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Need for pantothenic acid and its relation to ascorbic acid in nutrition of the guinea pig

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NEED FOR PANTOTHENIC ACID AND ITS RELATION TO ASCORBIC ACID IN NUTRITION OF THE GUINEA PIG

by

Cecelia Dolores Pudelkewicz

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Nutrition

Approved:

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In Charge of Major Work

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Head of Major Department

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Dean of Graduate College

Iowa State College
1955

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Pantothenic Acid

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INTRODUCTION

Limited information is available concerning the need for pantothenic acid in the nutrition of the guinea pig. Morgan and Simms (1940) have described what may have been a pantothenic acid deficiency in this species. The fur of three animals fed a diet consisting of casein, sucrose or cornstarch, agar, salts, Crisco, extracted wheat germ with daily supplements of wheat germ oil, ascorbic acid, nicotinic acid and cod liver oil, became dull and metallic, while that of a fourth animal turned completely gray. A crude preparation of the vitamin, however, failed to restore hair coloration or to improve the general condition of the gray animal. The fur of the other three guinea pigs remained dull and metallic but did not gray. Eventually all four animals died.

Mannering, in his excellent presentation of the vitamin requirements of the guinea pig as late as 1949, cited only the Morgan and Simms study with respect to the possible requirement of the guinea pig for pantothenic acid. This, then, seemed to be a fruitful field for experimentation. In the Fall of 1952, the present investigation was planned.

The purpose of the present investigation was two-fold: (1) to produce a pantothenic acid deficiency in the guinea pig in two ways, namely, by feeding an antimetabolite and by omitting the vitamin from the diet, and (2) to determine whether an interrelationship between pantothenic acid and ascorbic acid exists in this species similar to that reported in the rat.

Since pantothenic acid is widely distributed in foodstuffs and is very resistant to destruction by chemical, thermal, or hydrolytic agents in neutral solution, it is unlikely that either man or animal would develop a pantothenic acid deficiency. Hence, in order to study the importance of this vitamin in metabolism, it has been necessary either to feed simplified rations or to administer analogues which antagonize the action of this vitamin.

Early attempts to rear guinea pigs on simplified rations were very discouraging chiefly because of failure to attain satisfactory rates of growth. After the present investigation had been undertaken, Reid and Briggs (1953) described a semi-synthetic diet which supported a rate of growth in young guinea pigs equal to that obtained with commercial pellets. By omitting pantothenic acid from their diet, they were able to produce the first clear-cut symptoms of pantothenic acid deficiency to be reported in this species. The chief gross symptoms of deficiency were

a decrease in the rate of growth, loss of weight, roughening of the fur, diarrhea, anorexia, weakness, inactivity, and death. The conditions known as "spectacled eyes" and porphyrin staining, which occur in the rat, were absent. Black colored portions of the hair in the Beltsville strain became dull in color but showed no signs of loss of pigmentation. There was a reduction in the amount of fat deposited around the kidneys in the deficient animals but this was due to inanition since pair-fed controls showed a similar reduction in fat. The adrenals of the deficient animals were greatly enlarged and some became hyperemic. Several of the animals found dead had hemorrhagic adrenals, however, the authors did not identify the immediate cause of death (Reid and Briggs, 1954).

An investigation of pantothenic acid analogues revealed that of the many compounds which have been prepared, only one, omega-methylpantothenic acid acts as an antimetabolite in animals. It has been reported to have low acute toxicity in rats, mice, and chicks when given orally, intraperitoneally or intravenously. The simultaneous feeding of pantothenic acid during the administration of the analogue reversed its effects (Schinazi et al., 1950).

In 1951, Daft reported that when weanling rats were fed a pantothenic acid-deficient diet which was supplemented

with 2 per cent ascorbic acid, they showed either no pantothenic acid deficiency symptoms, or the development of the syndrome was "at the least greatly delayed or modified". Growth was better, porphyrin accumulation on fur or whiskers less, and length of life increased. The results of Daft's study are startling because normally the rat is able to synthesize as much ascorbic acid as it needs but requires a dietary source of pantothenic acid. Would the guinea pig, a species requiring a dietary source of ascorbic acid, also display a relationship between ascorbic acid and pantothenic acid when the intakes of these two vitamins were varied?

Recently Reid and Briggs (1954) have reported that "the sparing effect of large amounts of dietary ascorbic acid on pantothenic acid deficiency in the rat could not be duplicated in the guinea pig". However, Reid and Briggs reported no record of food intake and used growth and survival as their sole criteria. They conceded that

> Further studies are required, however, before drawing the conclusion that in diets containing suboptimum amounts of pantothenic acid no benefit is derived from the presence of dietary ascorbic acid in excess of the amount necessary to prevent scurvy. (p. 516.)

REVIEW OF LITERATURE

Omega-methylpantothenic Acid

Drell and Dunn synthesized omega-methylpantothenic acid in 1946. This compound contains a methyl group in place of a hydrogen atom on the terminal -CH_2OH group of the pantoic acid moiety of pantothenic acid. The growth of lactic acid bacteria was inhibited markedly by this antimetabolite and the inhibition was reversed, competitively, by addition of pantothenic acid over a wide range of concentrations.

A large number of structural analogues of pantothenic acid have been found to inhibit the growth of micro-organisms. However, only omega-methylpantothenic acid caused the appearance of symptoms of pantothenic acid deficiency in animals (Woolley, 1952, p. 45). Schinazi et al., (1950) found that the effects produced by the administration of the analogue could be reversed by the simultaneous feeding of pantothenic acid, and that, except for interference with the utilization of pantothenic acid, the analogue showed no apparent physiological activity. These toxicity tests were made on chicks, rats, and mice.

Drell and Dunn (1951) studied in great detail the effect of their antimetabolite on mice of the Bagg strain.

Weanling male mice were raised on a purified, pantothenic acid-free diet which was supplemented with the vitamin and the analogue in a variety of combinations. Survival time, weight gain and food consumption of the mice on the pantothenic acid-free diet with analogue decreased in proportion to the level of analogue fed, whereas animals on a diet containing neither pantothenic acid nor analogue gained some weight after weaning. The authors did not attribute failure to gain weight to low food intake. They based their assumption on the fact that when rats were pair-fed, those on the pantothenic acid-free diet supplemented with analogue lost about 25 per cent of their body weight, while those getting both pantothenic acid and analogue made small increases in body weight (2.5 per cent). When the pantothenic acid-free diet with analogue was supplemented with pantothenic acid, the survival time and weight gain of the mice increased. The appearance of the mice, their food consumption, body weight, and survival time depended upon the ratio of inhibitor to pantothenic acid in the diet, rather than upon the absolute amount of analogue.

At a ratio of 100:1 (analogue:pantothenic acid), two groups of mice increased in survival time comparable to that of the deficient group (0:0). At a ratio of less than 100:1 all of the animals survived and gained weight. The authors

labelled the ratio of 100:1 the critical or "interference ratio". Attention was called to the fact that greater weight increases occurred at the 100:1 ratio than at the 0:0 ratio which indicated that the vitamin was being utilized in the presence of the analogue for moderate growth although it exerted no effect on survival time.

The earliest deficiency symptoms observed on the diet with analogue were ruffling of the hair especially on the head and neck. The pattern of hair ruffling was characteristic but differed from that of mice whose diets were deficient in pantothenic acid. The skin became scaly but remained soft and pliable. During the final stages diarrhea developed, the animals became listless, and died. Mice of the CFW strain, deprived of pantothenic acid, developed taut, cracked and bleeding skin, inflammation of the eyelids, closing of one eye, and other symptoms which were completely reversed by the administration of pantothenic acid. Mice of the Bagg strain on the pantothenic acid-free diet displayed the same symptoms as a group receiving the analogue and in addition, exhibited alopecia to various degrees. From these differences in symptoms one could postulate that not all tissues are depleted to the same degree in an analogue-induced deficiency as they are in a dietary-induced deficiency.

Drell and Dunn felt that omega-methylpantothenic acid interfered with the utilization of pantothenic acid rather than caused any inherent toxicity. They support this view by calling attention to the following observations:

(1) The interference index to the levels of inhibitor held constant for levels as high as 0.6 per cent at which level death followed in less than 16 days if pantothenic acid was withheld.

(2) On diets at ratios below the interference ratio, there was a complete absence of all deficiency symptoms.

(3) The syndrome produced by the analogue was reversed when pantothenic acid was given.

Shils (1950) investigated the effect of omega-methylpantothenic acid on the ability of rats to acetylate sulfanilamide. He observed the same results with the analogue that Riggs and Hegsted (1948) had observed in rats with pantothenic acid deficiency induced by removal of the vitamin from the diet, namely, that acetylation was decreased in the pantothenic acid-deficient animals. After he withdrew the analogue and administered the vitamin, the animals showed a marked increase in acetylation.

Novelli and Lipmann (1948) found that omega-methylpantothenic acid interfered with the conversion of

pantothenic acid to Coenzyme A and with restoration of that function of Coenzyme A which was concerned with utilization of pyruvate.

Bean and Hodges (1954) have reported the effects of administration of omega-methylpantothenic acid to 4 human subjects. They fed, by stomach tube, a synthetic diet containing all known vitamins exclusive of pantothenic acid along with 500 mg. of the analogue each day. By the second week of analogue consumption, postural hypotension, dizziness and rapid heart rate on exertion were observed. The men became easily fatigued and slept in the daytime. By the third week they complained of epigastric distress, anorexia, and constipation. The fourth week found the subjects easily upset, quarrelsome, and discontented. They complained of numbness and tingling of the feet and hands. The symptoms became more severe during the fifth week and one subject experienced a constant burning of the feet while another developed an abnormal gait. By this time there was hyperactivity of the deep tendon reflexes, weakness of the extensor muscles of the fingers and impaired sense of balance. During the entire period of deficiency the men had frequent upper respiratory infections and one subject developed pneumonia.

