk 4.5 are T: 615 ng/ml, F: 550 ng/ml; at wk 6.5 are T: 650 ng/ml, F: 590 ng/ml.

the deficient diet. Advanced changes in the deficient group, such as significant weight loss when compared to the control group, were more evident by the end of five weeks (Hatano, 1962). Minks fed a pantothenate deficient diet for eight weeks appeared to be absent of fat especially in the coronary grove and kidney pelvis (McCarthy et al., 1966).

Since pantothenate is the precursor of CoA, it seems logical to assume that the level of one will correlate with the level of the other. However, this may not necessarily be the case. Tissue levels of CoA were found to be lower in weanling animals on a pantothenate deficient diet than adult rats on the same diet (Robinsharo and Needly, 1985). Adult rats fed a pantothenic acid deficient diet for 8 weeks had a reduced free pantothenate content by as much as 90% in the heart and 70% in the liver without ever affecting the level of CoA in these tissue. It may be possible that the need for pantothenic acid is decreased with adult animals because of slow growth and/or a larger pool of CoA. In young growing animals, tissue stores of pantothenate may not be adequate to support the high rate of CoA synthesis needed for development and therefore CoA level is affected along with free pantothenate level. Unna and Richards (1942) reported that the daily pantothenate maintenance requirement of the rats decreased from 100 ug at 3 weeks of age to 25 ug at 10 weeks. Maintenance of CoA level with simultaneously low pantothenate in the tissue

level during a deficient intake of pantothenic acid indicates that mechanisms other than dietary supply of free pantothenate may be responsible for control of CoA levels in mature animals. Karasawa et al. (1972) demonstrated that an excess intake of pantothenic acid (amount and duration of feeding not specified) did not increase the concentration of CoA in rat kidney or liver. However, the level of free pantothenic acid increased greatly (amounts not reported) in the kidney and liver.

Robinsharo and Neely (1985) reported that normal rats have the highest CoA contents in cardiac muscle and liver. Kidney, adrenal, and white skeletal muscle followed in concentration. The level of CoA in tissues in adult rats remained fairly constant.

In the study of Reibel et al. (1982), adult rats (250 - 300 grams) fed a pantothenate deficient diet for four week showed that the heart, kidney, gastrocnemius, diaphragm, adrenals, and testes contained only about 10% of the normal level of free pantothenate, and liver contained close to 30%. CoA content of the tissues and organs, however, was fairly similar between the deficient and normal groups for 8 weeks regardless of free pantothenate levels in tissues/organs. In addition, the level of free pantothenate at week eight was not significantly different from the values obtained at week four. Reibel et al. also examined if it was possible to increase CoA synthesis by fasting or inducing diabetes when

glucose was not available as the energy source. They used pantothenic acid deficient rats (consuming < 1 mg/kg diet) containing 10% of normal pantothenate in the heart and 30% in the liver as well as rats fed the control diet (supplemented with 50 mg/kg diet). They reported that heart and liver CoA content did increase in rats of both groups examined after a 48 hour fast or an injection with 60 mg alloxan/kg body weight to induce diabetes. They also reported that the pantothenate deficient rats were more responsive to the fast as well as diabetes than the control rats (an increase of 11-13 nmol pantothenate/g dry kidney tissue of deficient rats versus 10 nmol in the control). To determine the source of pantothenic acid for CoA synthesis, they examined kidney, gastrocnemius, testis, diaphragm, adrenal and serum of the rats fed the deficient and control diet. Pantothenic acid and CoA content increased in organs examined in the deficient group when fasted or diabetic. Whereas, no change in pantothenate or CoA except free pantothenic acid level in the kidney ($p<0.001$) was observed in the control rats. Thus, the source of pantothenic acid for the elevated CoA synthesis in the deficient pantothenate rats was not determined and could very well have come from a source not measured by the group. In addition, they did not specify housing condition and the possibility of coprophagia. Due to intestinal microbial synthesis of pantothenate, the practice

of coprophagy may result in extra source of pantothenic acid from feces.

Branca et al. (1984) demonstrated that pantetheine, not pantothenate, increase the total CoA content in the liver of 300 - 350g male Wistar rats. Liver perfusion was achieved by cannulation of the caval vein through the right atrium and the portal vein. Perfusion with one hundred uM pantetheine in perfusion medium increase CoA content by 20 - 30 percent. However, less than 100 uM of pantetheine did not increase CoA in the perfused medium. On the other hand, 100 uM of pantothenic acid in the same condition did not increase CoA content in liver.

Blood level of pantothenate in deficient rats showed significantly low level (Hatano, 1962). Hatano reported that feeding 28 day old rats with pantothenate deficient diet resulted in a reduction in blood value (43 ng total pantothenate/ml of whole blood) as early as the 18th day of the experiment. The blood value remained low for 32 days of the study; a reduction of 94% in total and 97% in the free pantothenate in the deficient group as compared to the control (37.5 ng total/ml and 15 ng free pantothenate/ml whole blood in the deficient rats versus normal values of 615 ng total and 550 ng free pantothenate/ml whole blood). When the deficient rats were supplemented, after 32 days, with 40 mg Ca-pantothenate/kg diet for 14 days, free pantothenate value in blood increased to level similar to that of the control (590 ng/ml compared

to the control of 550 ng/ml). However, the concentration of bound form of pantothenate was still reduced to a ratio of approximately 60% of the normal levels with no increase observed for the same period of supplementation. Hatano speculated that the reason for the bound form of pantothenate remaining low while the free form was similar to control rats' may be due to the heavy demand imposed on the bound (and active) form by various organs (Hatano, 1962).

Due to the wide variation cited in literature, controversy exists on the normal level of pantothenate in whole blood. Because of methodological difficulties and differences in microorganisms employed in the assay, the values for blood level of pantothenate for man range from 59 ng/ ml to 2622 ng/ml whole blood and 137 ng/ml to 1830 ng/ml for serum (Wyse et al., 1985). However, fasting whole blood and erythrocytes correlated significantly ($p<0.001$ using Pearson's correlation coefficients) with diet intake ($r=0.38$, 0.60 respectively) (Eissenstat et al., 1986). Only total pantothenate was determined in whole blood by the group, thus free pantothenate data was not available. Pearson (1941) has reported that pantothenic acid occurs in fairly equal concentration between plasma and cells in human. However the free form of pantothenate distribution varies with species. For instance, horse, human, rabbit and sheep was reported by Pearson as having a greater concentration of free pantothenate in the cells than plasma.

On the other hand, the opposite was observed in dogs and pigs. Pearson's study (1941) with rabbits showed a ratio of 1:1:1 in the distribution of free pantothenate in whole blood, plasma, and pack cells of controls. However, Pearson did not report any pantothenate blood values for rats which makes comparison of ratio to this study using rats difficult. Ono et al. (1974) reported that free pantothenate in rat blood comprised about 80% of total pantothenate.

DIETARY SOURCES OF PANTOTHENIC ACID: A NEED FOR CONCERN

Although pantothenate is available from many food sources, there may be a need for concern regarding consumption of enough pantothenate to meet the Estimated Safe and Adequate Daily Dietary Intake of 4 - 7 mg/day. Milk, chicken, potatoes, oats, tomato, whole grains are just a few of the items rich in pantothenate (Wyse et al., 1985). However, processed foods, foods made from refined grains, fruit products and fat contain low amount of the vitamin (Walsh et al., 1981). Eissenstat et al. (1986) calculated the dietary pantothenate intake from 4-day diet records of 63 adolescents. They reported 49% of the females and 15% of the males consumed less than 4 mg/day. The authors explained that the inadequate consumption was due to the poor food selection of the adolescents.

INTESTINAL MICROBIAL SYNTHESIS OF PANTOTHENIC ACID

There has been a speculation that pantothenate synthesized by intestinal microflora may contribute to the dietary requirement and reduce pantothenate requirement, therefore reducing the incidence of pantothenate deficiency. Henderson et al. (1941) determined in rats the amount of pantothenic acid ingested and excreted in the feces and found that the pantothenic acid in feces was almost independent of the diet. Twenty-one day old rats consuming a pantothenate deficient basal ration (contained 0.3 - 0.4 ug pantothenate/ kg diet) for six weeks excreted as much pantothenate per gram of feces as rats receiving the same diet but supplemented orally with $\geq$ 20 ug Ca-pantothenate/day. However, Henderson et al. did not report on the amount of diet consumed by the rats per day. The amount of diet consumed is influential to the amount excreted in the feces. The assumption that most of the pantothenate excreted in the feces was synthesized by intestinal bacterial action in cecum was also tested by Henderson et al. (1942) using three deficient rats. They found that intestinal content between stomach to cecum contained 1.5 - 5 ug of pantothenate. A large portion of fecal pantothenate arise between the cecum and colon (25 - 40 ug). However, they speculated that the pantothenate produced may not be available to the rat in an appreciable amount since deficient rats developed symptoms and even death, regardless of the amount of pantothenic acid present

in intestine and excreted in feces. However, researchers should be aware of the practice of coprophagia by rats and its possible influence on the study.

ABSORPTION OF PANTOTHENIC ACID

Pantothenate is present in foods mostly as CoA which cannot be directly absorbed through mucosal cells. Turner and Hughes (1962) investigated the absorption of CoA with everted sacs of rat intestine. They traced the path of CoA (8 x 10-5 M) which was incubated for an hour in the mucosal fluid and determined the amount of the coenzyme remaining on the mucosal side and amount appearing on the serosal side. Their results, as determined by microbiological assay using _Lactobacillus arabinosus_, showed that 42 - 71% of the CoA remained on the mucosal side and only 1 - 2% appeared on the serosal side after an hour. Most of the remaining CoA (8 -31%) appeared in serosal side as pantothenic acid. The fact that 100% of CoA was not accounted for in Turner and Houghes' study (1962) may be due to the low sensitivity of the microbiological assay. This means that the intestinal mucosa converts CoA to free pantothenate which is then passed to the serosal side. It is possible that CoA was converted to any intermediates such as pantothenine, which was not assayed for. Intestinal tissues contain a high level of alkaline phosphatase and pantotheinase which can hydrolyze CoA to pantetheine or pantothenate (Fenstermacher,

1987). In addition, the 1 -2% of CoA found on the serosal side might have been resynthesized in mucosal cells, absorbed intact without hydrolysis, or represented the range of experimental error.

Shibata et al. (1983) used rats to study the absorption of pantothenate by using radiolabelled CoA in vivo. They reported that the absorption occurred by simple diffusion. They suggested CoA was hydrolyzed in the intestinal lumen by the following reactions prior to its absorption in the form of pantothenate:

CoA--->dephospho-CoA--->4'-phosphopantetheine--->
pantetheine--- >pantothenate + cysteamine

Shibata et al. demonstrated that within 2 - 3 hours after the injection of C-14 labelled CoA into the intestine, 87% of the isotope appeared in the blood as pantothenic acid. The values are different from Turner and Hughes' (1962). This could simply be due to the assays employed, time of incubation, used of everted sacs versus live animal and labelled versus non-labelled CoA. There is, however, no experimental evidence to date, in human, on how CoA is digested and absorbed.

In an attempt to understand the metabolism and distribution of pantothenate among organs, Shibata et al. (1983) injected 110 nmol of [C-14] pantetheine (0.41 uCi) in 1.0 ml of 0.9% NaCl into the duodenal lumen of male Sprague-Dawley rats weighing 200 - 400 g. Five hours later,

they reported that 34.7% C-14 was largely taken up by muscle and 12.7% by liver.

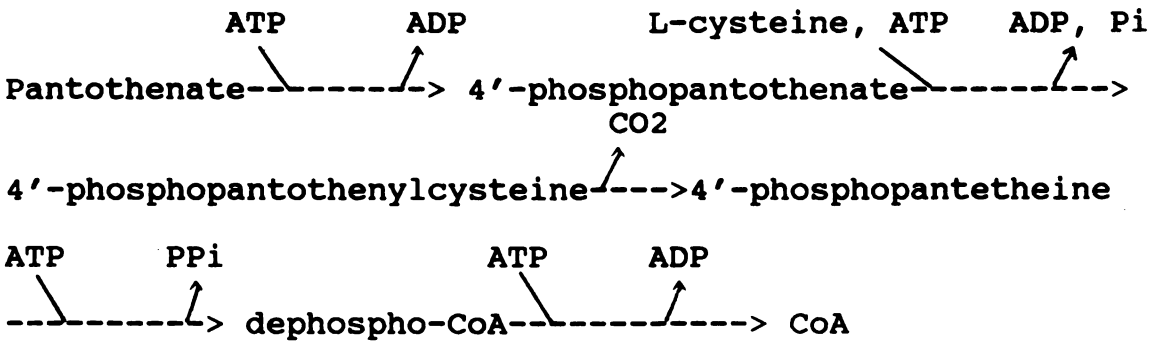
Hatano (1962) also examined the fate of subcutaneously injected pantothenic acid in rats previously fed pantothenate deficient or sufficient diet. Rats in the control group consumed a diet of 40 mg Ca-pantothenate/kg diet and the deficient group rats were fed a diet containing less than 0.8 ug/kg diet. Both groups were maintained on the respective diet for 32 days starting at 28 days after birth. Upon loading via subcutaneous injection of 4 mg Ca-pantothenate into the normal control rats, urinary elimination of pantothenate over the following 72 hour period was increased to 2136 ug/72 hours from the normal of 285 - 354 ug/72 hours, before the load. In the deficient group, the same dose subcutaneous injection caused a similar increase, however, it was about 1900 ug/72 hours or 10% less than that of the control.