Four grams of pantothenic acid fed along with the analogue failed to bring about a recovery. Only the

paresthesias improved, but the fatigue and malaise increased. One subject became somnolent and another vomited severely. The analogue was discontinued and the subjects were given cortisone along with a good general diet supplemented with vitamins including pantothenic acid, the combination of which brought about a complete and rapid recovery.

Biochemical changes observed during the period of deficiency included:

(1) an impaired ability to acetylate <u>para</u>-aminobenzoic acid

(2) a decline in blood levels of cholesterol and cholesterol esters

(3) adrenal cortical hypofunction as evidenced by the eosinophil response to ACTH, an increased sensitivity to the hypoglycemic effect of insulin, decreased urinary excretion of 17 ketosteroids and defective diuresis after water ingestion

(4) gastric hypochlorhydria

Use of Synthetic Diets for Guinea Pigs

One of the initial attempts to rear guinea pigs on purified diets included yeast as a source of the B-vitamins

(Cannon and Emerson, 1939). Later when crystalline vitamins were used, very poor rates of growth occurred (Woolley, 1942, Woolley and Sprince, 1945, Cannon et al., 1946).

The importance of bulk in a synthetic diet for the guinea pig was established in 1949 by Booth, Elvehjem, and Hart. They tested 14 different substances as sources of cellulose or hemicellulose and found that gum arabic gave the best response consistently. By replacing 15 per cent of the sucrose with gum arabic, the rate of growth over a 6-week period increased from 1.8 to 5.1 gm. per day.

When Booth et al., (1949) fed their synthetic ration with gum arabic along with 25 per cent alfalfa ash, they obtained growth comparable to that which they were getting with commercial stock ration supplemented with ascorbic acid. Further work proved that the active ingredients in the alfalfa ash was magnesium and potassium (Roine et al., 1949). Best growth was obtained when there was a total of 2.5 per cent potassium acetate and 0.5 per cent magnesium oxide in the diet. During 6 weeks on this ration 5 animals gained 7.2 gm. per day, which was the rate of weight gain that Cannon et al., (1945) advocated as "normal". They postulated that growth of less than 7 to 8 gm. per day, from the second to the eighth week of life, could not be considered "normal" in the guinea pig, if growth on natural foodstuffs was to be accepted as the standard.

Recently Reid and Briggs (1953) developed a semi-synthetic diet which promoted a rate of growth and over-all development (of 2 to 4 day-old animals kept on it up to 150 days) which was comparable to that obtained with commercial pellets, that is, 7.1 gm. per day. The basic features of their diet were: 30 per cent vitamin-free casein, 15 per cent cellophane spangles, 7.3 per cent corn oil, 2.5 per cent potassium acetate and 0.5 per cent magnesium oxide in addition to 6 per cent of a salt mixture, 3 sources of carbohydrate (cerelose, sucrose, and corn starch), and large amounts of the vitamins.

Reid and Briggs (1954) used this diet in routine growth experiments with reproducible results and are testing it for adequacy in reproduction. They have produced deficiencies of thiamine, riboflavin, folic acid, choline, pantothenic acid, pyridoxine, and niacin. The requirement of the guinea pig for pantothenic acid has been evaluated with this diet and the quantitative need for other B vitamins is being studied. Omission of corn oil resulted in symptoms characteristic of a deficiency of the essential fatty acids (Reid, 1954).

Pantothenic Acid Deficiency and the Adrenals

One of the characteristics of pantothenic acid deficiency is a fatal necrosis and hemorrhage of the adrenal gland (Daft and Sebrell, 1939). Gross examination of the adrenals reveals them to be hypertrophied and dark in color. Microscopically, they have been described by Ashburn (1940) as showing "congestion, hemorrhage, atrophy, necrosis, scarring, fibrosis, hemosiderin deposition and cortical fat depletion". Daft et al., (1940) have demonstrated the ability of pantothenic acid to prevent or correct adrenal necrosis, hemorrhage and other changes which they have observed. These results have been confirmed by several workers (Mills et al., 1940, Salmon and Engel, 1940, Unna, 1940).

Deane and McKibbin (1946) studied the sequence of events in the development of adrenal lesions. The first changes consisted of a disappearance of ketosteroids from the zona reticularis and zona fasciculata, accompanied and followed by the progressive depletion of sudanophilic droplets from the same area. Then, foci of necrosis and hemorrhage appeared in these zones. In severe cases this progressed until only a thin layer of intact cells remained in the zona glomerulosa.

Because the lipoid material of the adrenal cortex was depleted before necrosis and hemorrhage appeared, Ashburn (1940) postulated that the adrenals in a pantothenic acid-deficient rat might be insufficient functionally. Gaunt and coworkers (1946) supported this view because they were able to show that their deficient animals were more sensitive to water intoxication than the controls.

Deane and McKibbin (1946) took an opposing view. They postulated that the cortex might be overstimulated as the result of a stress reaction. This view has received considerable support. Dumm et al., (1953) have shown that the ability of the pantothenic acid-deficient rat to synthesize adrenal cholesterol is decreased. Winters et al., (1952), and Schultz et al., (1952) studied changes in the adrenals of weanling rats which had suckled females fed a pantothenic acid-deficient diet. Large doses of ACTH intensified the adrenal lesion whereas cortisone protected the rats completely from cortical necrosis and hemorrhage. The adrenals of the pantothenic acid-deficient, cortisone-treated animals could not be distinguished from those of the control rats given the cortisone. These investigators reasoned as follows: depletion of Coenzyme A in the adrenal cortex caused an increased secretion of ACTH with subsequent adrenal hypertrophy; with the progressing depletion of Coenzyme A, the cortex became increasingly

unable to produce and secrete steroid hormones; the sustained high level of circulating ACTH acting upon the glands, which were more and more unable to respond, led to "hemorrhagic necrosis" of the adrenal cortex. This was accompanied with many of the symptoms of pantothenic acid deficiency which were due to malfunction of the adrenal glands, such as hunger for salt, muscular weakness, gastritis, lowered liver glycogen, and hemorrhagic kidneys and thymuses.

Although there was a depletion of cholesterol and other steroids in the adrenal cortex of pantothenic acid-deficient rats, Guggenheim and Olson (1952) claimed that the cholesterol concentrations of the liver, heart, and blood serum in their deficient animals were normal. Bean and Hodges (1954) on the other hand reported a decrease in blood cholesterol and cholesterol esters in human subjects with pantothenic acid deficiency induced by an analogue in the presence of no dietary pantothenic acid.

The adrenal gland is believed to be involved in pigmentation. A pantothenic acid deficiency in black, hooded, or brown rats may be accompanied by the appearance of achromotrichia or graying of the hair. Some workers have shown that this condition is prevented or corrected by including calcium pantothenate in the diet (Unna, 1941,

Henderson et al., 1942). However, whether graying can be prevented or cured completely is controversial. Frost et al., (1941) found that the pure vitamin was only slightly effective in preventing graying in their animals, while Dimick and Lepp (1940) claimed that some graying in their dogs persisted even when they were given 50 mcg. of calcium pantothenate per day. This amount, no doubt, was insufficient for therapeutic purposes.

Emerson and Evans (1941) reported that pantothenic acid failed to restore the original color of the hair of their deficient animals but merely stippled it. Ralli and Graef (1945) found that adrenalectomy prevented gray hair in pantothenic acid-deficient rats and caused a reversal of the achromatrichia due to a lack of this vitamin. When desoxycortisone was injected, the deposition of melanin which had been brought about by the adrenalectomy was inhibited (Spoor and Ralli, 1944). Hence, it appeared that some cortisone was being produced during pantothenic acid deficiency. Two reports, one by Ralli (1946), the other by Dumm and Ralli (1948) have presented evidence that adrenalectomy increased the requirement for pantothenic acid as indicated by the prolonged survival rate of more than half the rats receiving 4 mg. or more of calcium pantothenate daily, after adrenalectomy. However,

removal of the adrenals did not modify the proportion of administered pantothenic acid which was excreted in the urine (Dumm and Ralli, 1949). What these findings signify is not clear, but they do show that the adrenal cortex is in some way concerned with either the production of melanin or its deposition.

It is also recognized that dietary deficiencies in addition to pantothenic acid may be involved in achromotrichia under appropriate experimental conditions. Richter and Clisby (1941) found that phenylthiocarbamide, a substance which inhibits the action of copper-containing oxidases, produces graying in rats. Therefore, in a copper deficiency, a graying of the hair may be due to decreased activity of tyrosinase or of dopa oxidase. Sealock and Goodland (1951) demonstrated that ascorbic acid also acted as a coenzyme in the oxidation of tyrosine; the velocity of the oxidation depended upon the concentration of ascorbic acid which was present. However, gray hair has not been reported in scurvy. Free (1940) and Henderson et al., (1942) were unable to relieve the graying of copper deficient rats with pantothenic acid. On the other hand, Singer and Davis (1950) report that they cured the graying of copper-deficient rats by using additional calcium pantothenate. They suggested that copper

and pantothenic acid may be metabolized together and that during a copper deficiency the pantothenic acid requirement may be accentuated.

Hundley and Ing (1951) did copper analyses on the skins of pantothenic acid-deficient rats, on litter mates receiving adequate pantothenic acid, and in similar rats on stock diet and they found that the deficient animals had about 5 times more copper in their skins than the other 2 groups. When weanling rats were fed the pantothenic acid-deficient diet until graying was well established and then treated with pantothenic acid for 2 weeks, the concentration of copper in the skin dropped down to normal levels. The authors interpreted their data as suggesting that pantothenic acid deficiency may produce gray hair by blocking the utilization of copper in hair growth and melanin formation.