The effect of a massive doses (500 mg Ca-pantothenate) on blood and the distribution between whole blood, plasma and blood cells was examined in rabbits by Pearson (1941). Rabbits were examined 60, 75, and 90 minutes after injection and only 90 minutes after ingestion of 500 mg Ca-pantothenate. Sixty minutes after injection, whole blood level of pantothenate increased 114 fold (6400 ug/100ml) from control. Coincidentally, the ratio of plasma pantothenate increased to 5.17 from normal ratio of about 1.0 (range: 0.81 - 1.29). Seventy-five minutes after

injection, blood drawn from the rabbits showed a even greater increase (10,000 ug/100ml) in whole blood. The plasma/blood cell ratio had increased to 12.00. However, ninety minutes after ingestion of 500 mg of Ca-pantothenate, the whole blood pantothenate concentration increased only 10-fold over the normal level of 48 ug/100 ml. Also at this time, most of the excess pantothenate was located in the plasma (680 ug/100 ml from normal level of 50 ug/ml). The maximum rate of excretion of pantothenate in urine occurred during the first two hours after ingestion of the massive dose. The level in the urine was maintained fairly high for approximately 72 hours of collection. Amount excreted was similar for rabbits injected or ingested 500 mg Ca-pantothenate (296 mg/24 hours, 211 mg/24 hours, respectively).

SYNTHESIS OF CoA

Lipmann and Kaplan (1948) reported that CoA is synthesized in rat liver cells from pantothenic acid as foll ws:



The mechanisms regulating CoA synthesis have not been completely elucidated. CoA synthesis may possibly be regulated by dietary supply of pantothenate, uptake of the vitamin by tissue, or combination of nutritional and hormonal factors such as fasting or diabetes as demonstrated by Reibel et al. (1982).

FUNCTIONS OF CoA

CoA is required in many different metabolic reactions as a transient carrier of acyl groups through a reactive thiol group (Lehninger, 1982). During acyl-group transfer reactions, the thiol group is covalently linked to an acyl group to form thioesters. For example, CoA functions as an acyl acceptor for pyruvate dehydrogenase complexes and alpha-ketoglutarate dehydrogenase complexes in the citric acid cycle. It then forms acetyl CoA and succinyl CoA, respectively.

Acetyl-CoA is an important intermediary metabolite. It is formed in the first reaction of oxidative decarboxylation of pyruvate. Fatty acid can also yield acetyl-CoA via beta oxidation. Other sources of acetyl-CoA are the carbon skeleton of ten amino acids (alanine, threonine, glycine, serine, cysteine, phenylalanine, tyrosine, leucine, lysine, and tryptophan). These acetyl-CoA can go through the citric acid cycle where the acetyl group of acetyl-CoA can then be transferred to oxaloacetate to yield citrate. The acetyl-CoA can also be used as a

carbon source in fatty acid synthesis or incorporated in cholesterol, steroid hormones, prostagladins synthesis.

TOXICITY OF PANTOTHENIC ACID

Schwartz and Bagdon (1964) examined the acute toxic effects of pantothenate derivatives. They injected large intramuscular doses (2.0g/kg and 0.2 g/kg) of sodium pantothenate, d-panthenol, and d-pantetheine, separately, into two groups of Sprague-Dawley rats: one group weighed 110 - 120 g, the other weighed 225 - 275 g. The injections of all derivatives, when examined 96 hours after administration, did not significantly affect body weight, liver weight, nor produce evidence of hepatotoxicity. Hepatotoxicity was determined by histology stained sections with hematoxylin-eosin or hematoxylin-sudan IV. However they did notice a slight but not significant increase in liver lipid deposition in rats weighing 110 - 120g when given either 0.2g or 2.0g of d-panthenol per kg body weight. But this was not observed in heavier rats weighing 220 - 275 g (Schwartz and Bagdon, 1964). Alhadeff et al. (1984) reported that, though pantothenate has minimal toxicity in human, intake of 10 - 20 g per day may lead to diarrhea and water retention.

MATERIALS AND METHODS

PRELIMINARY EXPERIMENTS

Two preliminary experiments were carried out to establish valid methodologies. The purpose of the first experiment was to determine the optimal activities of enzymes necessary to release all bound forms of pantothenate to free form in various organs. The purpose of the second experiment was to determine the extent of conversion of bound pantothenate to free form in various sample preparation methods.

Due to different concentrations of bound pantothenate and endogenous enzymes activities of each organ, we decided to determine the enzyme activity levels for each organ sample to maximally release bound pantothenate for the assay of total pantothenate. To achieve the stated objective, various organs were excised from normal rats. Pantetheninase (provided by Dr. Carl Wittwer, Univ. of Utah), in varying activities (0 to 60 units) was added to an organ homogenate prepared from approximately 0.5 grams of wet organ. Simultaneously 0 to 30 units of alkaline phosphatase (type VII-S, P-5521, Sigma Chemical Co.) were also added. The samples were incubated (37o C) over night (10-15 hr), then deproteinized with equimolar of saturated Ba(OH)2 and 10% ZnSO4 and later analyzed for content of

pantothenate. We found that a combination of 15 units of alkaline phosphatase and 30 units of pantetheninase resulted in the maximal release of pantothenate in all organs. Additional amount of enzymes to the sample did not increase the amount of pantothenate released.

Many organs contain alkaline phosphatase and pantothenase which release pantothenate from its bound derivatives. It was therefore speculated that post-mortem autolysis of bound pantothenate from compounds such as CoA to pantothenate would occur. Many studies have reported the content of free and bound forms of pantothenate in various organs, however, the extent of autolysis in various organs which may have occurred prior to quantification has not been reported in these studies. The objective of this preliminary experiment was to determine the extent of autolysis of bound pantothenate to free pantothenate at time 0, 1, 2, 6, 12 and 24 hours when organ samples were held on ice and also after 24 hours, 48 hours, and 2 weeks when samples were frozen (-4.4o C). Fresh liver was excised from two 7 week old female Sprague-Dawley rats weighing an average of 126 g and maintained on a stock diet (Wayne Lablox) ad libitum. Liver was chosen because it is the largest organ found in the rat that many samples could be prepared from. Rats were anesthetized with ether and sacrificed by bleeding via cardiac puncture. Each fresh liver was rinsed in saline and divided into equivalent size (approximately 0.5 gram each) for analysis at different

intervals. Liver samples were kept on ice bath or samples frozen and homogenized at the intervals described earlier and analyzed for both free and total pantothenate. Results from this experiment showed that a minimal conversion of bound pantothenic acid to free pantothenic acid occurred before 2 weeks of storage at -4.4o C. Therefore, in the main study, samples were homogenized and prepared within 2 weeks to eliminate possible conversion of bound pantothenate to free pantothenate.

RESEARCH DESIGN

Forty-eight weanling (21 day old) Sprague-Dawley male rats (Harlan-Davis, Indianapolis, IN) weighing an average of 42g were housed in individual hanging stainless steel cages. Upon receipt, the animals were weighed and maintained on Wayne Lablox nonpurified stock diet ad libitum for 24 hours. The rats were maintained on a schedule of 12 hours light, 12 hours dark and at a controlled temperature (20-22o C). Animals were randomly divided into four groups (12 per group) to receive for maximum of 8 weeks: 1) group A, AIN-76 purified diet (U.S. BioChemical, see Table 1, 1a, 1b) with an excessive amount of pantothenate (+120 mg d-Ca-pantothenate /kg diet), ad libitum; 2) group B, an adequate control diet (+12 mg d-Ca-pantothenate/kg diet), ad libitum; 3) group C, a pantothenate deficient diet (+0 mg/kg diet), ad libitum; and 4) group D, an adequate control diet, pair-fed with

Table 1. Purified pantothenate deficient basal diet composition (AIN -76TM, U.S. BioChem Co.)

Ingredient	%
Casein (vitamin free)	20.0
DL-Methionine	0.3
Corn starch	15.0
Sucrose	50.0
Fiber	5.0
Corn oil	5.0
AIN mineral mix*	3.5
AIN vitamin mix** (without pantothenate)	1.0
Choline bitartrate	0.2

* see Table 1a
** see Table 1b

Table 1a. AIN-76 TM mineral mixture

Ingredient	g/kg
Calcium phosphate, dibasic	500.00
Sodium chloride	74.00
Potassium citrate, monohydrate	220.00
Potassium sulfate	52.00
Magnesium oxide	24.00
Manganous carbonate	3.50
Ferric citrate	6.00
Zinc carbonate	4.60
Cupric carbonate	0.30
Potassium iodate	0.01
Sodium selenite	0.01
Chromium potassium sulfate	0.55
Sucrose	to make 1000.00

Table 1b. AIN-76 TM vitamin mixture

Vitamin	per kg mixture
Thiamin- HCl	600mg
Riboflavin	600mg
Pyridoxine-HCl	700mg
Nicotinic acid	3000mg
Folic acid	200mg
D-Biotin	20mg
Cyanocobalamin	1mg
Vitamin A	400,000IU
Vitamin E	5,000IU
Vitamin D-3	2.5mg
Vitamin K	5mg
Sucrose	to make 1000g

group C. Adequate pantothenate has been recommended by the National Academy of Sciences-National Research Council as 8.0 mg Ca-pantothenate/kg diet (National Academy of Sciences, 1972) while the American Institute of Nutrition recommended 16 mg Ca-pantothenate/kg diet for laboratory rats. Thus as a compromise of the two recommendations, 12 mg Ca-pantothenate/kg diet, was used in this study. Tap water was readily available for all rats.

DATA COLLECTION

Food intake was measured weekly except for the pair-fed rats and those fed the deficient diet. Intake for each deficient rat was measured daily so that the same amount of pantothenate adequate control diet could be fed to its pair-fed rat the next day. Spillage of food was collected and accounted for in calculations of food intake. Body weight was also measured weekly. Clinical observations such as hair loss, hair color change, behavior changes were made and recorded by the main investigator daily for the entire study. However no measurement of these clinical symptoms was performed.

Five days prior to sacrifice, at week 2, 5 and 8, four rats/group were housed individually in stainless steel metabolic cages for collection of urine for two 24 hour periods. At week two of the experiment, however, only one 48 hour sample was collected from each rat because of the small volume of collectable urine. An antibacterial agent,

1% thimersol (500 ul), was added into each glass collection vial of urine collected daily. The complete 24 or 48 hour samples were diluted to 50 ml with deionized water and stored frozen until analysis.

The same four rats per group were fasted overnight then anesthetized with ether before sacrifice by bleeding via cardiac puncture. Blood was collected into vacutainers containing ethylenediaminetetraacetate (EDTA). Freshly collected blood was later divided into two portions: one portion was separated into plasma and packed cells by centrifugation at 4000 rpm for 10 minutes and the other left as whole blood.

COLLECTION AND TREATMENT OF ORGANS

Organs (heart, spleen, kidneys) were excised, rinsed in normal saline, weighed and frozen (-4.4o C) until ready to be analyzed. Approximately 0.5 grams of wet organ samples were used for analysis of pantothenate. Since there were two kidneys from a rat, each kidney (including the capsule) was weighed and used separately. One entire kidney was used for determination of free and the other for total pantothenic acid. This procedure was necessary because in a preliminary study we found that pantothenate was not equally distributed within the kidney. We further tested this by removing the three major parts of the kidney: cortex (including the capsule), medulla and pelvis, with the aid of a magnify glass, and analyzed pantothenate content.

We discovered that pantothenate concentration differed in these three major parts of the kidney. As shown in Figure 3, the cortex and pelvis contained a larger concentration of pantothenic acid than the medulla. Interestingly, the ratio of bound to free was lower in the cortex compared to the medulla and pelvis.