Sodium, chloride, and potassium balances are known to be upset by adrenal cortical hypofunction. Ralli et al., (1941) found that "filtrate factor" deficient rats showed graying of the fur much earlier and more severely when given a low salt intake than when given a normal or high salt intake. A study by Gaunt et al., (1946) revealed a failure of water metabolism in pantothenic acid deficient rats. The animals were unable to excrete water and to

resist water intoxication but administration of either adrenal cortical extract or of pantothenic acid restored this ability.

METHOD OF PROCEDURE

General Experimental Plan

Experiment A

An attempt was made to produce a pantothenic acid deficiency in growing male guinea pigs by feeding the analogue, omega-methylpantothenic acid, along with a complete natural ration. For the first 15 days of the study the antimetabolite was fed as 0.15 per cent of the intake, then it was increased to 0.30 per cent for the next 18 days, and finally increased to 0.40 per cent for the remaining 14 days of the study. Each experimental animal had a litter mate as its control. Three pairs of guinea pigs were sacrificed after the first 15 days and the last of the remaining 6 pairs of pigs were sacrificed on the 47th day of the experiment. The two groups were compared on the following bases:

(1) weight gain
(2) ration consumed and food efficiency
(3) red blood cell count
(4) red blood cell volume and hemoglobin concentration
(5) ascorbic acid concentration in blood serum

(6) pyruvic acid level in the blood

(7) condition at autopsy

(8) histological examination of testes and adrenal glands of 2 animals from each group at the conclusion of the experiment

Experiment B

The possibility of an interrelationship between ascorbic acid and pantothenic acid in the guinea pig was investigated by feeding a complete semi-synthetic ration with 4 levels of ascorbic acid and 4 levels of calcium pantothenate according to the following plan:

		Ascorbic acid levels			
		0	/	///	
Calcium pantothenate levels	0				
	/				
	///				// // Control

/ indicates minimal amounts

// indicates normal or average amounts

/// indicates excess amounts

The experiment was replicated 3 times, Study I, Study II, and Study III. In Study I, for the first 5 days, all

9 pigs were given 10 mg. ascorbic acid and 0.3 mg. calcium pantothenate per day. On the sixth day they were given neither ascorbic acid nor calcium pantothenate. On the seventh day the experimental regimen was started. The 2 guinea pigs getting maximal calcium pantothenate received a total of 180 mg. of the vitamin during the first 2 days of experimental feeding. Beginning with the third day they were given 8 mg. calcium pantothenate per day. In Study II and Study III the experimental dietary regimen was begun immediately upon the arrival of the animals, except that the 3 pigs scheduled to receive no ascorbic acid were given a single 10 mg. dose of the vitamin on the first day. In each study the following data were considered:

(1) weight gain

(2) ration consumed and food efficiency

(3) red blood cell volume and hemoglobin concentration

(4) blood plasma ascorbic acid concentration measured at weekly intervals and at conclusion of study

(5) weight of adrenal glands and concentration of ascorbic acid in the adrenals at the conclusion of the study

(6) pyruvic acid level in blood at conclusion of the study

(7) amount of pantothenic acid excreted in the urine during a 3-day collection period each week

(8) concentration of pantothenic acid in the blood and liver at the conclusion of the study

(9) condition at autopsy.

Preliminary Work

Confirmation of Daft's study

First, the author repeated the experiment which Daft (1951) had published. Thirty, 28-day-old albino rats of the Wistar strain were studied for a period of 12 weeks. Fifteen male and 15 female rats were randomly distributed into one of three groups. Group I received a semi-synthetic diet with neither pantothenic acid nor ascorbic acid, Group II, a semi-synthetic diet containing 2 per cent ascorbic acid but with no pantothenic acid, and Group III, a semi-synthetic diet with one mg. calcium pantothenate per day but no ascorbic acid.

The experiment confirmed the results of Daft's study with the following added observations:

(a) The differences in the rate of growth among the 3 groups of male rats were much more striking

than among the 3 groups of more slowly growing female rats.

(b) Ascorbic acid seemed to exert a beneficial effect on the pantothenic acid concentration in the blood of both male and female animals receiving no pantothenic acid, thus emphasizing the possibility of an interrelationship between the two vitamins.

(c) Blood serum ascorbic acid levels seemed unaffected by either the presence or absence of 2 per cent ascorbic acid in the diet of both male and female rats.

Upon the completion of this experiment, plans were made for a similar study using the guinea pig as the experimental animal.

Guinea pig stock colony

In order to supply the very young guinea pigs which would be required for a pantothenic acid study, as well as to supply animals whose nutritional backgrounds were known, a guinea pig stock colony was started on November 18, 1952. Ten male and 10 female animals of breeding stock were obtained from the Gopher State Caviary in St. Paul, Minnesota.

The animals were housed in 18" x 12" x 8" wire cages which were placed over wood shavings. The shavings were

changed daily. The adult animals were weighed twice weekly, the offspring daily. The stock diet consisted of commercial rabbit pellets, 10 mg. ascorbic acid per day, 10 to 15 gm. lettuce three times per week, and one drop of oleum percomorphum per week. Salt licks were also provided.

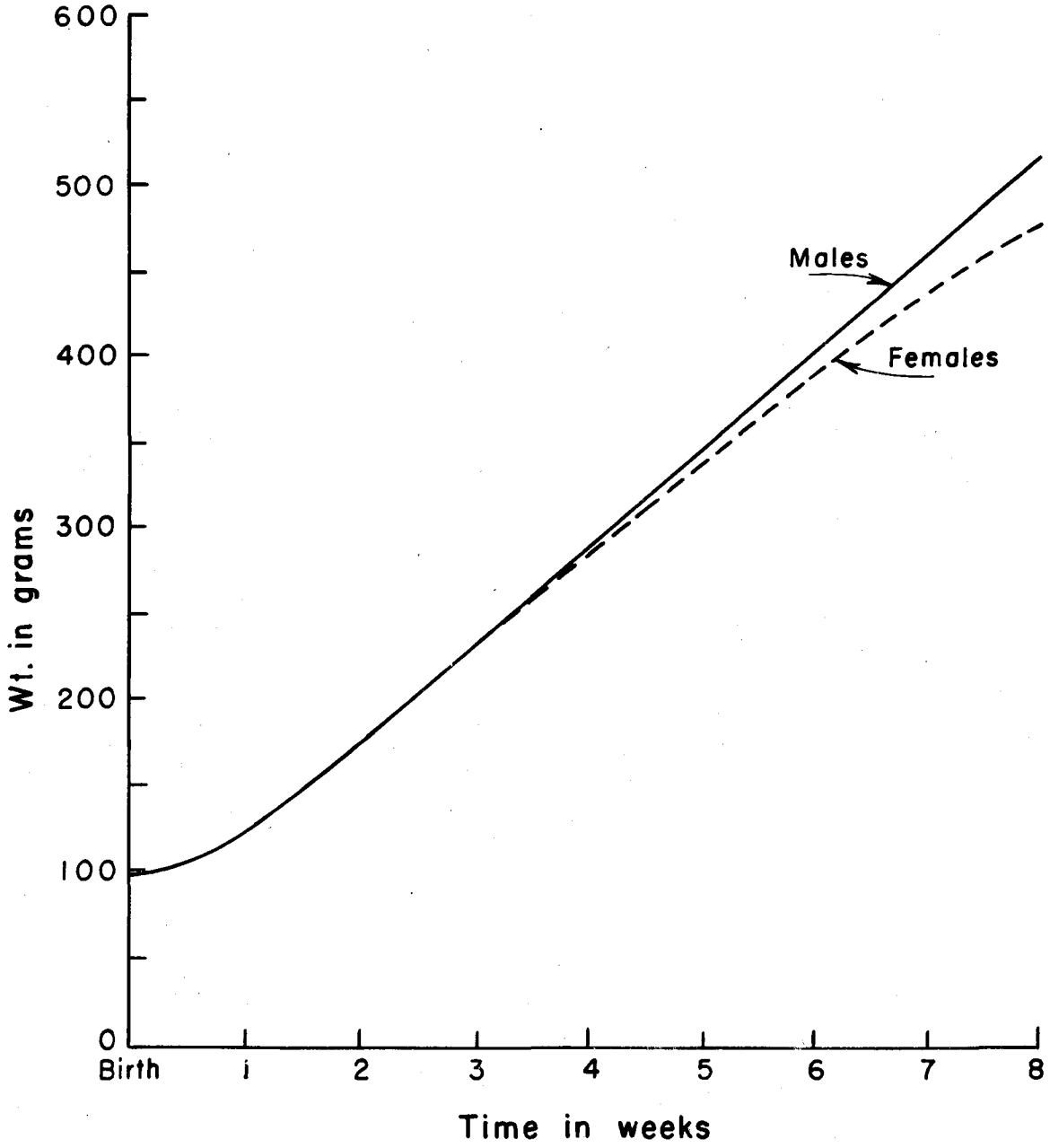
From November until the following July, a total of 76 young pigs had been born. Of these, 21 pigs (28 per cent) were either born dead or died soon after birth. Average growth curves of 34 male and 13 female animals which were maintained on stock diet from birth until 8 weeks of age are plotted in Figure 1. The young were generally weaned at 23 days of age. Individual weights for each pig as well as the average weight gain per animal per day during this 8-week period may be found in Appendix Table A.

Some of the new-born young were used for experimental purposes such as testing synthetic diets, trying out various "harnesses", and in urine collecting experiments. The stock colony was discontinued in July, 1953, due to lack of space and because of the inability of the animals to withstand a hot Iowa summer.

Problem of coprophagy

Since the guinea pig is notorious for coprophagy, and since this habit would be a detriment to an experiment in

Figure 1. Normal growth curve of stock colony guinea pigs fed rabbit pellets, 10 mg. ascorbic acid per day, and 1 drop oleum percomorphum per week


600
500
400
300
200
100
0
Wt. in grams
Males
Females
Birth
1
2
3
4
5
6
7
8
Time in weeks

which one of the B-vitamins was being studied, several attempts were made to design a "harness" which might prevent this practice. It was essential that the "harness" be light in weight and cause the animal a minimum of discomfort.