Both free and bound pantothenate were measured in organs and blood using RIA. In urine and plasma only the free form was estimated because the bound form was not found. The pre-weighed organs (approximately 0.5 grams) were later homogenized with distilled water (1000 ul for all organs) using a Polytron (Brinkmann Instruments Co., type PT 1020 3500) at speed 3.5 for one minute for all organs. For free pantothenate determination, the organ homogenates were immediately deproteinized with approximately 0.5 ml each of equimolar amounts of saturated Ba(OH)2 and 10% ZnSO4. Equimolar ratio was determined by titrating 10 ml of 10% ZnSO4 diluted with an equal volume of distilled water against saturated Ba(OH)2 with phenolphthalein as an indicator. The homogenate was then centrifuged (Sorvall Superspeed RC2-B Automatic Refrigerated centrifuge set at 0o - 5o C) at 4000 rpm for 10 minutes and the resulting clear supernate was separated and frozen until used in the radioimmunoassay. For determination of total pantothenate in organs, the homogenized organ (approximately 0.5 gram of wet organ) was incubated at 37o C with 15 units of bovine intestine alkaline phosphatase,

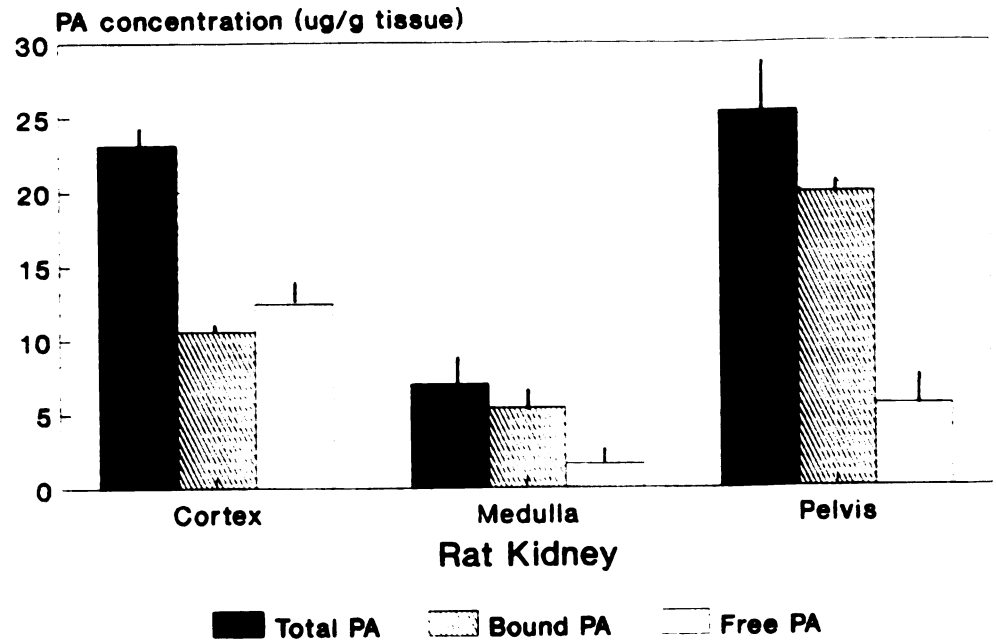


Figure 3. Pantothenate content in cortex, medulla and pelvis of rat kidney. Total and free pantothenate analyzed via RIA from 2 normal adult female rats. Bound pantothenate determined by [total]-[free]. Values represent mean ± std dev.

type VII-S (P-5521, Sigma Chemical Co.) and 30 units of pantetheinase (Dr. Carl Wittwer, Univ. of Utah). Deproteinization of the digested homogenate was in the same manner as for free pantothenate determination as described earlier.

Whole blood sample was hemolyzed via three quick thaw/freeze cycles. For free pantothenate determination, fifty ul of the hemolyzed blood was immediately deproteinized with equimolar saturated Ba(OH)2 and 10% ZnSO4. Blood samples for total pantothenate determination were subjected to the enzyme treatment of 20 units of pantetheinase and 10 units of alkaline phosphatase following hemolysis and incubated overnight for release of the bound form (amount determined previously in a preliminary study in our lab). After incubation, the blood samples were deproteinized as described earlier and analyzed for total pantothenate. Blood samples for free pantothenate were deproteinized as described earlier and analyzed via RIA.

Plasma was deproteinized in the same fashion and analyzed for free pantothenate only. Plasma from pair-fed control rats at week 8 of the study was not analyzed due to collection of an inadequate amount of whole blood. Only the whole blood was used for analysis. Urine samples, diluted with distilled water, were analyzed for free pantothenate by RIA. All supernatants and samples were completely thawed before analysis by RIA.

PANTOTHENIC ACID DETERMINATION

Pantothenate was determined by radioimmunoassay (RIA) as described by Wyse et al.(1979). Fifty ul of supernat (of organ, whole blood, plasma or urine) was pipetted into a vial (5 ml polypropylene, Denville Scientific, Inc., #V9951). To each vial, two hundred-fifty ul of 1.2% rabbit serum albumin in phosphate buffered saline (PBS) solution mixed with antisera. Rabbit antisera were added to the vial with ranges from 1:100 - 1:35 dilution to obtain the desired binding of 30-50% in the blank. Approximately 5000 dpm of labelled 14-C pantothenate (Sodium [1-C14]-D-pantothenate, specific activity of 3.7 Ci/mole, from New England Nuclear, Boston, MA) was also pipetted to each mixture. The final mixture was agitated and incubated for 15 minutes at 37o C. To each vial, three hundred-twenty ul (a volume equivalent to the sum of supernat, rabbit serum albumin in PBS, antisera and radiolabelled pantothenate) of saturated ammonium sulfate were added to result in a 50% saturated ammonium sulfate in the final medium. After thorough mixing by vortexing each vial, the vials were centrifuged (Sorvall Superspeed RC2-B Automatic Refrigerated Centrifuge set at 0o - 5o C) at 68,000 x g (or 85,000 rpm) for 15 minutes. The supernatant was then suctioned off leaving the precipitate pellet and, again, 500 ul of 50% saturated ammonium sulfate was added to the precipitate pellet, resuspended and centrifuged at 68,000 x g for 15 minutes. Supernatant was again suctioned off. Tissue

solubilizer (soluene 350, Packard Instrument Co, Inc., #6003038) was then added (30 ul) to the pellet remaining in the sample vial. Vials were allow to sit in 60o C water bath to for 30 minutes to enhance dissolving of the pellet. Scintillation cocktail (Safety-Solve, Research Product International Corp., #111177), in the amount of 3.5 ml, was added to the vials for counting in liquid scintillation. Radioactivity of the samples was read by the Packard 4000 series liquid scintillation counter (Packard Instrument, Inc.) for five minute per vial. Standards used for assays ranged from 0 to 350 ng d-Ca-Pantothenate. The standard curve was constructed by plotting the percent bound as a function of ng pantothenic acid on log-probit paper.

STATISTICAL ANALYSIS

Statistical analyses of the data were performed using two-way analysis of variance (ANOVA) to test for treatment effect, time effect, and the interaction effect between the ad lib control and the deficient group, and the pair-fed control and the deficient group. The variables examined were diet intake, growth, organ weight, organ pantothenate concentration (total and free), whole blood (total and free), plasma and urine (free only) pantothenate concentration. The difference between the treatments was further tested by Dunnett t-test selecting the deficient group as the control group to be compared to the ad lib

control and the pair-fed control. Correlation within the deficient group of organ pantothenate concentration with bodily fluid was calculated using product-moment correlation. The predictive equation for each organ was estimated using bodily fluid variables whose simple correlation with each organ was greater than "0.3". The predictive equation for bodily fluid was estimated using variables which displayed the highest simple correlations with bodily fluid.

RESULTS

Rats fed the diet deficient in pantothenic acid (group C) exhibited pantothenate deficiency signs similar to those reported by others (Hatano, 1962; Henderson et al., 1941; Reibel et al., 1982). The deficient rats in this study demonstrated anorexia and consumed less (in grams per day) than the ad lib control (group B) or the group fed 120 mg pantothenate per kg diet (group A). The deficient rats also failed to grow as well as their counterparts fed a pantothenate supplemented diet ad lib. In addition, the deficient group rats also experienced occasional nasal discharge of blood-like fluid. Because albino Sprague-Dawley rats were used in this study, graying of the hair was not detected. However a rusty tinge was noted in the deficient rats by the 3rd week of the study and they lost more hair. The other groups exhibited no overt abnormalities.

There was a significant time and diet effect ($p<0.01$) on daily intake of the rats in the study. The average daily intake of the deficient group was significantly ($p<0.01$) lower than ad lib control as early as week 2 of the study. The rats of deficient group consumed a range of 7 to 11 grams of diet per day over the eight weeks of the study, whereas rats of ad lib control consumed 11 to 22

grams of diet per day as shown on Figure 4.

Growth, measured by weight gain, also showed time, diet as well as the interaction effect. Weight gain of the deficient rats, were significantly lower ($p<0.05$) than the ad lib control rats at week 4 to week 8 of the study as shown in Figure 5 (group B weight: 197 ± 75; group C weight: 122 ± 34). Over all, the pair-fed control (group D) rats gained more weight than the rats of the deficient group which consumed the same amount of diet but absent of pantothenate. However the weight difference between the deficient group and pair-fed control was not statistically significant using the Dunnett t-test.

The rats fed a pantothenate deficient diet had lower organ weight in the heart, spleen and kidney than the ad lib control as shown on Table 2. Significant difference ($p<0.05$) in heart weight between the deficient and the ad lib was observed at weeks 2 and 8 but not 5. Spleen weight was significantly different ($p<0.01$) between the deficient and the ad lib control at all time points. Difference in kidney organ weight was significant ($p<0.10$) near the end of the study (weeks 5 and 8) between the deficient and the ad lib control. However, there were no significant differences in organs weights when presented as percent body weight among the different groups of the organs examined.

Overall, the rats in group fed 120 mg Ca-pantothenate per kg diet (+120 mg/kg diet) grew comparable to ad lib

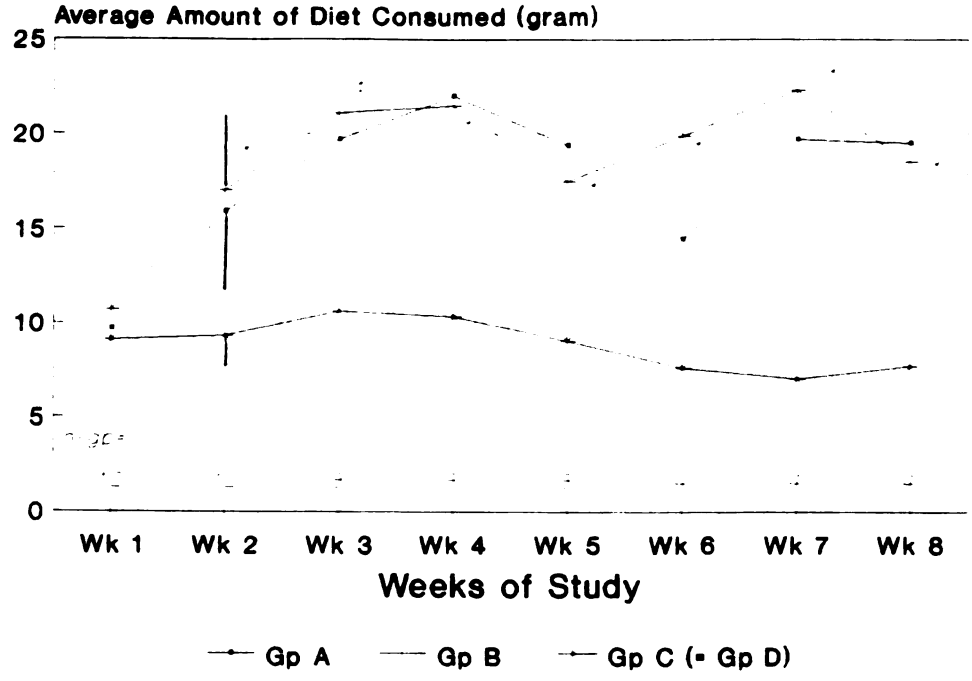


Figure 4. Diet intake of rats.

Group A (Gp A) rats were fed 120 mg Ca-pantothenate/kg diet ad lib, group B (Gp B) rats were fed the ad lib control diet, ad lib, and group C (Gp C) rats were fed the pantothenate deficient diet. Same amount consumed by group C was pair-fed to rats of group D (pair-fed control) with the control diet. Values represent mean $\pm$ SEM. Statistical significance between Gp B and C: * = $p<0.001$.