Many variations of cardboard collars and fastenings were attempted, but as might be expected, the possibility of strangulation was a definite hazard. The most nearly satisfactory "harness", one which seemed to cause the animal the least discomfort, consisted of a very light piece of wood (such as a wooden spoon, broken in two, crosswise) which was covered with adhesive tape and fastened over the abdomen lengthwise. The adhesive tape prevented the wood from splintering when holes were punched for inserting a cord. The cord was crisscrossed across the animal's back. So long as an animal remained in the "harness", it was very effective, but by stretching the body, together with wiggling motions, the pigs eventually managed to free themselves. There was always the danger of tying the cord too tightly and thereby preventing normal circulation or uninhibited swallowing.

It was noted that while in a collar or "harness", the pigs consumed less food and required constant attention because of their attempts to free themselves. Therefore, it was decided to study the guinea pig under usual conditions.

Pilot study using the analogue

In order to ascertain the most suitable levels at which to feed the analogue, omega-methylpantothenic acid, a preliminary study was made in which one, 12-day-old guinea pig was fed rabbit pellets into which the analogue had been incorporated. The pellets were supplemented with 10 mg. ascorbic acid per day and one drop of oleum percomorphum per week. For the first 14 days the analogue was fed at 0.15 per cent and for the following 19 days, it was increased to 0.30 per cent of the intake. By the 33rd day of analogue consumption, the guinea pig was in such a state of debility that it was necessary to sacrifice it.

It was interesting to note that the animal had a good appetite and continued to gain weight at approximately 4.5 gm. per day while ingesting the antimetabolite. Some of the observations noted during the final three days of the study included rapid respiration, lassitude, paleness of the ears, eyelids, nose, and paws and a coldness of the extremities. The fur of the animal showed a decided change in texture. It became very soft and woolly and stood on end. Lacrimation, which consisted of a thick viscid substance, also was observed. At autopsy, the adrenals were found to be hemorrhagic and the liver showed evidence of

fatty infiltration. The blood was pink in color and clotted very slowly.

Equipment for balance study

Cages. Round rat metabolism cages were converted into metabolism cages for the guinea pigs by the Instrument Shop of the Physics Department. A portion of one side of the cage was cut away and a rounded screen extension which housed the feed cup was added. This extension was on hinges and had a fastening, thus making the feed cup, which was easily removed by sliding upward and out, conveniently accessible. By housing the feed cup, the extension also gave the animal a little more space for movement. Three metal strips which served as feet were soldered to the bottom of the cage so that the cage would stand firmly over a large collecting funnel. The animals were maintained in metabolism cages and occupied the same relative position on top of the metabolism tables during the entire course of each metabolism study. Preliminary work had indicated a decreased food consumption with a corresponding decrease in weight when the pigs were changed from the more spacious guinea pig cages to the smaller metabolism cages.

Metabolism tables. A metabolism table was especially constructed by the Physical Plant, according to specifications, and was made to accommodate 6 pigs at one time.

It had 6 holes, about 10 inches in diameter, conveniently spaced across the top of the table. About 20 inches below, there was a shelf which held the collection bottles. This table proved to be very satisfactory for our purposes. A temporary metabolism table, which was available in the department, was used in making collections from the 3 pigs in each metabolism study which received no ascorbic acid. Thus, facilities were available for collections from 9 pigs at one time.

Method of urine collection. At first, urine had been collected by placing a round rat metabolism cage over a large Pyrex pie plate. The cage was held in place with metal holders. To catch feces and spilled food, some screening was cut the size and shape of the cage and large tacks, about one inch long, were soldered to the screening in several places, thereby supporting the screen over the Pyrex plate. This method proved unsatisfactory for several reasons. The relatively large amount of urine excreted warranted the use of a preservative, which, because of its proximity to the animal was irritating to it; also, guinea pigs tended to play with their water bottles, and excessive spillage could result in the loss of part of a collection.

A modification of the procedure used by Storvick (1933) was adopted finally and a picture of the equipment can be

seen in Plate 1. A large funnel was fitted into the opening in the metabolism table. Into the funnel was placed a round piece of fine screening to catch spilled food and feces. The round metabolism cage stood firmly on its three feet over the funnel. The tip of the funnel touched the top of a pear-shaped glass bubble which was supported by glass hooks over the edge of a small funnel which in turn fitted into the collection bottle. As a precautionary measure, the collection flask was placed into a wide-mouthed glass container (Heinz mayonnaise jar). The urine followed the walls of the large funnel to the glass bubble, around which it dripped into the collection flask. At the beginning of each balance period, 100 ml. of distilled water and 5 ml. of toluene were placed into each collection flask.

To test this method of collection a one-day sample of urine was collected and an aliquot removed and frozen. The remainder of the sample was allowed to stand at room temperature for 2 days before freezing. The aliquot which was frozen immediately assayed 17.6 mcg. of pantothenic acid per day, while that allowed to stand at room temperature assayed 18.2 mcg. per day. These results are within experimental error and indicate no loss of the vitamin by this method of collection.

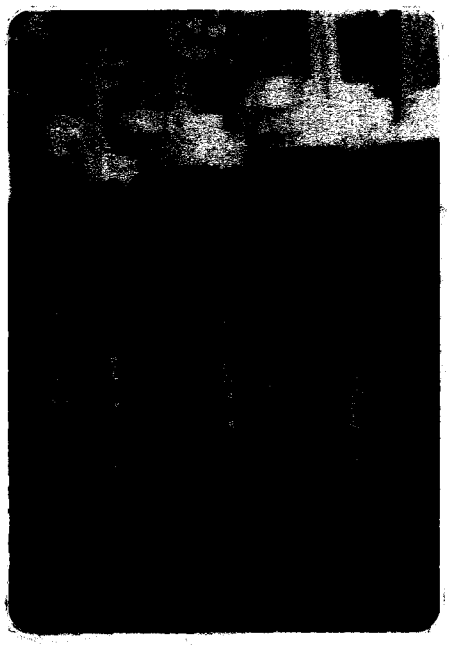


Plate 1. Equipment used in urine collections

Animals Used and Their Care

Experiment A

Nine pairs of weanling male albino guinea pigs were obtained for the experiment from the Gopher State Caviary, St. Paul, Minnesota. They weighed, on the average, 169 gm. (range, 138 to 232 gm.) and were housed in individual cages during the entire experiment. The pigs were weighed daily and fed their ration and distilled water ad libitum. Three pairs of pigs were sacrificed after the first 15 days of the study.

Experiment B

Study I. Nine male guinea pigs were kindly supplied for Study I by Dr. Paul Bennett of the Veterinary Medicine Division. The animals varied widely in weight, from 91 to 210 gms. Four of the animals had not been weaned. One of these served as a control and the other 3 were placed on the diet containing no pantothenic acid. The smallest of these animals was 3 days old and weighed 91 gms. The other 5 animals had been weaned and the 2 largest ones were assigned to the scorbutic diet. The remaining 3 pigs were assigned one of the remaining diets at random. In

short, the pigs were distributed according to their body weights after the following plan:

Study I

Ascorbic acid intake per day

		0	2 mg.	40 mg.	10 mg.
		gm.	gm.	gm.	gm.
Calcium	0	146	150	91	
pantothenate	0.06 mg.	207	179	171	
intake per day	8 mg.	210	191		
	0.2 mg.				167

The animals were weighed daily and fed a semi-synthetic diet and distilled water ad libitum.

Study II and Study III. Two to four-day-old male guinea pigs were ordered from the Gopher State Caviary for both studies. The animals were placed on a train in Minneapolis at 4:00 P.M. one day and picked up at the station in Ames the following morning at 5:00 A.M. For Study II, 10 animals ranging in weight from 84 to 116 gm. were received, while for Study III, 12 pigs whose weights ranged from 68 to 110 gm. were received. The animals were assigned diets according to their body weights after the following plan:

Study II

Ascorbic acid intake per day

		0	2 mg.	40 mg.	10 mg.
		gm.	gm.	gm.	gm.
Calcium	0	112	84	85	
pantothenate	0.06 mg.	103	98	109	
intake per day	8 mg.	112	99		
	0.2 mg.				101

Pellet control 116 gm.

Study III

Ascorbic acid intake per day

		0	2 mg.	40 mg.	10 mg.
		gm.	gm.	gm.	gm.
Calcium	0	110	83 85	68 78	
pantothenate	0.06 mg.	85	95	107	
intake per day	8 mg.	93	99		
	0.2 mg.				100

The pigs were weighed daily and fed a semisynthetic diet and distilled water ad libitum.

The Ration

Experiment A

The guinea pigs were fed a basal diet of Nutrena rabbit pellets supplemented with 10 mg. of ascorbic acid per day and one drop of oleum percomorphum per week. Both supplements were administered *per os*, the ascorbic acid as a solution in 70 per cent glucose. Approximately 1250 units of vitamin A and 180 units of vitamin D were provided by one drop of oleum percomorphum. Nine experimental animals were fed this diet along with calcium *omega*-methylpantothenate which was incorporated into the rabbit pellets.

The analogue was purchased from the H. M. Chemical Co., Ltd., Santa Monica, California in 3 lots, one of 87 per cent purity and the other 2 of 83 per cent purity. Allowances were made for the impurities. The analogue was incorporated into the rabbit pellets after they had been ground in a Wiley mill. The ration was then moistened with a small amount of distilled water, re-pelleted[1], and dried under an infra red lamp for approximately 2 hours at a temperature under 55° C.

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[1]Appreciation is expressed to the Mechanical Engineering Department for use of their plastic mold for re-pelleting the ration.

Experiment B

The semi-synthetic ration used in Experiment B was a modification of that used by Reid and Briggs (1953). Its composition is given in Table 1. The fact that this diet is semi-synthetic is recognized, but for the sake of convenience, it will be referred to hereafter as a synthetic diet.

The ration was made up in 4 kg. batches and kept frozen at -4° F. As needed, 400 gm. lots were mixed with 35 to 40 ml. of distilled water, rolled out, and cut into small bite size pieces. The ration was spread out on trays and dried, with frequent turning, in an air oven for approximately 3 hours at a temperature below 55° C. When thoroughly dried, it was cooled, placed in tightly covered containers in a freezer for storage.

Vitamin B_{12}, calcium pantothenate, and ascorbic acid were fed to each pig *per os* daily while on experiment. One ml. of a solution of vitamin B_{12}, containing 30 mcg. per ml. of sterile saline (Eli Lilly), was diluted with 5 ml. of distilled water. One drop, which contained approximately 0.25 mcg. of the vitamin, was given to each animal per day while on experiment.

Ascorbic acid was weighed out each morning on the Roller-Smith balance (accurate to 0.2 mg.) and dissolved

Table 1. Composition of the synthetic ration

Ingredients	Amount (gm./kg.)	Ingredients	Amount (mg./kg.)
Vitamin-free casein	300	Thiamine HCl	16.00
Corn oil	73	Riboflavin	16.00
Gum arabic	150	Pyridoxine HCl	16.00
Salts[a]	50	Niacin	200.00
Magnesium oxide	5	Biotin	0.60
Potassium acetate	23	Folic acid	10.00
Glucose	116	Alpha tocopherol acetate	20.00
Sucrose	116		
Dextrin	117	2-Methyl-1,4-naphthoquinone	2.00
Choline chloride	2	Oleum percomorphum[b]	300.00
Inositol	2		

[a]Phillips and Hart (1935).

[b]18,000 U.S.P. units Vitamin A
2,550 U.S.P. units Vitamin D

in a 70 per cent solution of glucose (20 mg./ml.). The animals receiving the minimal amount, which was 2 mg. per day, were given 0.1 ml. from a pipette; the control received 10 mg. per day in one 0.5 ml. dose, and the animals receiving the excess amount were fed 40 mg. per day in four 0.5 ml. doses. Between feedings, the beaker

containing the ascorbic acid solution, was covered tightly with aluminum foil and refrigerated.

Calcium pantothenate was also weighed out each morning on the Roller-Smith balance. It was dissolved in distilled water and fed in Study I and Study II according to the following plan: 20 mg. calcium pantothenate were dissolved in 5 ml. of distilled water. Animals receiving the excess amount were given 0.5 ml. of this solution, 4 times each day, or a total of 8 mg. per day. Of the above solution, 0.5 ml. was mixed with 9.5 ml. of water. The control was given two, 0.5 ml. doses each day of this solution, or a total of 0.2 mg. per day. The animals scheduled for the minimal amount were given 0.3 ml. of this second solution, or 0.06 mg. per day.

In Study III, the pigs receiving the excess amount of calcium pantothenate were given 1 mg. per day instead of 8 mg. which had been fed in Study I and Study II. The dilution was made in this manner: 20 mg. calcium pantothenate were dissolved in 5 ml. of water. Of this solution, 0.5 ml. was further diluted with 4.5 ml. of water. The 2 animals getting the excess amount of calcium pantothenate were each fed this solution in 5 doses of 0.5 ml. per day.

Sacrifice and Autopsy of the Animals

Experiment A

Three experimental and three control animals were sacrificed on the 14th day of the study, because preliminary work had indicated this time as the probable mid-point of the experiment. On the 35th day of the study one of the six remaining experimental animals and its litter-mate control were sacrificed. The animals were sacrificed (in both Experiments A and B) when it was feared that they might die before a blood sample could be obtained. On the 45th day another pair of litter-mates were sacrificed, and on the 47th day of the experiment the study was terminated.

Gross observations of the physical condition of the guinea pigs were made before they were injected intraperitoneally with about 1 ml. of a nembutal solution (4-1/2 grains of nembutal in 10 ml. of water). Blood samples were obtained from the portal vein. Aliquots of the blood were measured immediately for pyruvic acid determinations and the rest was mixed with an appropriate amount of dried double oxalate, tightly stoppered, and placed in the refrigerator. During autopsy, the condition of the lungs, liver, kidneys, adrenal glands, intestinal

tract, and visceral fat stores was observed and recorded.

The testes and adrenal glands of 2 experimental animals and of their litter-mate controls, namely, guinea pigs 1E and 1C, and 2E and 2C, were removed and placed in Bouin's fixative for histological examination.

Experiment B

The animals were anesthesized with an intraperitoneal injection of a nembutal solution prepared as for Experiment A. Blood was obtained from the portal vein. Part of the blood was measured immediately for pyruvic acid determinations and the rest was mixed with an appropriate amount of dried double oxalate, tightly stoppered, and refrigerated. Immediately after the pigs were bled the adrenal glands were removed, weighed to the nearest 0.2 mg., covered with a cold solution of 5 per cent metaphosphoric acid and placed in the freezer. The liver was removed, blotted with filter paper, weighed to the nearest 0.1 gm., wrapped in aluminum foil and frozen. The intestinal tract was removed and weighed. Records were made of the condition of the lungs, liver, kidneys, adrenal glands, intestinal tract, and fat stores. All animals were carefully examined for scorbutic symptoms.

Methods and Procedures

Hematological

Red blood cell counts. Red blood cell counts were made in Experiment A only. Oxalated blood was diluted 1:200 with Hayem's diluting fluid using N.B.S. Trenner automatic pipettes. Two complete dilutions were made with pipettes which had been matched and duplicate counts from each dilution agreeing within 200,000 cells per $mm.^3$ were averaged. The counts were made on a Bright-Line counting chamber.

Packed red blood cell volume. Determinations of packed red blood cell volume were made, in duplicate, with the Van Allen hematocrit tube. The blood was diluted with a 1.3 per cent solution of sodium oxalate.

Hemoglobin. Hemoglobin determinations were made by a modification of the procedure of Wintrobe (1946, p. 255) which measures oxyhemoglobin. Twenty $mm.^3$ of oxalated blood were diluted with 8.0 ml. of a 0.5 per cent solution of ammonium hydroxide and read in the Beckman spectrophotometer at a wave length of 540 mu. When small amounts of blood were obtained, 10 $mm.^3$ of oxalated blood were diluted with 4.0 ml. of the ammonium hydroxide solution. The Beckman spectrophotometer was standardized by the oxygen capacity

method of Peters and Van Slyke (1932, p. 263). Since it has been demonstrated that oxyhemoglobin from different sources may differ in both spectroscopic characteristics and in affinity for oxygen (Hawk, Oser, and Summerson, 1951, p. 424), iron determinations by the Wong procedure (Hawk et al., 1951, p. 564) were also made. They substantiated the hemoglobin values obtained by measuring the oxyhemoglobin formed.

Chemical analyses

Blood serum or plasma ascorbic acid. Blood serum or plasma ascorbic acid determinations were estimated by the Lowry, Lopez, and Bessey (1945) microadaptation of the dinitrophenylhydrazine method of Roe and Kuether (1943). Ten mm.^{3} of blood serum or plasma were deproteinized with 40 mm.^{3} of 5 per cent trichloroacetic acid. A 30 mm.^{3} aliquot of the clear supernatant solution was transferred to another 6 x 50 mm. tube; the tubes were tightly stoppered and stored immediately at -4° F. Later 10 mm.^{3} of thiourea, copper sulfate-dinitrophenylhydrazine reagent was added to each tube with tapping. This reagent was also added to tubes which contained 30 mm.^{3} aliquots of trichloroacetic acid and of standard ascorbic acid solutions. The tubes were capped and incubated in air for 4 hours at 37° C. They were then chilled in ice water and

50 mm.^{3} of ice cold 65 per cent sulfuric acid were added slowly. The tubes were well shaken, cooled again, and removed from the ice bath. After standing for 30 minutes to 3 hours at room temperature, they were again mixed by tapping. The solution was transferred to cuvettes and the light absorption measured at 520 mu in the Beckman spectrophotometer. Six determinations were made on each blood sample.

In this procedure all of the ascorbic acid is oxidized to dehydroascorbic acid in the presence of copper sulfate. The resulting dehydroascorbic acid upon incubation with 2,4-dinitrophenylhydrazine produces a derivative which when treated with 65 per cent sulfuric acid develops a red color.

Adrenal ascorbic acid. The method of Roe and Kuether (1944) was also used to determine the ascorbic acid concentration of the adrenal glands. The adrenals were weighed on a Roller-Smith balance to the nearest 0.2 mg. and then ground in a cold mortar containing cold 5 per cent metaphosphoric acid. The suspension was transferred to a 25 ml. volumetric flask, made to volume and centrifuged at 3000 r.p.m. for 10 minutes. At this point the supernatant was removed, placed in a small Erlenmeyer flask, tightly stoppered and frozen. Then 5, 10, or 15 ml. aliquots of the supernatant were transferred into a 25 ml. volumetric

flask and oxidized with bromine water dropwise until a faint yellow color indicative of an excess persisted. Thiourea was added to decolorize the bromine and the solution made to volume with 5 per cent metaphosphoric acid. Then 4 ml. aliquots were removed and placed into 3 test tubes. To 2 tubes, 1 ml. of 2,4-dinitrophenylhydrazine was added and the mixture incubated for 3 hours in a water bath at 37° C. The third tube was left at room temperature and served as a blank. At the completion of the incubation period, both tubes were immersed in an ice bath and to each was added 5 ml. of 85 per cent sulfuric acid drop by drop from a burette. To the blank tubes 1 ml. of 2,4-dinitrophenylhydrazine was added. Then both tubes were removed from the ice bath and allowed to stand at room temperature for 30 minutes. Solutions were read in the Beckman spectrophotometer at 520 mu.