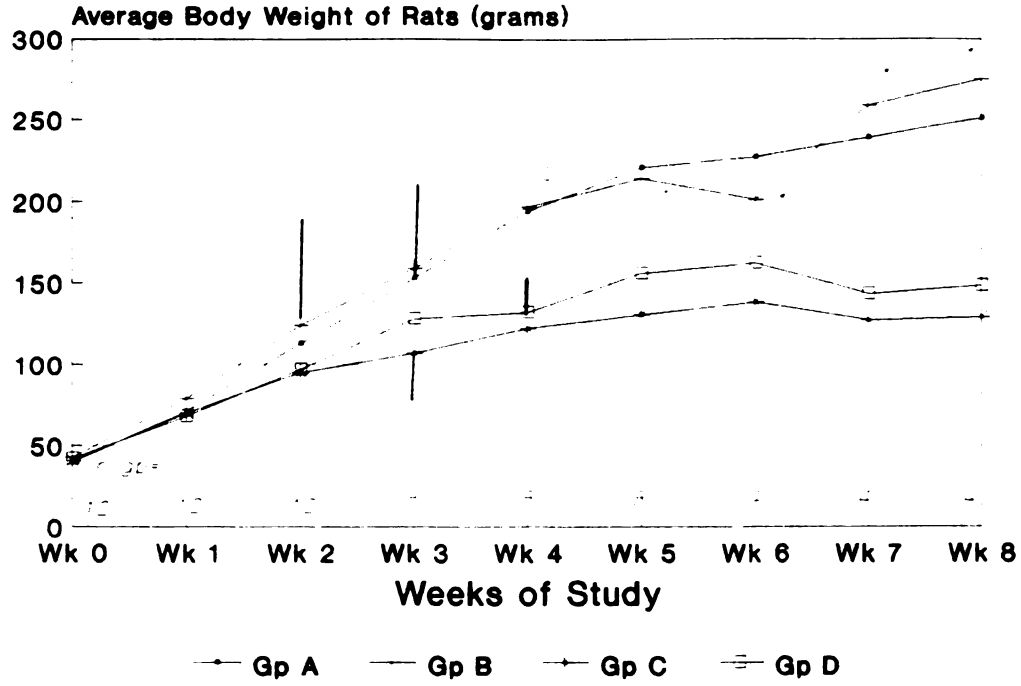


Figure 5. Growth pattern of rats.
Weight gain of group A (Gp A) rats fed 120 mg Ca-pantothenate/kg diet, ad lib, group B (Gp B) rats fed the ad lib control diet, ad lib, group C (Gp C) rats fed the pantothenate deficient diet, ad lib, and group D (Gp D) pair-fed the control diet. Values represent mean $\pm$ SEM. Statistical significance between Gp B and C: o = $p<0.10$, * = $p<0.05$.

Table 2. Total weight and percent of body weight of rats in the study.

Group A1	Group B	Group C	Group D
HEART: gram weight (percent body weight)			
Wk 2			
0.53±0.20 (.005±.0006)	0.70±0.17* (.006±.002)	0.41±0.11 (.004±.0005)	0.41±0.09 (.005±.0004)
Wk 5			
0.87 (.004)	0.76 (.004)	0.55 (.005)	0.51 (.004)
Wk 8			
0.98 (.004)	0.98* (.004)	0.64 (.005)	0.59 (.004)
SPLEEN			
Wk 2			
0.44±0.08 (.004±.0009)	0.46±0.10** (.004±.0007)	0.27±0.05 (.003±.0004)	0.33±0.07 (.004±.0007)
Wk 5			
0.58 (.003)	0.50** (.003)	0.28 (.002)	0.30 (.002)
Wk 8			
0.55 (.002)	0.57** (.002)	0.31±0.07 (.003)	0.37 (.003)
KIDNEY			
Wk 2			
1.07±0.36 (.01±.001)	1.15±0.33 (.01±.001)	0.97±0.19 (.01±.0009)	0.79±0.24 (.008±.001)
Wk 5			
1.68 (.008)	1.52o (.008)	1.05 (.009)	1.03 (.008)
Wk 8			
1.88 (.008)	1.87* (.007)	1.20 (.01)	1.22 (.008)

Values represents mean ± SEM

1Group A= rats fed 120 mg Ca-pantothenate/kg diet, ad lib.
Group B= rats fed control, ad lib.
Group C= rats fed deficient diet, ad lib.
Group D= pair-fed the control diet.

Statistical significance between Gp B and C:
o = $p<0.10$, * = $p<0.05$, ** = $p<0.01$.

control (+12 mg/kg diet) throughout the 8 weeks. No toxic signs or symptoms of feeding 10 times the recommended amount of pantothenate for rats were observed. The excess amount of pantothenic acid in the diet of group A did not result in a higher pantothenate concentration in any of the organs examined compared to the rats fed the control diet ad lib fed and pair-fed. The pantothenic level in whole blood, plasma and urine of the group fed 120 mg Ca-pantothenate per kg diet, however, was significantly greater ($p<0.01$) than the ad lib control, suggesting that excess pantothenic acid was absorbed and excreted. The pair-fed control group did not differ in the organs examined nor in blood and urine pantothenate concentration of total and free pantothenic acid from the ad lib control. Although the pair-fed rats had lower body weights than the rats fed ad lib control, the pair-fed control still maintained organ pantothenic acid concentration comparable to the ad lib control group.

Of the organs examined, the largest concentration of total pantothenic acid was found in the order of kidney, heart and spleen. The free to total ratio of pantothenic acid was similar in groups fed 120 mg Ca-pantothenate per kg diet, ad lib control, and the pair-fed control as well as the deficient group in all organs examined (Table 3). Within each group, no consistent changes were noted with increase age of the rat.

At a confidence interval of 90%, the total pantothenic acid concentration (ug of pantothenate/g organ) in the heart

Table 3. The ratio of free (F) to total (T) pantothenate in heart, spleen and kidney of rats in the study.*

	Group A	Group B	Group C	Group D
		Heart, ug/g organ		
wk 2				
F	12.90±5.48	17.83±2.02	10.16±3.15	15.96±3.03
T	39.64±7.00	47.25±8.59	32.69±8.06	44.14±7.99
F:T	0.32±0.11	0.40±0.07	0.34±0.12	0.37±0.09
wk 5				
F	19.99	16.32	12.03	14.76
T	43.19	51.84	37.49	50.29
F:T	0.47	0.32	0.32	0.29
wk 8				
F	17.85	17.61	11.02	17.44
T	52.40	56.68	39.68	57.53
F:T	0.34	0.31	0.30	0.32
		Spleen		
wk 2				
F	7.61±2.37	9.28±3.88	0.40±0.50	11.37±4.39
T	16.78±4.26	16.09±5.36	8.58±2.74	18.75±5.81
F:T	0.47±0.14	0.65±0.28	0.05±0.15	0.61±0.27
wk 5				
F	5.98	1.68	1.19	3.12
T	15.98	15.44	6.89	11.97
F:T	0.39	0.13	0.23	0.26
wk 8				
F	4.02	3.24	1.17	4.12
T	12.08	15.74	4.27	7.40
F:T	0.35	0.25	0.28	0.65
		Kidney		
wk 2				
F	47.81±10.56	33.67±7.44	21.36±5.57	35.50±4.97
T	72.15±11.37	54.98±4.68	39.78±13.90	49.01±13.18
F:T	0.68±0.15	0.62±0.15	0.55±0.14	0.75±0.10
wk 5				
F	38.04	48.52	30.73	37.80
T	49.75	56.86	66.78	67.37
F:T	0.76	0.86	0.47	0.58
wk 8				
F	49.06	44.64	30.58	30.07
T	64.34	53.19	42.03	52.92
F:T	0.72	0.85	0.73	0.58

Values represent mean±SEM

*Group A= rats fed 120 mg Ca-Pantothenate/kg diet, ad lib.
Group B= rats fed control diet, ad lib.
Group C= rats fed deficient diet, ad lib.
Group D= pair-fed the control diet.

showed only a diet effect. Result of the interaction contrast shows that there appears to be a slight ordinal interaction. The total pantothenate concentration was lower in the deficient group than in the pair-fed control and ad lib control (Figure 6). The total concentration in the heart was significant ($p<0.05$) at weeks 2, 5 and 8 between the ad lib control and deficient. Significance in heart total pantothenate concentration was seen later at weeks 5 and 8 between the deficient and the pair-fed group ($p<0.10$). At the same confidence interval of 90%, the free pantothenate concentration also only showed a diet effect. However, interaction contrast shows almost no effect (interaction contrast = -0.34) between deficient and pair-fed as well as deficient and ad lib control. The concentration of the free form of pantothenic acid in the heart of the deficient group was significantly different ($p<0.05$) from pair-fed control and ad lib control at weeks 2 and 8 but not 5, similar to the trend seen in the difference of heart weight.

Content of pantothenate in the whole organ was determined by multiplying the concentration (ug of pantothenate per gram of organ) times the gram weight of the whole organ (Table 4). The heart pantothenate content of rats fed the pantothenate deficient diet did increase (12 ug of pantothenate) throughout the 8 weeks of the study. The pair-fed control group had an increase of 16 ug of pantothenate, that is 4 mg more than the deficient

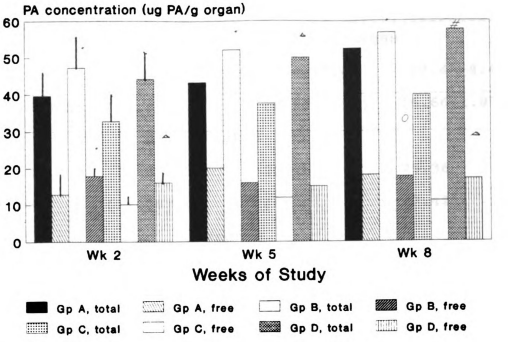


Figure 6. Concentration of pantothenate in rat heart. Total and free pantothenate concentration of group A (Gp A) rats fed 120 mg Ca-pantothenate/kg diet, ad lib, group B (Gp B) rats fed the ad lib control, ad lib, group C (Gp C) rats fed the pantothenate deficient diet, ad lib, and group D (Gp D) pair-fed the control diet. Values represent mean $\pm$ SEM. Statistical significance between Gp B and C:
o = $p<0.10$,
* = $p<0.05$. Statistical significance between Gp D and C:
$\triangle$ = $p<0.10$, # = $p<0.05$.

Table 4. Pantothenate content in whole rat heart.

Group A*	Group B	Group C	Group D
Week 2: Total (Free), ug/organ			
21.21±13.03	31.81±11.19	13.48±7.63	18.46±8.45
(6.89±6.25)	(12.27±7.90)	(4.19±1.95)	(6.66±1.97)
Week 5			
37.43	39.43	20.26	25.36
(17.47)	(12.28)	(6.50)	(7.42)
Week 8			
51.29	55.26	25.81	34.26
(17.27)	(17.312)	(6.97)	(10.11)

Values represent mean±SEM.
*Group A= rats fed 120 mg Ca-pantothenate/kg diet, ad lib.
Group B= rats fed control diet, ad lib.
Group C= rats fed deficient diet, ad lib.
Group D= pair-fed the control diet.

group, in total heart content. However, the content of pantothenate in the heart of the rats in ad lib control increased 23 ug in the eight weeks of the study. The rats in the group fed 120 mg pantothenate per kg diet and the pair-fed control had heart pantothenate concentration and content similar to the ad lib control group. Thus, an excess supplementation of pantothenate in the diet (as was for the group fed 120 mg pantothenate per kg diet rats) did not increase pantothenate concentration nor content in the heart.

In the spleen, at the confidence interval of 90%, diet also seems to have an effect. The interaction contrast shows a strong ordinal interaction between the deficient and the two control diet fed groups (ad lib and pair-fed). The deficient group had significantly lower total pantothenate concentration than ad lib control ($p<0.10$) at weeks 2, 5 and 8 of the study (Figure 7). Free pantothenate concentration had significant ($p<0.10$) diet, time and the interaction of the two effect between the deficient and the two groups fed the control diet. Interaction contrast shows an association between the two groups. Upon graphing, it appeared to be a disordinal response. Significance was seen only at week 2 between the deficient group and the two control diet fed groups ($p<0.01$). Pantothenate content in whole spleen was lower in the deficient group than pair-fed control, ad lib

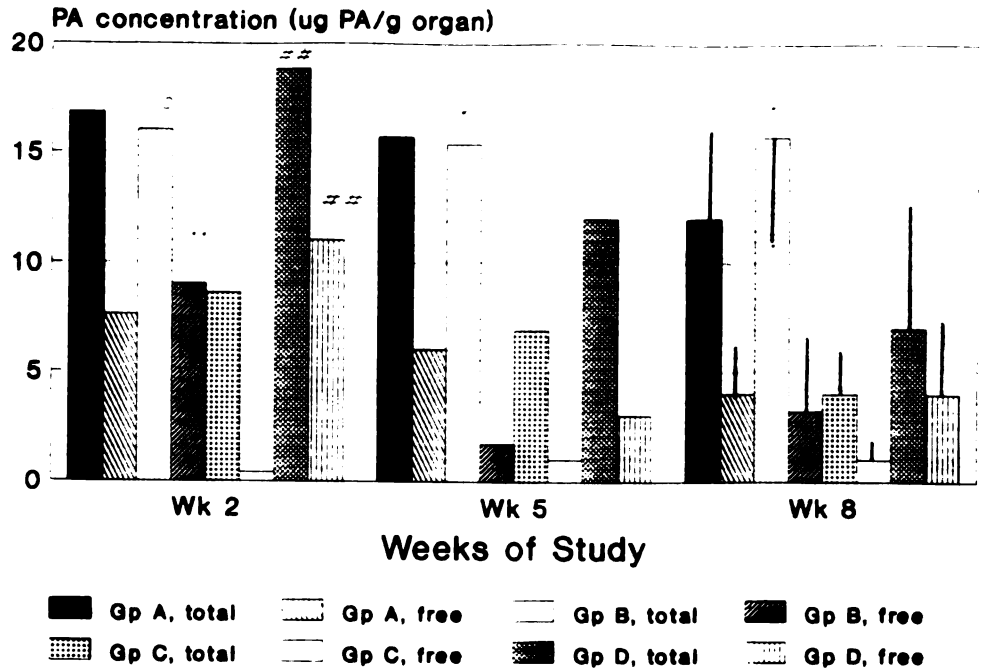


Figure 7. Concentration of pantothenate in rat spleen. Total and free pantothenate concentration of group A (Gp A) rats fed 120 mg Ca-pantothenate/kg diet, ad lib, group B (Gp B) rats fed the ad lib control, ad lib, group C (Gp C) rats fed the pantothenate deficient diet, ad lib, and group D (Gp D) pair-fed the control diet. Values represent mean ± SEM. n=4/group. Statistical significance between Gp B and C:
o = $p<0.10$, * = $p<0.05$, ** = $p<0.01$. Statistical significance between Gp D and C: ^ = $p<0.10$, # = $p<0.05$, ## = $p<0.01$.

control, as well as rat fed 120 mg pantothenate per kg diet in all weeks examined as shown in Table 5.