Ascorbic acid standard was prepared by dissolving 25 mg. of pure crystalline ascorbic acid in approximately 200 ml. of 5 per cent metaphosphoric acid contained in a 250 ml. volumetric flask. The solution was treated with bromine water until an excess was present, decolorized with thiourea, and made to volume. From this solution convenient aliquots were diluted in order to prepare a standard curve. From these dilutions, 4 ml. aliquots were

pipetted into each of 2 test tubes and treated in the same manner as the unknown solutions. Three blank determinations were made for the standard.

Blood pyruvic acid. Pyruvic acid concentrations of the blood were determined by the micromethod of Tsao and Brown (1950). One-tenth ml. of unoxalated blood was deproteinized with 0.5 ml. of 10 per cent metaphosphoric acid. The original blood samples were pipetted in duplicate and four 100 $mm.^3$ aliquots of supernatant solution were removed from each tube and treated with 33 $mm.^3$ of a 2,4-dinitrophenylhydrazine solution. The solutions were mixed by shaking. To this mixture, 100 $mm.^3$ of xylene were added and the tubes were shaken in a Kahn shaker for 2 minutes, followed by centrifugation at 2500 r.p.m. for 5 minutes. The bottom layer was completely removed and discarded. The hydrazone of pyruvic acid was then extracted from the xylene by adding 100 $mm.^3$ of 10 per cent sodium carbonate solution and shaking for 3 minutes. Again the tubes were centrifuged, and 75 $mm.^3$ of the lower carbonate layer were transferred to a microcuvette, 18 $mm.^3$ of approximately 7 N sodium hydroxide added, and the contents of the cuvette were mixed. All readings were made in the Beckman spectrophotometer at a wave length of 520 mu between 2 and 3 minutes after the color had developed.

Reagent blanks and standard pyruvic acid solutions, all in triplicate, were treated in a similar manner.

Histological procedure

Experiment A. The testes and adrenal glands of guinea pigs 1E, 1C, 2E and 2C were fixed in Bouin's fixative for 24 hours, washed in 70 per cent alcohol (24 hours) and stored in 70 per cent alcohol. They were dehydrated in 3 changes of dioxane and then embedded in paraffin (Altman's mixture) after 3 changes in melted paraffin (56 - 58° C.). Sections were made at 10 u thickness and stained with Delafield's hematoxylin and eosin.

Balance procedure

Experiment B. A group of 3 animals was set up on funnels for 3 successive days once each week. Except for the first collection in Study I which lasted for 4 days, all collections consisted of 3-day periods.

In Study I, feed cups were removed 5 to 6 hours before the animals were placed on balance. Two to three hours after removal of the feed cups blood samples from an ear vein were collected for microascorbic acid determinations. Since there was not enough fat on the blood plasma to interfere with measurement of plasma aliquots and since

the pantothenic acid and ascorbic acid were omitted from the ration, this procedure was not followed in Study II and Study III.

On the first day of collection, the ascorbic acid and calcium pantothenate were withheld until after the animals had been set up on funnels. Likewise, on the last day of balance, the vitamins were withheld until after they came off balance. Urine collection was completed by washing the cage bottom, screening, funnel, and glass bubble with a fine spray of warm distilled water. The urine and washings were filtered through glass wool and made to known volume. The pH was adjusted to 7 and the samples covered with a thin layer of toluene and stored in a freezer until assays were completed.

Microbiological assays

Pantothenic acid assays of liver, blood, rabbit pellets, and urine were made by microbiological assay with Lactobacillus arabinosus. Lactic acid produced by the bacteria was titrated against 0.1 N NaOH with brom thymol blue as the indicator.

Basal medium used was essentially that of the American Association of Agricultural Chemists and United States Pharmacopoeia (1945, p. 362) with the following modifications per 100 ml. double strength media:

Sodium acetate was increased from 2.0 to 4.0 gm.

50 mg. of l-asparagine and 40 mg. of glutamic acid were added.

Thiamine, riboflavin, niacin, and para-aminobenzoic acid were doubled in amount.

Biotin was increased from 0.08 to 0.2 mcg., pyridoxine was increased from 20 mg. to 100 mg. and 4 mcg. of folic acid were added.

Liver. Livers were homogenized with acetate buffer (pH 4.6 to 4.8) in a Waring blendor, diluted to a known volume, covered with a thin layer of toluene and frozen. The frozen tissues were thawed at room temperature. One ml. aliquots were treated with 0.1 ml. of a freshly prepared solution of intestinal phosphatase (containing 100 mg. dissolved in 10 ml. of distilled water), 0.05 ml. of chicken liver enzyme preparation, and 0.1 ml. of sodium bicarbonate (0.1M) according to the procedure of Novelli et al., (1949). The samples were incubated in a water bath at a pH of 8.5 for 4 hours at 37° C. Adjustment of pH to 6.6 to 6.8 and dilution to known volume completed the treatment.

Blood. Duplicate one ml. aliquots of oxalated blood were pipetted into 9 ml. of distilled water. Of this 1:10 dilution, 3 ml. aliquots were incubated at pH 8.5 with the

same amounts of the enzymes in exactly the same manner as the liver samples, except that the incubation period was shortened to 3 hours. After incubation the samples were diluted to approximately 10 ml., 4 to 5 drops of 0.1N hydrochloric acid added and the samples autoclaved at 15 pounds for 10 minutes to coagulate the protein. Dilution to 50 ml. followed by filtration completed the treatment for the blood samples.

Rabbit pellets. The pellets were ground in a Wiley mill, mixed with acetate buffer, and diluted to approximately 200 ml. The pH was adjusted to 6.6 to 6.8 and the mixture autoclaved at 15 pounds for 10 minutes. After cooling the volume was adjusted to 250 ml. and then the samples were filtered through glass wool. Of the filtrate, 2 ml. aliquots were incubated with the 2 enzymes used for liver and blood at a pH of 8.5 for 4 hours. After incubation the pH was adjusted to 6.6 to 6.8 and the samples diluted to 250 ml.

The amount of pantothenic acid present in the enzymes used for liberation of bound pantothenic acid from the liver, blood, and rabbit pellets was determined and the appropriate corrections were applied.

Urine. Two ml. aliquots of urine were incubated with the 2 enzymes used for liver and blood at a pH of 8.5 for

4 hours. The concentration of pantothenic acid after such treatment was not increased above that obtained when no enzyme treatment was used. For example, when the concentration without enzyme was 22.8 mcg., that with enzyme was 21.7; 12.4 mcg. without enzyme corresponded to 12.2 mcg. with enzyme. These values are within experimental error. Therefore, aliquots of urine were pipetted into a volumetric flask, the pH adjusted to 6.6 to 6.8, and when the samples made to a known volume, free pantothenic acid was measured microbiologically.

RESULTS AND DISCUSSION

Weight Gain, Ration Consumed, and Food Efficiency

Experiment A

Daily weight gain, food intake, and food efficiency data of the experimental guinea pigs and of their litter mate controls are summarized by the week in Table 2 and Figure 2. During the first week of analogue (omega-methylpantothenic acid) feeding, there was no difference in average weight gain, food intake, or food efficiency between the two groups. During the second and third weeks, the experimental group was eating 2 gm. more of feed each day but gaining 2 gm. less in weight each day than the control group, with a corresponding decrease in food efficiency. During the fourth week, the pigs receiving the analogue were eating 2 gm. less of food each day than the controls while gaining 4 gm. less each day. In the fifth, sixth, and seventh weeks of the experiment, the difference in food intake between the two groups increased to 7, 12 and 11 gm. respectively, while the difference in weight gain per day remained constant at 6.6 or 6.7 gm.

Table 2. Summary of weekly weight gain, food intake, and food efficiency of guinea pigs fed omega-methylpantothenic acid and of their litter mate controls