The total pantothenate concentration in kidney showed a time effect at the confidence interval of 90%. Interaction contrast shows almost zero effect. The deficient group, the pair-fed control and ad lib control was not significantly different at any time in kidney pantothenate concentration. Free pantothenate concentration, between the deficient and the ad lib control, had a time and diet effect at the confidence interval of 90%. Interaction contrast shows a strong ordinal interaction. The deficient group was significantly lower ($p<0.05$) than ad lib control at weeks 2, 5 and 8. However, this significance was not observed between the deficient and the pair-fed control (Figure 8). Content of pantothenic acid in whole kidney increased 8 ug (from 38 ug to 46 ug) while the organ weight increased 0.23 gram in the deficient group. Content of pantothenate in the kidney of the rats of the pair-fed control increased 25 ug which is 3 times the increased seen in the deficient group. Weight of the kidney in the pair-fed control rats increased 0.43 grams. When the rats are allowed to consume as much of the control diet as desired, as with the rats of the ad lib control, pantothenate content in the kidney increased 37 ug (from 62 ug to 99 ug) which is close to 5 times the increase seen in the deficient group. In addition, the organ weight of the ad lib control increased 0.72 grams which is 3 times

Table 5. Pantothenate content in whole rat spleen.

Group A*	Group B	Group C	Group D
	Week 2: Total (Free), ug/organ		
7.27±2.20	7.27±2.47	2.36±0.87	6.17±1.81
(3.30±1.07)	(4.18±1.65)	(0.11±0.15)	(3.73±1.45)
	Week 5		
9.00	5.65	1.98	3.54
(3.44)	(0.76)	(0.33)	(0.92)
	Week 8		
6.69	8.64	1.37	2.67
(1.92)	(1.93)	(0.37)	(1.53)

Values represent mean±SEM.
*Group A= rats fed 120 mg Ca-pantothenate/kg diet, ad lib.
Group B= rats fed control diet, ad lib.
Group C= rats fed deficient diet, ad lib.
Group D= pair-fed the control diet.

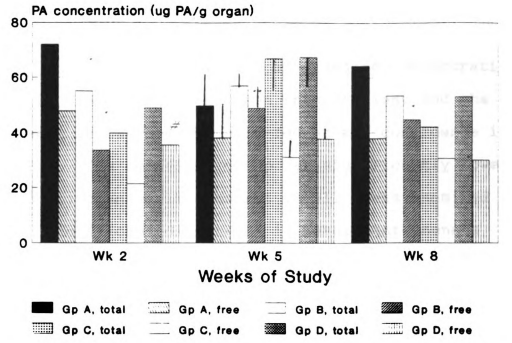


Figure 8. Concentration of pantothenate in rat kidney. Total and free pantothenate concentration of group A (Gp A) rats fed 120 mg Ca-pantothenate/kg diet, ad lib, group B (Gp B) rats fed the ad lib control, ad lib, group C (Gp C) rats fed the pantothenate deficient diet, ad lib, and group D (Gp D) pair-fed the control diet. Values represent mean $\pm$ SEM. n=4/group. Statistical significance between Gp B and C:
* = $p<0.05$, ** = $p<0.01$. Statistical significance between Gp D and C: # = $p<0.05$.

the weight increase seen in the deficient rats (Table 6). Without pantothenate in the diet, the growth of the kidney appeared hindered.

The whole blood total pantothenate concentration showed a diet effect between the deficient and the ad lib as well as between the pair-fed at the confidence interval of 90%. The deficient group was significantly lower ($p<0.05$) than ad lib control in all weeks examined (Figure 9). Significant difference in total pantothenate concentration ($p<0.01$) between the deficient group and the pair-fed control was found only at week 2. At the same confidence interval of 90%, the free pantothenate concentration had a diet effect. Free pantothenate concentration of whole blood in the deficient group was not significantly lower compared to the pair-fed control nor the ad lib control at any time point. The group fed 120 mg Ca-pantothenate per kg diet had the largest pantothenate concentration in whole blood, followed by ad lib control, pair-fed control and finally the deficient. The ratio of free to total pantothenate in whole blood was about 1 in all the groups examined.

At the confidence interval of 90%, the plasma pantothenate concentration had a diet and time effect between the deficient and the two control diet fed groups. Plasma free pantothenate concentration was significantly lower ($p<0.05$) in the deficient group compared to the ad lib control at weeks 5 and 8 of the study as shown on Figure

Table 6. Pantothenate content in whole rat kidney.

Group A*	Group B	Group C	Group D
Week 2: Total (Free), ug/organ			
76.91±23.03	62.35±17.42	38.46±14.26	40.70±20.21
(50.39±22.27)	(38.53±21.33)	(20.78±7.78)	(28.84±8.75)
Week 5			
83.82	86.67	69.69	69.33
(64.00)	(74.01)	(32.28)	(38.72)
Week 8			
120.77	99.34	46.34	65.62
(92.53)	(83.87)	(35.95)	(37.18)

Values represent mean±SEM.
*Group A= rats fed 120 mg Ca-pantothenate/kg diet, ad lib.
Group B= rats fed control diet, ad lib.
Group C= rats fed deficient diet, ad lib.
Group D= pair-fed the control diet.

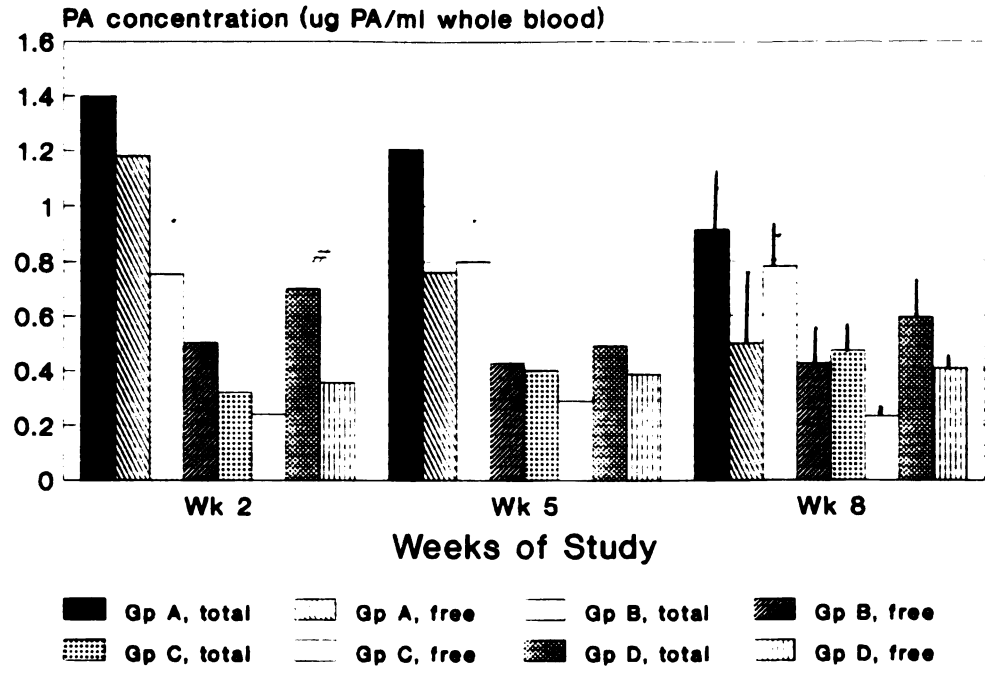


Figure 9. Concentration of pantothenate in rat whole blood. Total and free pantothenate concentration of group A (Gp A) rats fed 120 mg Ca-pantothenate/kg diet, ad lib, group B (Gp B) rats fed the ad lib control, ad lib, group C (Gp C) rats fed the pantothenate deficient diet, ad lib, and group D (Gp D) pair-fed the control diet. Values represent mean ± SEM. n=4/group. Statistical significance between Gp B and C:
* = $p<0.05$. Statistical significance between Gp D and C:
# = $p<0.05$.

10. No plasma was collected at week 8 of the pair-fed group, thus comparison could not be made. The rats of group fed 120 mg pantothenate per kg and the ad lib control contained the highest plasma pantothenate concentration, followed by the pair-fed control and lastly the deficient group in all weeks examined.

Urine pantothenate excretion was significantly lower ($p<0.01$) in the deficient group than the pair-fed and ad lib control rats at weeks 5 and 8 (Figure 11). The group fed 120 mg pantothenate per kg diet, consistently, had significantly higher ($p<0.01$) concentration (ug pantothenate/24 hours) of free pantothenate excretion (334 - 375 ug/24 hours) than the ad lib control (range 18 - 121 ug/24 hours). The ad lib control and the pair-fed control had comparable amount of free pantothenate excreted at week 2 and 5. However, by week 8 of the study the effect of limited intake on the pair-fed rats was apparent (the pair-fed excreted less than the ad lib control). By week 8, the pair-fed group was fed approximately 7 grams of diet per day (compared to about 20 grams consumed by the ad lib control rats). It appeared that the pair-fed rats were conserving pantothenate stores by a decreasing urinary pantothenate excretion when the amount of diet was restricted. The deficient group had significantly lower ($p<0.01$) pantothenate excreted than either control group. This was noted at week 2 (4.05 ± 1 ug/24 hours compared to pair-fed control: 21.6 ± 23 ug/24 hours and ad lib

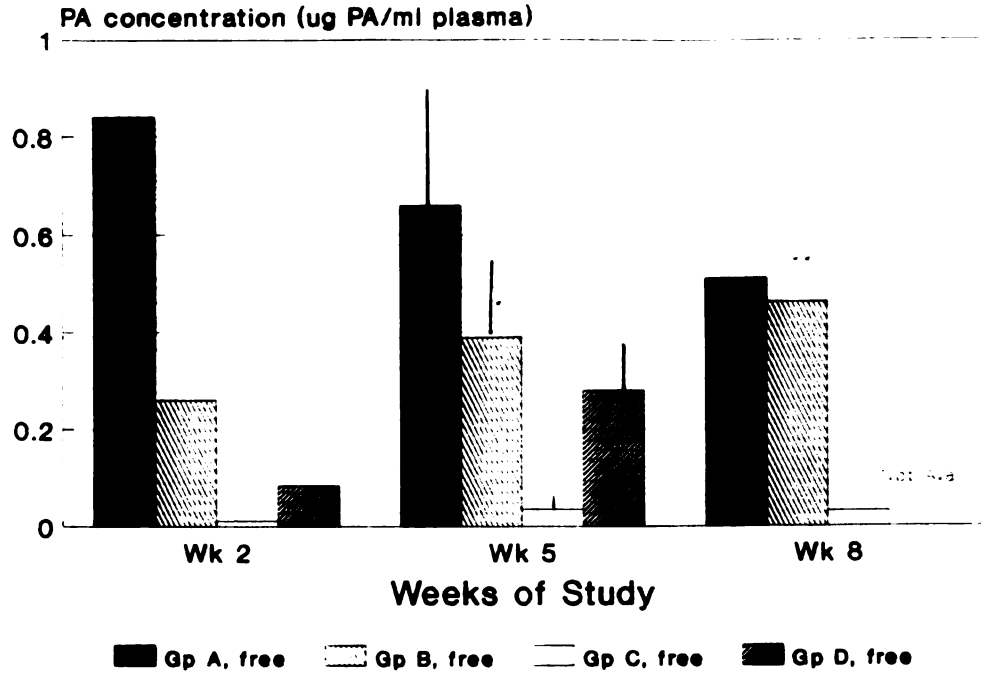


Figure 10. Concentration of pantothenate in rat plasma. Free pantothenate concentration of group A (Gp A) rats fed 120 mg Ca-pantothenate/kg diet, ad lib, group B (Gp B) rats fed the ad lib control, ad lib, group C (Gp C) rats fed the pantothenate deficient diet, ad lib, and group D (Gp D) pair-fed the control diet. n=4/group. Values represent mean ± SEM. Statistical significance between Gp B and C:
* = $p<0.05$,
** = $p<0.01$.

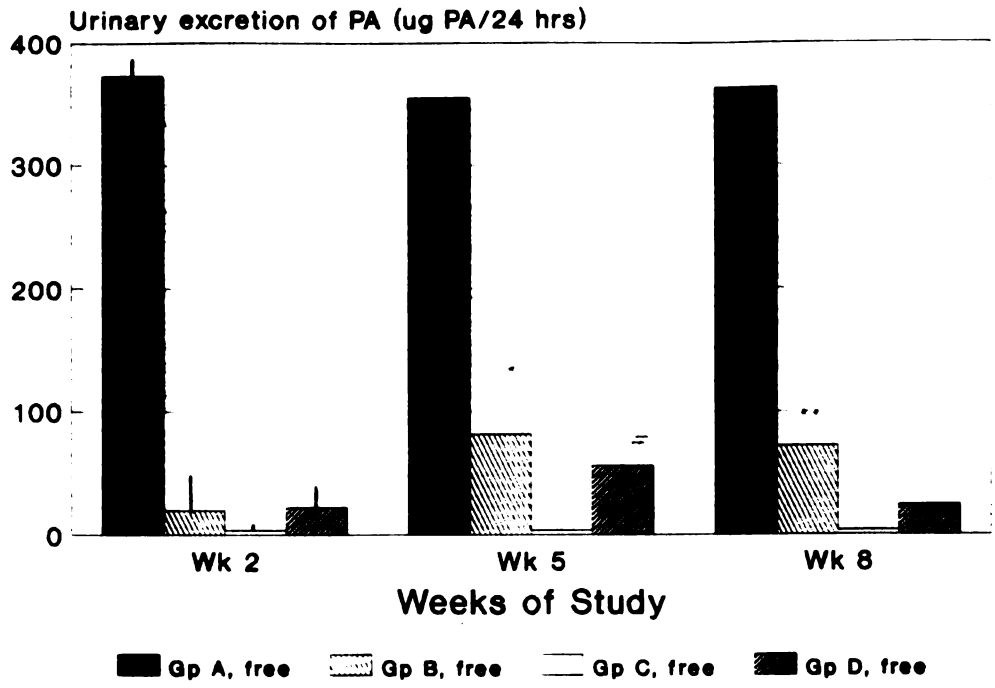


Figure 11. Concentration of pantothenate in rat urine. Free pantothenate excreted in urine per 24 hours of group A (Gp A) rats fed 120 mg Ca-pantothenate/kg diet, ad lib, group B (Gp B) rats fed the ad lib control, ad lib, group C (Gp C) rats fed the pantothenate deficient diet, ad lib, and group D (Gp D) pair-fed the control diet. n=4/group. Values represent mean ± SEM. n=4/group. Statistical significance between Gp B and C: * = $p<0.05$, ** = $p<0.01$. Statistical significance between Gp D and C: # = $p<0.05$.

control: 19.8 ± 37 ug/24 hours). The low level of urinary pantothenate remained constant in the deficient group for the duration of the study. However, rats consuming adequate pantothenic acid (ad lib control and pair-fed control) increased the concentration of pantothenate excreted over the weeks of the study. During period of fast growth, at 5 weeks of age or at week 2 of the study, more pantothenate may be needed for organ growth and increased metabolism than during a period of less growth. Thus, due to conservation of pantothenate, less pantothenate was excreted at week 2 in the ad lib and pair-fed control than the following weeks.

Correlation of the pantothenate concentration of whole blood, plasma and urine to organs concentration was important in this study to determine if one could predict the other. It was also important to note if easily accessible bodily fluids were good indicators of pantothenate status. Pantothenate values in whole blood, plasma and urine were correlated to organ pantothenate concentration. The deficient group was the population at risk for pantothenate deficiency and was focused on. Levels of pantothenic acid in organs, blood and urine may have reached saturation and plateaued in the rats of the groups fed the pantothenate supplemented diet. Thus, normal rat values of pantothenate concentration were not considered for determining the best pantothenate indicator and predictor. The results of the correlations are shown on

Table 7. Under the experimental conditions, the highest correlation was seen between plasma pantothenate concentration and free pantothenic acid concentration in spleen (r = 0.80). Total pantothenate concentration in whole blood correlated best with free pantothenate concentration in kidney (r = 0.48). Free pantothenate concentration of whole blood correlated best with heart (r = 0.60). Urine was poorly correlated with organ pantothenate concentration and was more reflective of the diet and this supports Eissenstat et al.'s (1986) study with human subjects.

Since the variables of interest (pantothenate concentration in organs) were determined this study, the concern of the equation formulated is the ability (of plasma pantothenate concentration) to predict future random values. The variables with the highest correlation were used in determining the predictive equations. In predictive equation 1, actual free pantothenate concentrations of heart (x1), spleen (x2) and kidney (x3) were inserted into the equation from the independent observations made from the rats of the deficient group (n=12). Organ free pantothenate concentration (x1, x2, x3) was paired to plasma pantothenate concentration (y). These pairs were then used to estimate partial regression coefficients. We assumed a linear function existed between the pairs so that the data points were fitted into a hyper-plane where Y1 = b0 + b1[x1] + b2[x2] + b3[x3]. The estimates of parameters determined

Table 7. Correlation results of organ pantothenate (PA) concentration vs bodily fluid of group C rats (r).

	Whole Blood		Free PA	
	Total PA	Free PA	Plasma	Urine
Total PA				
Heart	-0.06	-0.18	0.22	0.04
Spleen	-0.40	-0.12	-0.49	0.25
Kidney	0.01	0.52	0.49	-0.42
Free PA				
Heart	0.37	0.60	0.78	-0.41
Spleen	0.44	0.56	0.80	0.12
Kidney	0.48	0.37	0.68	-0.04

should produce a sum of squares that is minimized by the method of least square. Predictive equation 1 formulated to predict pantothenate status of the rat is as follows:

(Free pantothenate in plasma) =
b0 + [b1(free pantothenate in heart)
+ b2(free pantothenate in spleen)
+ b3(free pantothenate in kidney)]

where,

b0 = -0.0183
b1 = 0.00216
b2 = 0.0186
b3 = 0.000299

Table 8 shows the residual results of the predictive equation 1. The coeffcient of determination (R2) of predictive equation 1 is 0.80. This means that 80% of the variation in Y is explained by the variation in x1, x2 and x3.

Prediction equations 2, 3 and 4 are the reverse of prediction equation 1. In 2, 3 and 4 we determined the ability to predict organ pantothenate concentration when the concentration of pantothenate in bodily fluids (whole blood, plasma, and/or urine) is known. These equations will be more realistic in a clinical setting because rarely is a biopsy performed to determine nutritional status of the individual. Again, only highly correlated variables were used ($r > 0.30$) to determined predictive equations 2, 3 and 4.

Predictive equation 2 uses the free pantothenate concentration found in whole blood, plasma, and urine to calculate the free pantothenate concentration in the heart

Table 8. Residual results of the predictive equation 1*.

Actual Y Value (ug/ml)	Predicted Y (ug/ml)	Residual Value
0.017	0.010	0.007
0.010	0.015	-0.005
0.015	0.025	-0.010
0.004	-0.000	0.004
0.029	0.029	-0.000
0.033	0.025	0.008
0.040	0.031	0.009
0.074	0.071	0.003
0.023	0.029	-0.006
0.018	0.028	-0.010
0.029	0.044	-0.015
0.059	0.045	0.014

*Predictive equation 1: $Y1 = b0 + [b1(x1)+b2(x2)+b3(x3)]$

where: Y1 = Free PA in plasma (ug/ml)
x1 = Free PA in heart (ug/g)
x2 = Free PA in spleen (ug/g)
x3 = Free PA in kidney (ug/g)

and

b0 = -0.0183
b1 = 0.0022
b2 = 0.0186
b3 = 0.0003

of a deficient rat. The predictive equation 2 is as follows:

(Free PA in heart) =
b0 + [b4(free PA in whole blood)
+ b5(free PA in plasma)
+ b6(free PA in urine)]

where,

b0 = 6.73
b4 = 19.73
b5 = 78.85
b6 = -0.74

The residual results are shown on Table 9. The R2 for predictive equation 2 is 0.63.

Predictive equation 3 estimates the free pantothenate concentration in the spleen of a deficient rat from total and free pantothenate concentration in whole blood as well as free pantothenate concentration in plasma. Urine was not highly correlated with spleen (r = 0.12) and thus, not used in the equation. The residual results are shown on Table 10 and the R2 for this equation is 0.65. Predictive equation 3 is as follows:

(Free PA in Spleen) =
b0 +[b7(total PA in whole blood)
+ b8(free PA in whole blood)
+ b9(free PA in plasma)]

Where,

b0 = 0.097
b7 = -0.013
b8 = 0.122
b9 = 19.263

The total and free pantothenate concentration in whole blood along with the free pantothenate concentration in plasma were used to formulate the predictive equation (4)

Table 9. Residual results of the predictive equation 2*.

Actual Y Value (ug/g)	Predicted Y (ug/g)	Residual Value
6.73	9.17	-2.44
9.33	8.87	0.46
14.45	10.15	4.30
10.13	9.24	0.89
8.19	9.54	-1.35
10.23	12.80	-2.57
10.96	13.27	-2.31
18.74	17.19	1.55
9.94	10.81	-0.87
8.80	8.05	0.74
10.97	10.42	0.55
14.37	13.34	1.03

*Predictive equation 2: $Y2 = b0 + [b4(x4)+b5(x5)+b6(x6)]$

where: Y2 = Free PA in heart (ug/g)
x4 = Free PA in whole blood (ug/ml)
x5 = Free PA in plasma (ug/ml)
x6 = Free PA in urine (ug/24 hr)

and

b0 = 6.73
b4 = 19.72
b5 = 78.85
b6 = -0.74

Table 10. Residual results of predictive equation 3*.

Actual Y Value (ug/g)	Predicted Y (ug/g)	Residual Value
0.43	0.68	-0.25
0.34	0.48	-0.14
0.31	0.64	-0.33
0.51	0.48	0.03
1.14	0.93	0.21
0.70	0.99	-0.29
0.93	1.16	0.23
1.99	1.90	0.09
0.86	0.79	0.07
0.92	0.61	0.31
1.65	0.91	0.74
1.25	1.45	-0.20

* Predictive equation 3: $Y3 = b0 + [b7(x7)+b8(x8)+b9(x9)]$

where: Y3 = Free PA in spleen (ug/g)
x7 = Total PA in whole blood (ug/ml)
x8 = Free PA in whole blood (ug/ml)
x9 = Free PA in plasma (ug/ml)

and

b0 = 0.097
b7 = -0.013
b8 = 0.122
b9 = 19.263

for free pantothenate concentration in the kidney of the deficient rat. The residual results are shown on Table 11. The R2 (= 0.48) of predictive equation 4 is not as strong as the earlier equations but it is worthwhile to note. Predictive equation 4 is as follows:

(Free PA in Kidney) =
b0 + [b10(total PA in whole blood)
+ b11(free PA in whole blood)
+ b12(free PA in plasma)]

Where,

b0 = 21.63
b11 = 0.63
b12 = -0.76
b13 = 183.46

Table 11. Residual results of predictive equation 4*.

Actual Y Value (ug/g)	Predicted Y (ug/g)	Residual Value
20.56	24.92	-4.36
22.86	24.00	-1.14
20.78	24.59	-3.81
21.24	22.16	-0.92
29.13	27.76	1.37
26.26	28.23	-1.97
28.61	28.64	-0.03
38.91	35.41	3.50
34.14	27.38	6.76
33.34	25.53	7.81
25.86	27.31	-1.45
28.99	34.76	-5.77

*Predictive equation 4:

$$Y4 = b0 + [b10(x10)+b11(x11)+b12(x12)]$$

where: Y4 = Free PA in kidney (ug/g)
x10 = Total PA in whole blood (ug/ml)
x11 = Free PA in whole blood (ug/ml)
x12 = Free PA in plasma (ug/ml)

and

b0 = 21.63
b7 = 0.63
b8 = -0.76
b9 =183.46

DISCUSSION

It is evident from this study that pantothenic acid is an essential nutrient for male Sprague-Dawley rats. The Ca-pantothenate supplementation of 12 mg/kg diet was sufficient to prevent deficiency signs as observed in rats of group C, fed the pantothenate deficient diet. Supplementation of 120 mg Ca-pantothenate/kg diet (group A) in rats showed no deviation from the rats supplemented with 12 mg Ca-pantothenate/kg diet (group B). Similar results were seen by Barboriak et al. (1956). They reported that weanling rats fed 100 mg Ca-pantothenate/kg diet ad libitum also failed to gain as much weight, also not significantly, as their counterparts fed 8 mg Ca-pantothenate/kg diet ad libitum. Thus, Ca-pantothenate greater than 8 mg/kg in the diet did not result in greater growth or weight gain for the 680 days of their study. In fact, the reverse was seen in this study as well as in the study of Barboriak et al. Barboriak et al. also fed weanling rats Ca-pantothenate levels of 2 mg/kg and 4 mg/kg ad libitum for 680 days. The growth of rats fed these diets was lower than the growth of rats fed 8 mg Ca-pantothenate/kg. The difference was significant in the first year for the rats consuming the 2 mg Ca-pantothenate/kg diet but not for rats consuming 4 mg

Ca-pantothenate/kg. However, after one year, weight gain of all rats studied (consuming pantothenate supplemented diet) plateaued and significant difference was not observed among any groups. Weanling rats in this study on a deficient diet for 8 weeks, weighed 40% less than rats fed diet supplemented with 12 mg Ca-pantothenate/kg. Thus, there is an optimal level of pantothenate required for maximum growth of weanling rats possibly greater than 20 mg Ca-pantothenate as suggested by Henderson et al. (1942).