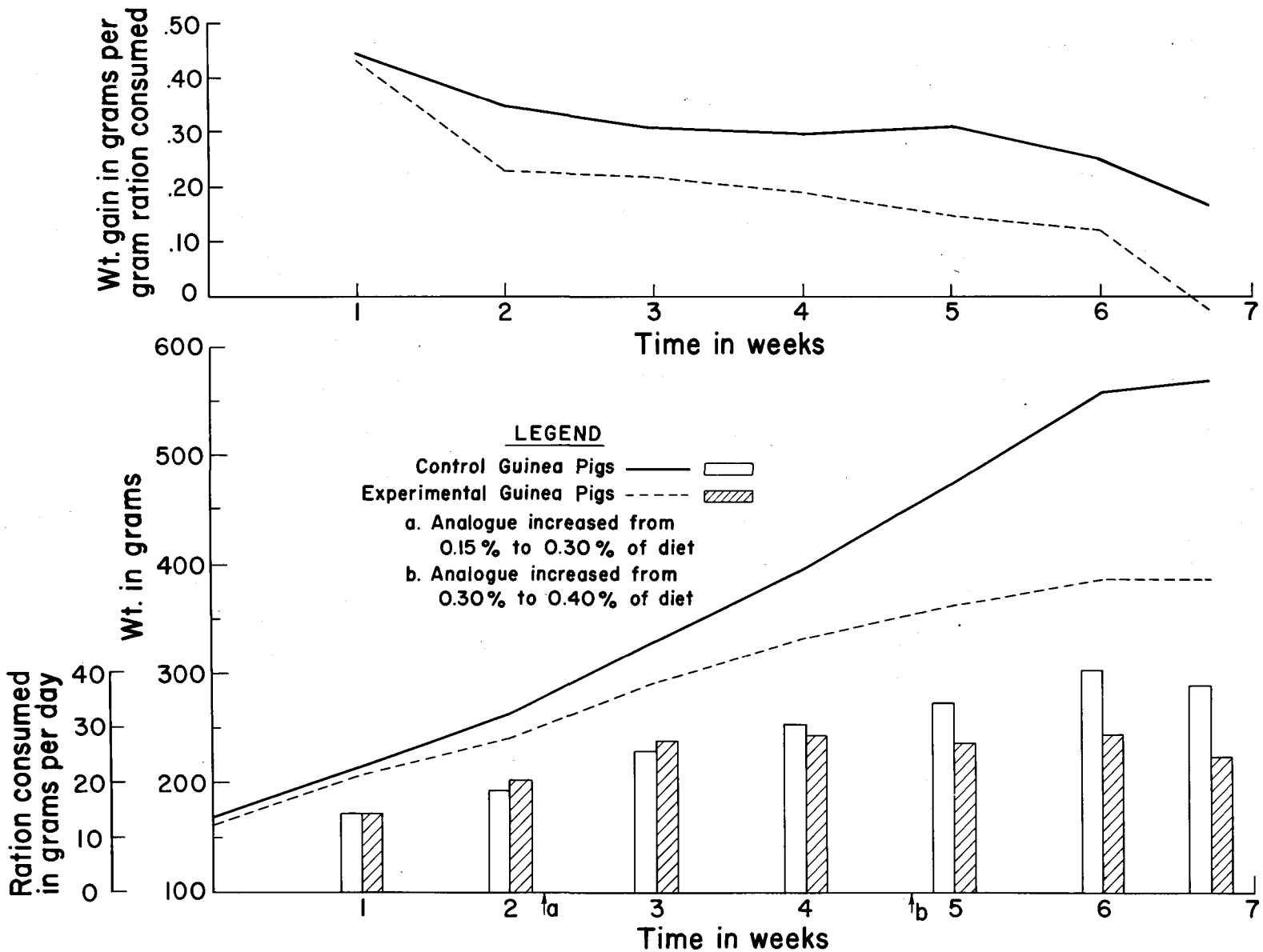
Week of study		Control	Analogue
1	No. of animals	9	9
	Ave. initial wt. (gm.)	169	164
	Ave. wt. at end of week (gm.)	216	209
	Ave. wt. gain (gm./day)	6.6	6.5
	Ave. food intake (gm./day)	15	15
	Ave. wt. gain per gram food eaten (gm./day)	0.44	0.43
2	No. of animals	9	9
	Ave. wt. at end of week (gm.)	264	243
	Ave. wt. gain (gm./day)	6.8	4.8
	Ave. food intake (gm./day)	19	21
	Ave. wt. gain per gram food eaten (gm./day)	0.35	0.23
3	No. of animals	6	6
	Ave. wt. at end of week (gm.)	333	294
	Ave. wt. gain (gm./day)	7.9	6.0
	Ave. food intake (gm./day)	26	28
	Ave. wt. gain per gram food eaten (gm./day)	0.31	0.22
4	No. of animals	6	6
	Ave. wt. at end of week (gm.)	398	332
	Ave. wt. gain (gm./day)	9.3	5.4
	Ave. food intake (gm./day)	31	29
	Ave. wt. gain per gram food eaten (gm./day)	0.30	0.19
5	No. of animals	6	6
	Ave. wt. at end of week (gm.)	474	362
	Ave. wt. gain (gm./day)	10.9	4.3
	Ave. food intake (gm./day)	35	28
	Ave. wt. gain per gram food eaten (gm./day)	0.31	0.15

Table 2 (Cont'd)

Week of study		Control	Analogue
6	No. of animals	5	5
	Ave. wt. at end of week (gm.)	558	388
	Ave. wt. gain (gm./day)	10.2	3.5
	Ave. food intake (gm./day)	41	29
	Ave. wt. gain per gram food eaten (gm./day)	0.25	0.12
7	No. of animals	4[a]	4[a]
	Ave. wt. at end of expt. (gm.)	568	388
	Ave. wt. gain (gm./day)	6.3	-0.4
	Ave. food intake (gm./day)	36	25
	Ave. wt. gain per gram food eaten (gm./day)	0.18	-0.02

[a]On experiment for 5 days of 7th week.

Figure 2. Average weekly weight, food consumption and food efficiency of guinea pigs receiving omega-methylpantothenic acid and of their litter mate controls


Wt. gain in grams per gram ration consumed
.50
.40
.30
.20
.10
0
1
2
3
4
5
6
7
Time in weeks
600
500
400
300
200
100
Wt. in grams
LEGEND
Control Guinea Pigs
Experimental Guinea Pigs
a. Analogue increased from 0.15 % to 0.30 % of diet
b. Analogue increased from 0.30 % to 0.40 % of diet
Ration consumed in grams per day
40
30
20
10
0
1
2
a
3
4
b
5
6
7
Time in weeks

The data for the individual animals by pairs (Figure 3) showed that the weight for 4 pairs of pigs ran parallel for the first three weeks of the experiment with the difference between the experimental pig and its litter mate control gradually increasing after the third week of the experiment. Between guinea pigs 2E and 2C and 3E and 3C, however, the difference in weight began after the first week of the experiment. This early difference probably indicates poorer tissue saturation or a higher requirement in 2E and 3E than in the other experimental pigs.

An assay of the rabbit pellets indicated the concentration of pantothenic acid to be 47.2 mcg./gm. When the analogue was incorporated at 0.15, 0.30, and 0.40 per cent, the ratio of analogue to pantothenic acid was 32:1, 64:1, and 85:1, respectively.

When Drell and Dunn (1951) fed mice of the Bagg strain a ratio of 33:1 (analogue to pantothenic acid), they found that the mice gained almost the same amount of weight as the controls, but had to eat one gram more each week in order to do so. The 9 guinea pigs in the present study (Table 3, Figure 2) gained one gram less in weight while eating one gram more food per day than the controls, while being fed the 32:1 ratio of analogue to pantothenic acid (0.15 per cent level of intake). A correspondingly small

Figure 3. Average weekly weight and food consumption of individual guinea pigs receiving omega-methylpantothenic acid and of their litter mate controls

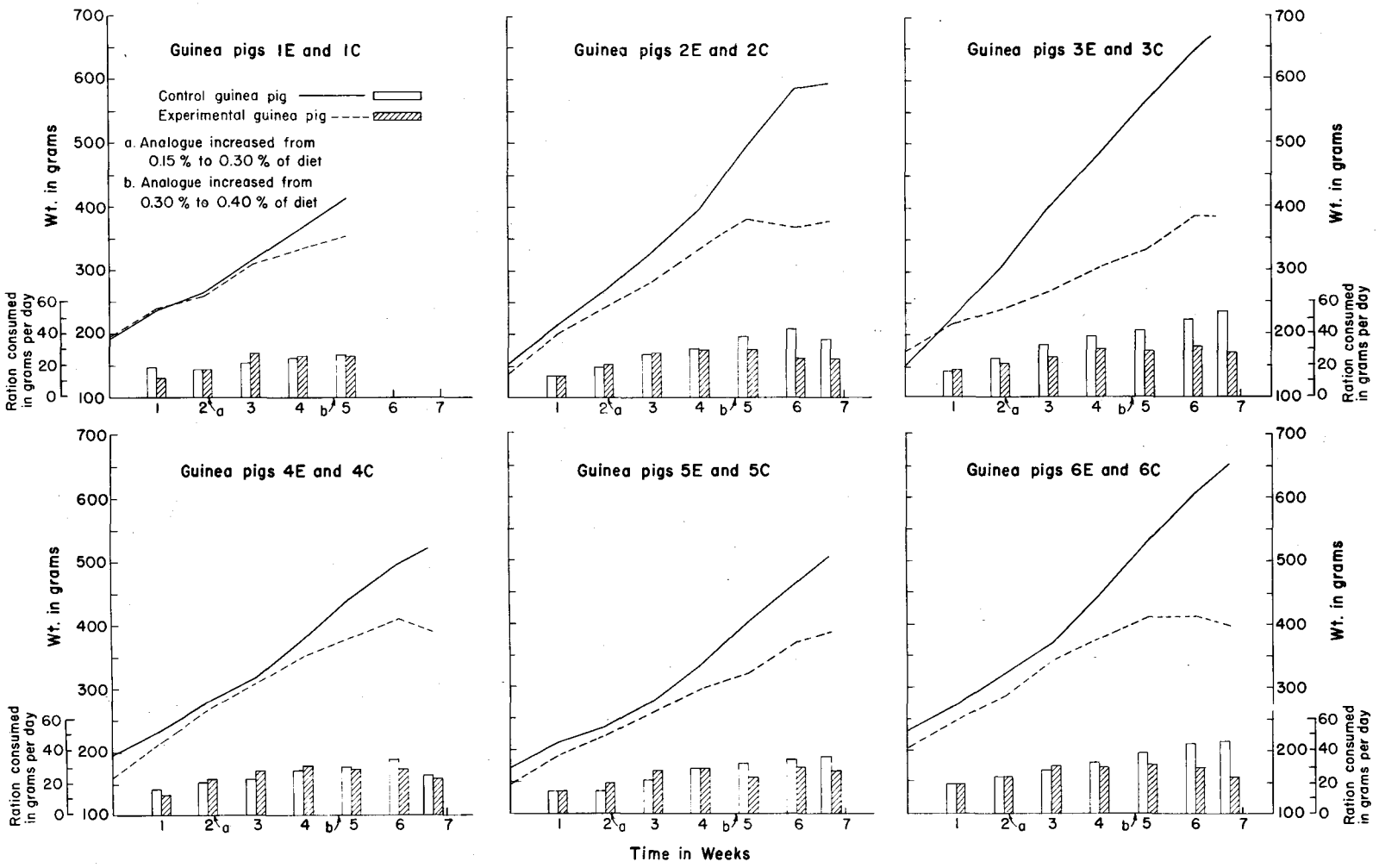

Guinea pigs 1E and 1C
Guinea pigs 2E and 2C
Guinea pigs 3E and 3C
Guinea pigs 4E and 4C
Guinea pigs 5E and 5C
Guinea pigs 6E and 6C
Control guinea pig
Experimental guinea pig
a. Analogue increased from 0.15 % to 0.30 % of diet
b. Analogue increased from 0.30 % to 0.40 % of diet
Wt. in grams
Ration consumed in grams per day
Time in Weeks