Results from this study also suggest that the requirement for pantothenic acid may be higher at the period of growth spurt (birth to 5 weeks of age) than adulthood of the rat. Rats fed the diet supplemented with 12 mg Ca-pantothenate/kg ad lib excreted only 20 ug pantothenate per 24 hours in the urine at week 2 of the study compared to 82 ug and 72 ug per 24 hours at week 5 and 8 respectively. The ad lib control group retained in their body 90% of the pantothenate they obtained from the diet. Thus, during period of fast development and organ growth, 12 mg Ca-pantothenate/kg may not be sufficient for rats. At week 5 of the study or 8 weeks of age the requirement for pantothenate is lower and therefore the rats excreted more pantothenate. By week 5, they excreted over four times the amount than they excreted than week 2 while consuming approximately the same amount of diet as week 2. At week 5 and 8, ad lib control retained 61% and 65%, respectively, of the pantothenate consumed from diet. This was more

evident in rats of the deficient group. At week 2, they only excreted 4 ug per 24 hours and remained close to that level for the duration of the study. They received no pantothenate from their diet; thus their body retained what little pantothenate they had stored from birth and excreted minimally. Rats consuming the diet supplemented with 120 mg Ca-pantothenate/kg did not experience a fluctuation in urine pantothenate excretion since they were consuming more than an optimal amount of pantothenate in their diet. These rats excreted a consistent level close to 400 ug per day at weeks 2, 5 and 8 of the study.

The question addressed is how the rats in deficient group grew (42 grams at week 0 to 125 grams at week 8), though at a significantly lower rate than the rats of the ad lib control. As stated earlier, the rats consuming a pantothenate deficient diet excreted very little pantothenate in the urine (4 ug/24 hours from week 2 to 8). In addition very little was found in blood throughout the study. This suggests that much of the pantothenate remained in its respective organ.

The heart of rats in the deficient group was one organ examined that increased in total pantothenate concentration over time. However, total content of whole heart in rats of the deficient group was lower than that of the ad lib control at all weeks examined. The increase of pantothenate concentration observed over the weeks of the study in the deficient group rats could have come from two

possible sources. The heart may be siphoning pantothenate from other organs. Spleen, for example was one organ that decreased in pantothenate concentration in rats of the deficient group. This decrease was not observed in rats fed ad lib control. Muscle may have contributed much of the pantothenate to the heart. However, pantothenate concentration of skeletal muscle was not determined in this study. Second possible source of pantothenic acid may come from the feces through the practice of coprophagy. In an unpublished study by Dr. Won Song of Michigan State University, and also observed by Henderson et al. (1942), the pantothenic acid content (ug/g feces) of feces was similar in deficient pantothenic acid rats and rats fed an adequate diet (12 mg Ca-pantothenate/kg diet). Consumption of feces of rats in this study was reduced by housing rats in screen bottom cages, however, coprophagy could not be prevented. It is very possible that these rats practiced coprophagy consistently. Barnes et al. (1957) reported that rats consumed 50 - 65% of excreted feces when housed in wire bottom cages. In future studies, it would be interesting to note any difference in pantothenate deficient state with and without the use of tail cups.

The prediction equation formulated was to determine if the pantothenate level in an easily accessible body fluid was reflective of organ pantothenate status of the rat. Nevertheless, plasma was identified to be the best indicator

of pantothenate status in the rat. In a study by Cheryl Bates of Michigan State University, she examined the pantothenate concentration in the different blood cell components of rats. She concluded that the total and free pantothenate concentration in whole blood were reliable indicator of pantothenate intake. However, she did not examine how well correlated the blood values were to intake nor tissue/organ level. Similarly, Eissenstat et al. (1986) reported that total pantothenate in fasted whole blood in human was well correlated (r=0.38) with intake. Again, no correlation was performed on blood concentration and organ concentration. Predictive equations 2, 3 and 4 include whole blood, especially the free pantothenate concentration, in the calculation of the organ (heart, spleen and kidney) free pantothenate concentration. However, in this study, plasma pantothenate concentration was better correlated to the organ pantothenate concentration than whole blood. This suggests that plasma free pantothenate concentration is a good indicator of pantothenate status in the rat.

CONCLUSION

Rats require pantothenate in their diets to achieve normal growth. As demonstrated in this study, rats fed a diet supplemented with at least 12 mg Ca-pantothenate grew significantly better than those without pantothenate. Those rats fed an excess of 12 mg Ca-pantothenate/kg diet, overall, did not demonstrate greater growth than rats fed ad lib control.

Organ pantothenate concentration was lower in rats fed the pantothenate deficient diet compared to the control. It is unsure from this study if the decreased level of pantothenate concentration in the rats of the deficient group had impaired functions. The rats fed 120 mg Ca-pantothenate per kg of diet did not result in a higher concentration or storage of pantothenate in their organs compared to the control in the eight weeks of the study.

Plasma pantothenate concentration was identified as the best indicator of organ pantothenate status. Plasma pantothenate concentration of the rats in the deficient group showed the highest correlation with the organ pantothenate concentration. The formulated predictive equations 2, 3 and 4 can be used to estimate the organ pantothenate status of the rat. The coefficient of

determination, R2, which represents the proportion of variation in the dependent variable (Y) explained or associated with the variation in the independent variable, is used as a criterion in searching for a good (predictive) estimator. The R2 for the predictive equations were fairly high.

LIMITATIONS

In this study, limitations were set in part by what was feasible. All sample preparations were performed by the principal investigator to decrease variation. It was only possible to examine a few organs in this study with each sacrifice. Had more organs or tissues been examined, the speed of sample preparation by one individual would have decreased and therefore increased the chance of autolysis in the samples. Thus, we were selective in choosing our "target" organs. The heart, spleen and kidney were chosen in this study because of their role in circulation, blood, or urine production. All RIA were performed by the principal investigator. Precision of the RIA was assured prior to the onset of the study. Internal standards were used consistently with each RIA; however, external standard (for example, yeast preparation) were used sporadically and randomly to assure accuracy. It may be advised to consistently used both internal and external standard with each RIA. Behavioral changes were not monitored in rats 24 hours a day. Perhaps videotaping the rats may document the neurological changes associated with human subjects deficient in pantothenate.

Only a 24 hour adaptation period was used before starting weanling rats on the diet regimes. This was

necessary due to the rapid development of rats and for fear of missing fast growth stage of rats. Perhaps a longer adaptation period may be more appropriate and accurate. A longer adjustment period will allow rats to accustom themselves to the new environment instead of subjecting them to more stress (of being separated from others, place in a new cage, and fed a new diet). Regardless, all rats were exposed to the same "stress" and differed only in diet fed.

Diet used in this study contained 69% kcal from carbohydrates. Fat provides 10% of kcal while protein provides 21%. Thus, organ, blood and urine pantothenate concentration reported from other groups may differ from this study due to the difference in the composition of the diet.

RECOMMENDATION

The accuracy of the predictive equation must be further tested. In future studies, other tissues and organs such as skeletal muscle, lung, liver and even body pool of pantothenate should be examined along with plasma and whole blood concentration. The possibility of coprophagy should also be limited. With the proper use of a tail cup, the act of coprophagy may be limited. The use of a tail cup will decrease consumption of intestinal synthesized pantothenate from the feces and perhaps enhance the on-set of pantothenate deficiency.

Pantothenate was fed as mg pantothenate per kg diet instead of mg per kg body weight. Though this would not have affected the deficient group, it may influence the other pantothenate supplemented groups. The rats were similar in body weight at the start of the study so part the amount of pantothenate consumed per kg body weight would have been similar. However, as the rats grew, the amount of pantothenate consumed per unit of body weight would vary widely.

APPENDIX

APPENDIX A: FOOD INTAKE OF RATS.*

Week	Group A	n	Group B	n	Group C**	n
1	9.7±2.4	12	10.7±1.0	12	9.1±1.2	12
2	15.9±1.5	12	17.0±1.4	12	9.3±1.1	12
3	19.7±3.4	8	21.1±2.6	8	10.6±1.0	8
4	22.0±2.0	8	21.5±3.7	8	10.3±0.8	8
5	19.4±3.3	8	17.5±6.9	8	9.0±0.6	8
6	14.4±3.3	4	19.9±6.0	4	7.6±1.1	4
7	19.7±1.4	4	18.5±2.1	4	7.1±1.4	4
8	19.5±1.5	4	18.5±2.1	3	7.7±0.4	4

values represent mean grams of diet ± stand. dev.
*Group A=rats fed 120 mg Ca-pantothenate/kg diet, ad lib.
Group B=rats fed the control diet, ad lib.
Group C=rats fed deficient diet, ad lib.
** Same amount consumed by group C was fed to group D (pair-fed control)

APPENDIX B: WEIGHT GAINS OF RATS.*

Week	Group A	n	Group B	n	Group C	n	Group D	n
0	40±5	12	43±5	12	41±6	12	42±4	12
1	71±10	12	79±8	12	70±7	12	68±6	12
2	113±12	12	124±12	12	99±9	12	97±7	12
3	153±19	8	163±17	8	112±13	8	129±13	8
4	192±28	8	194±27	8	122±14	8	132±16	8
5	215±25	8	213±40	8	133±17	8	154±22	8
6	213±29	4	220±54	4	135±19	4	149±22	4
7	229±24	4	269±26	4	129±21	4	137±21	4
8	244±23	4	284±27	3	135±25	4	141±22	4

values represent mean grams weight ± stand. dev.
*Group A=rats fed 120 mg Ca-pantothenate/kg diet, ad lib.
Group B=rats fed the control diet, ad lib.
Group C=rats fed deficient diet, ad lib.
Group D=rats pair-fed the control diet.

APPENDIX C: PANTOTHENATE (PA) CONCENTRATION OF RAT HEART.*

Rat I.D.	Total PA	Free PA	Total PA	Free PA
		Week 2		
	Group A		Group B	
1	33.51	7.61	37.66	15.29
2	42.05	20.91	45.36	20.85
3	46.20	10.80	66.88	17.01
4	36.78	12.26	39.09	18.16
mean	39.64	12.90	47.25	17.83
std dev	5.62	5.69	13.51	2.33
	Group C		Group D	
1	36.93	6.73	36.95	20.00
2	35.47	9.33	48.54	11.30
3	23.73	14.45	41.75	17.24
4	34.62	10.13	49.31	15.29
mean	32.69	10.16	44.14	15.96
std dev	6.04	3.21	5.87	3.66
		Week 5		
	Group A		Group B	
5	36.86	20.42	51.14	19.36
6	46.18	21.63	57.17	16.02
7	41.06	17.98	48.83	15.93
8	48.65	19.93	50.22	13.97
mean	43.19	19.99	51.84	16.32
std dev	5.27	1.52	3.68	2.24
	Group C		Group D	
5	32.80	8.19	46.85	11.02
6	31.67	10.23	51.19	14.82
7	42.92	10.96	50.80	16.43
8	42.56	18.74	52.32	16.76
mean	37.49	12.03	50.29	14.76
std dev	6.08	4.62	2.38	2.63

Appendix C continues

	Week 8			
	Group A		Group B	
9	54.97	12.85	62.44	16.33
10	53.15	27.32	50.70	19.96
11	55.78	19.24	56.94	16.55
12	45.71	11.97	NA	NA
mean	52.40	17.85	56.68	17.61
std dev	4.60	7.10	5.85	2.04
	Group C		Group D	
9	34.38	9.94	66.72	15.45
10	57.07	8.80	47.74	18.30
11	28.49	10.97	65.08	14.18
12	38.76	14.37	50.59	21.82
mean	39.68	11.02	57.53	17.44
std dev	12.34	2.40	9.75	3.39

values represent ug pantothenate/g organ ± stand. dev.
*Group A=rats fed 120 mg Ca-pantothenate/kg diet, ad lib.
Group B=rats fed the control diet, ad lib.
Group C=rats fed deficient diet, ad lib.
Group D=rats pair-fed the control diet.

APPENDIX D: PANTOTHENATE (PA) CONCENTRATION OF RAT SPLEEN.*

Rat I.D.	Total PA	Free PA	Total PA	Free PA
		Week 2		
	Group A		Group B	
1	17.66	6.00	8.90	6.32
2	16.44	11.06	15.15	12.81
3	21.39	5.56	14.85	11.25
4	21.39	7.83	25.47	6.73
mean	16.78	7.61	16.09	9.28
std dev	4.04	2.50	6.88	3.25
	Group C		Group D	
1	7.04	0.43	11.55	5.76
2	10.25	0.34	23.68	10.41
3	5.41	0.31	19.10	15.51
4	11.63	0.51	20.68	13.78
mean	8.58	0.40	18.75	11.37
std dev	2.86	0.09	5.16	4.3
		Week 5		
	Group A		Group B	
5	23.28	8.51	9.44	3.13
6	15.03	6.26	12.16	1.01
7	11.90	5.32	18.10	0.51
8	12.74	3.82	22.05	2.07
mean	15.74	5.98	15.44	1.68
std dev	5.20	1.96	5.70	1.17
	Group C		Group D	
5	10.27	1.14	12.08	2.89
6	8.35	0.70	8.90	2.32
7	5.26	0.93	9.56	2.44
8	3.67	1.99	17.35	4.84
mean	6.89	1.19	11.97	3.12
std dev	2.98	0.56	3.84	1.17

Appendix D continues

	Week 8			
	Group A		Group B	
9	9.46	4.46	21.01	4.17
10	12.10	6.54	8.98	3.91
11	11.90	2.35	17.22	1.65
12	14.85	2.73	NA	NA
mean	12.08	4.02	15.74	3.24
std dev	2.20	1.91	6.15	1.39
	Group C		Group D	
9	3.99	0.86	8.22	5.11
10	5.02	0.92	10.64	1.40
11	4.59	1.65	5.47	5.03
12	3.46	1.25	5.27	4.95
mean	4.27	1.17	7.40	4.12
std dev	0.68	0.36	2.55	1.82

values represent ug pantothenate/g organ ± stand. dev.
*Group A=rats fed 120 mg Ca-pantothenate/kg diet, ad lib.
Group B=rats fed the control diet, ad lib.
Group C=rats fed deficient diet, ad lib.
Group D=rats pair-fed the control diet.

APPENDIX E: PANTOTHENATE (PA) CONCENTRATION OF RAT KIDNEY. *

Rat I.D.	Total PA	Free PA	Total PA	Free PA
		Week 2		
	Group A		Group B	
1	65.80	61.30	49.01	29.32
2	84.21	40.17	54.56	34.54
3	73.58	48.71	52.82	40.13
4	65.00	41.05	63.54	30.70
mean	72.15	47.81	54.98	33.67
std dev	8.92	9.78	6.16	4.84
	Group C		Group D	
1	33.40	20.56	59.73	38.26
2	36.95	22.86	58.25	40.83
3	39.83	20.78	30.06	27.98
4	48.93	21.24	48.01	34.92
mean	39.78	21.36	49.01	35.50
std dev	6.64	1.04	13.66	5.57
		Week 5		
	Group A		Group B	
5	51.10	47.60	53.73	53.07
6	59.78	46.85	56.51	51.05
7	49.17	27.79	54.81	46.09
8	38.95	29.91	62.39	43.87
mean	49.75	38.04	56.86	48.52
std dev	8.55	10.65	3.86	4.27
	Group C		Group D	
5	58.23	29.13	60.57	32.49
6	68.33	26.26	52.64	40.38
7	78.89	28.61	80.05	38.39
8	61.68	38.91	76.21	39.92
mean	66.78	30.73	67.37	37.80
std dev	9.10	5.60	12.94	3.64
		Week 8		
	Group A		Group B	
9	64.59	51.44	56.84	41.62
10	57.98	55.91	55.78	47.27
11	67.90	50.33	46.95	45.03
12	66.89	38.57	NA	NA
mean	64.34	49.06	53.19	44.64
std dev	4.46	7.40	5.43	2.85

Appendix E continues

	(Week 8)			
	Group C		Group D	
9	51.37	34.14	47.15	30.66
10	40.79	33.34	42.49	29.18
11	34.76	25.86	60.93	34.51
12	41.18	28.99	61.12	25.94
mean	42.03	30.58	52.92	30.87
std dev	6.89	3.88	9.55	3.55

values represent ug pantothenate/g organ

* Group A=rats fed 120 mg Ca-pantothenate/kg diet, ad lib.
Group B=rats fed the control diet, ad lib.
Group C=rats fed deficient diet, ad lib
Group D=rats pair-fed the control diet.

APPENDIX F: PANTOTHENATE (PA) CONCENTRATION OF RAT WHOLE BLOOD. *

Rat I.D.	Total PA	Free PA	Total PA	Free PA
		Week 2		
	Group A		Group B	
1	1.07	0.90	1.01	0.79
2	1.66	1.19	0.43	0.27
3	1.51	1.43	0.86	0.64
4	1.36	1.21	0.71	0.32
mean	1.40	1.18	0.75	0.50
std dev	0.25	0.22	0.25	0.25
	Group C		Group D	
1	0.32	0.24	0.50	0.41
2	0.32	0.19	0.75	0.42
3	0.32	0.24	0.68	0.35
4	0.32	0.29	0.88	0.25
mean	0.32	0.24	0.70	0.36
std dev	0.002	0.04	0.16	0.08
		Week 5		
	Group A		Group B	
5	1.26	0.72	0.66	0.29
6	1.57	0.81	0.74	0.49
7	1.39	0.92	0.76	0.49
8	0.81	0.59	1.04	0.43
mean	1.26	0.76	0.80	0.43
std dev	0.32	0.14	0.17	0.09

Appendix F continues

(Week 5)

	Group C		Group D	
5	0.46	0.27	0.41	0.31
6	0.39	0.25	0.66	0.46
7	0.28	0.27	0.35	0.34
8	0.47	0.36	0.54	0.45
mean	0.40	0.29	0.49	0.39
std dev	0.09	0.05	0.14	0.08

Week 8

	Group A		Group B	
9	0.75	0.44	0.80	0.45
10	1.11	0.52	0.54	0.43
11	0.67	0.42	1.00	0.39
12	1.11	0.62	NA	NA
mean	0.91	0.50	0.78	0.42
std dev	0.24	0.09	0.19	0.03

	Group C		Group D	
9	0.56	0.26	0.80	0.40
10	0.31	0.17	0.48	0.40
11	0.36	0.25	0.54	0.47
12	0.66	0.25	0.54	0.37
mean	0.47	0.23	0.59	0.41
std dev	0.17	0.04	0.14	0.04

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values represent ug pantothate/ml wh bld

*Group A=rats fed 120 mg Ca-pantothenate/kg diet, ad lib.
Group B=rats fed the control diet, ad lib.
Group C=rats fed deficient diet, ad lib.
Group D=rats pair-fed the control diet.

APPENDIX G: FREE PANTOTHENATE CONCENTRATION OF RAT PLASMA. *

Rat I.D.	Group A	Group B	Group C	Group D
		Week 2		
1	0.86	0.26	0.02	0.04
2	1.12	0.29	0.01	0.11
3	1.08	0.32	0.02	0.08
4	0.31	0.13	0.004	0.11
mean	0.84	0.25	0.01	0.09
std dev	0.37	0.08	0.006	0.03
		Week 5		
5	0.56	0.30	0.29	0.34
6	0.92	0.67	0.03	0.36
7	0.83	0.47	0.04	0.22
8	0.33	0.11	0.07	0.20
mean	0.66	0.39	0.04	0.28
std dev	0.27	0.24	0.02	0.08
		Week 8		
9	0.45	0.32	0.02	NA
10	0.50	0.59	0.02	NA
11	0.58	0.46	0.03	NA
12	0.50	NA	0.06	NA
mean	0.51	0.46	0.03	NA
std dev	0.05	0.11	0.02	NA

values represent ug pantothenate/ml plasma

*Group A=rats fed 120 mg Ca-pantothenate/kg diet, ad lib.
Group B=rats fed the control diet, ad lib.
Group C=rats fed deficient diet, ad lib.
Group D=rats pair-fed the control diet.

APPENDIX H: FREE PANTOTHENATE CONCENTRATION EXCRETED IN URINE BY RATS. *

Rat I.D.	Group A	Group B	Group C	Group D
		Week 2		
1	364.30	20.40	4.62	24.49
2	379.07	17.92	3.26	29.10
3	387.19	18.16	3.33	16.03
4	360.46	23.23	4.71	16.73
mean	372.76	19.93	3.98	21.59
std dev	12.53	2.47	0.79	6.31
		Week 5		
5	364.31	55.39	6.51	21.38
6	369.16	71.68	2.01	52.34
7	343.68	107.19	2.62	52.62
8	343.70	93.82	3.38	98.43
mean	355.21	82.02	3.63	56.19
std dev	13.46	23.01	2.00	31.75
		Week 8		
9	376.09	56.53	3.92	24.14
10	378.10	103.36	4.67	17.57
11	369.14	121.13	4.74	37.93
12	327.72	NA	4.04	15.58
mean	362.76	93.67	4.34	23.81
std dev	23.67	27.25	0.42	10.10

values represent ug pantothenate excreted in urine/24 hours

*Group A=rats fed 120 mg Ca-pantothenate/kg diet, ad lib.
Group B=rats fed the control diet, ad lib.
Group C=rats fed deficient diet, ad lib.
Group D=rats pair-fed the control diet.

APPENDIX I: ORGAN WEIGHTS OF RATS.*

Rat I.D.	Group A	Group B	Group C	Group D
		Heart (grams)		
		Week 2		
1	0.53	0.73	0.39	0.43
2	0.54	0.50	0.46	0.44
3	0.54	0.59	0.40	0.41
4	0.53	0.96	0.41	0.39
mean	0.53	0.70	0.41	0.42
std dev	0.01	0.20	0.03	0.02
Week 5				
5	0.90	0.62	0.67	0.54
6	1.02	0.86	0.61	0.48
7	0.86	0.84	0.40	0.51
8	0.70	0.71	0.53	0.49
mean	0.87	0.76	0.55	0.51
std dev	0.13	0.11	0.12	0.03
Week 8				
9	1.03	0.86	0.57	0.54
10	0.94	1.01	0.73	0.54
11	0.94	1.07	0.62	0.74
12	1.02	NA	0.62	0.54
mean	0.98	0.98	0.64	0.59
std dev	0.05	0.11	0.07	0.10
		Spleen (grams)		
		Week 2		
1	0.37	0.50	0.24	0.35
2	0.40	0.42	0.30	0.32
3	0.57	0.45	0.23	0.31
4	0.44	0.45	0.29	0.34
mean	0.44	0.46	0.27	0.33
std dev	0.09	0.03	0.03	0.02
Week 5				
5	0.54	0.44	0.32	0.33
6	0.62	0.62	0.28	0.28
7	0.61	0.60	0.25	0.32
8	0.54	0.35	0.27	0.27
mean	0.58	0.50	0.28	0.30
std dev	0.05	0.13	0.03	0.03

Appendix I continues

		(Spleen in grams)		
		Week 8		
9	0.56	0.55	0.25	0.24
10	0.60	0.70	0.41	0.38
11	0.54	0.48	0.31	0.54
12	0.53	NA	0.30	0.33
mean	0.55	0.57	0.31	0.37
std dev	0.03	0.11	0.07	0.13
		Kidney (grams)		
		Week 2		
1	0.94	1.36	0.89	0.88
2	1.05	1.11	1.20	0.93
3	1.08	1.16	0.81	0.46
4	1.20	0.96	0.97	0.89
mean	1.07	1.15	0.97	0.79
std dev	0.10	0.17	0.17	0.22
Week 5				
5	1.56	1.27	1.17	1.12
6	1.84	1.67	1.08	0.97
7	1.62	1.75	0.93	1.08
8	1.69	1.41	1.03	0.94
mean	1.68	1.52	1.05	1.03
std dev	0.12	0.22	0.10	0.08
		Week 8		
9	2.06	1.68	1.05	1.08
10	1.82	2.03	1.04	1.12
11	1.91	1.90	1.60	1.52
12	1.72	NA	1.10	1.17
mean	1.88	1.87	1.20	1.22
std dev	0.14	0.17	0.27	0.20

*Group A=rats fed 120 mg Ca-pantothenate/kg diet, ad lib.
Group B=rats fed the control diet, ad lib.
Group C=rats fed deficient diet, ad lib.
Group D=rats pair-fed the control diet.

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