Table 3. Weight gain, food consumption, and food efficiency of guinea pigs fed omega-methylpantothenic acid and of their litter mate controls summarized by periods according to the intake of analogue

Guinea pig	Initial wt. (gm.)	Actual wt. (gm.)	Ave. gain in wt. (gm./day)	Ave. food intake (gm./day)	Wt. gained per gm. food eaten (gm./day)
	After 15 days on 0.15 per cent analogue				
1C[a]	191	263	4.8	19	0.26
2C	153	273	8.0	17	0.47
3C	146	310	10.9	21	0.53
4C	196	285	5.9	19	0.31
5C	172	235	4.2	13	0.32
6C	232	329	6.5	23	0.29
7C	149	240	6.1	15	0.40
8C	148	232	5.6	15	0.38
9C	138	247	7.3	17	0.44
Average	169	268	5.9	18	0.38
1E[a]	195	265	4.7	16	0.29
2E	139	242	6.9	18	0.38
3E	171	240	4.6	19	0.24
4E	160	261	6.7	19	0.36
5E	146	225	5.3	17	0.31
6E	206	286	5.3	22	0.25
7E	165	252	5.4	20	0.28
8E	147	237	6.0	20	0.31
9E	145	222	5.1	19	0.28
Average	164	248	5.0	19	0.30
	After 18 days on 0.30 per cent analogue				
1C[a]	191	399	7.6	25	0.31
2C	153	465	10.7	32	0.34
3C	146	530	12.2	38	0.32
4C	196	421	7.6	27	0.28
5C	172	381	8.1	27	0.30
6C	232	507	9.9	32	0.30
Average	169	450	9.3	30	0.31

[a]C designates control- E designates experimental animal.

Table 3 (Cont'd)

Guinea pig	Initial wt. (gm.)	Actual wt. (gm.)	Ave. gain in wt. (gm./day)	Ave. food intake (gm./day)	Wt. gained per gm. food eaten (gm./day)
1E[a]	195	369	5.8	27	0.21
2E	139	371	7.2	30	0.24
3E	171	331	5.1	28	0.18
4E	160	370	6.1	29	0.21
5E	146	313	4.9	26	0.19
6E	206	399	6.3	31	0.20
Average	164	359	5.9	28	0.21
	After 14 days on 0.40 per cent analogue				
1C[a]	191	414[b]	7.5	28	0.27
2C	153	591	9.0	40	0.22
3C	146	668[c]	11.5	39	0.23
4C	196	521	5.7	31	0.23
5C	172	507	8.6	34	0.26
6C	232	653	9.7	44	0.23
Average	169	568	8.9	38	0.24
1E[a]	195	353[b]	-8.0	24	-0.33
2E	139	375	0.3	26	0.01
3E	171	379[c]	4.0	31	0.13
4E	160	389	1.4	28	0.05
5E	146	388	5.4	28	0.19
6E	206	399	0	28	0
Average	164	388	1.8	27	0.06

[b]Sacrificed on 3rd day of 0.40 per cent analogue.

[c]Sacrificed on 12th day of 0.40 per cent analogue.

decrease in food efficiency occurred at the same time, from 0.38 gm. gain per gram of food eaten per day for the controls to 0.30 for the experimental group. The analogue intake was then increased from 0.15 per cent to 0.30 per cent.

After 18 days on the 0.30 per cent level of analogue (64:1), the difference in weight gain and food intake between the two groups of guinea pigs increased so that the experimental pigs ate two grams less food per day and gained 3.4 gm. less weight than the controls. At a ratio of 50:1, the mice in Drell and Dunn's study ate 7 per cent more food but gained 7 per cent less weight than their controls, while at a ratio of 75:1, they ate only 3 per cent more food and gained 40 per cent less weight. In the present investigation, the 0.30 per cent level of analogue (64:1) was succeeded by the 0.40 per cent level (85:1).

After 14 days on the 0.40 per cent level of analogue (85:1), the difference between the two groups of pigs increased in both food intake and weight gain. The controls gained an average of 8.9 gm. per day while eating 38 grams of food, whereas the experimental group gained only 1.8 gm. per day while eating 27 grams of ration. This difference amounted to 30 per cent less food per day and a gain in weight which was 80 per cent less than that of the controls.

Drell and Dunn showed that at a ratio of 75:1, which lies between two of the levels mentioned above, three mice gained 40 per cent less weight while eating 3 per cent more food than the controls. At a ratio of 100:1, the mice ate considerably less food and gained 67 per cent less weight than the controls. These data lead one to speculate that a ratio of 100:1 in the Bagg mouse would probably be the equivalent of a ratio between 64:1 and 85:1 in the male albino guinea pigs of this study.

To summarize the above observations, as the analogue to pantothenic acid ratio was increased from 32:1 to 64:1 to 85:1, guinea pigs ate less and less food, gained less and less weight and became more and more inefficient in utilization of food. Only at the 32:1 ratio was the food intake of experimental and control animals comparable. On the other hand, mice of the Bagg strain ate more food than the controls at ratios of 33:1, 50:1, and 75:1 but also gained less weight and also became less efficient in food utilization.

It is interesting that Bean and Hodges (1954) found no loss of weight in their four human subjects during the period when omega-methylpantothenic acid was fed to them. Their subjects were adult, whereas, the guinea pigs of the present investigation, as well as the mice in Drell and

Dunn's study, were growing animals. Although the caloric intake of Bean's subjects was kept constant by tube feeding, the men did complain of anorexia during the third week of analogue consumption. It is very likely that had they been fed ad libitum, they would have consumed less food from the third week until the end of the study.

Experiment B

Study I. In Study I there was considerable variation in the age of the animals. Four of them had not been weaned and five had been weaned a few days prior to the beginning of the study. For five days before the experimental feeding began, the animals were given synthetic diet along with 10 mg. ascorbic acid and 0.3 mg. calcium pantothenate each day.

It was apparent from the data in Table 4 that the pigs adjusted to a synthetic diet with considerable variability during the first week. The youngest animal (40 A.A., 0 P.A.)[1] made the smallest weight gain and consumed the least food each day. The least thrifty animal of the lot seemed to be pig 3 (0 A.A., 0.06 P.A.) which was the least efficient in utilization of food; it gained only 2.7 gm. per day

[1]A.A. and P.A. will be used as abbreviations for ascorbic acid and pantothenic acid, respectively. The number preceding the abbreviation indicates the amount of the supplement in milligrams.

Table 4. Weekly food consumption, gain in weight and food efficiency of guinea pigs fed synthetic diet supplemented with four levels of ascorbic acid and four levels of calcium pantothenate

Study I

Week of study	Guinea pig	Supplement		Initial wt. (gm.)	Wt. at end of week (gm.)	Ave. wt. gain (gm./day)	Ave. food intake (gm./day)	Wt. gain per gm. food eaten (gm./day)
		Asc. A. (mg./day)	Ca Panto. (mg./day)					
1	8	0	0	146	173	3.9	10.9	0.36
	3	0	0.06	207	226	2.7	12.7	0.21
	6	0	8	210	241	4.4	13.1	0.34
	7	2	0	158	198	5.7	12.7	0.45
	2	2	0.06	179	213	4.9	14.7	0.33
	5	2	8	191	215	3.4	11.4	0.30
	1	40	0	91	103	1.7	5.3	0.32
	9	40	0.06	171	217	6.6	11.6	0.57
	4	10	0.2	167	207	5.7	15.7	0.36
2	8	0	0	146	209	5.1	12.3	0.42
	3	0	0.06	207	258	4.6	13.9	0.33
	6	0	8	210	300	8.4	19.7	0.43
	7	2	0	158	237	5.6	12.4	0.45
	2	2	0.06	179	273	8.6	14.7	0.58
	5	2	8	191	257	6.0	14.1	0.42

Table 4 (Cont'd)

Week of study	Guinea pig	Supplement: Asc. A. (mg./day)	Supplement: Ca Panto. (mg./day)	Initial wt. (gm.)	Wt. at end of week (gm.)	Ave. wt. gain (gm./day)	Ave. food intake (gm./day)	Wt. gain per gm. food eaten (gm./day)
	1	40	0	91	155	7.4	8.6	0.87
	9	40	0.06	171	255	5.4	13.4	0.40
	4	10	0.2	167	267	8.6	17.3	0.50
3	8	0	0	146	178	-4.4	6.6	-0.67
	3	0	0.06	207	220[a]	-5.4	7.4	-0.73
	6	0	8	210	286	-2.0	13.4	-0.15
	7	2	0	158	291	7.7	14.7	0.52
	2	2	0.06	179	289	2.3	14.4	0.16
	5	2	8	191	301	6.3	17.3	0.36
	1	40	0	91	187	4.6	9.7	0.47
	9	40	0.06	171	287	4.6	14.6	0.31
	4	10	0.2	167	311	6.3	18.0	0.35
4	8	0	0	146	156[b]	-5.5	6.2	-0.88
	6	0	8	210	233[b]	-13.2	4.5	-2.94

[a]Final weight.

[b]Final weight--on experiment 4 days of 4th week.

Table 4 (Cont'd)

Week of study	Guinea pig	Supplement Asc. A. (mg./day)	Supplement Ca Panto. (mg./day)	Initial wt. (gm.)	Wt. at end of week (gm.)	Ave. wt. gain (gm./day)	Ave. food intake (gm./day)	Wt. gain per gm. food eaten (gm./day)
	7	2	0	158	251	-5.7	9.7	-0.59
	2	2	0.06	179	317	4.0	14.9	0.27
	5	2	8	191	293	-1.1	12.3	-0.09
	1	40	0	91	245	8.3	12.9	0.64
	9	40	0.06	171	339	7.4	17.3	0.43
	4	10	0.2	167	364	7.6	19.1	0.40
5	7	2	0	158	316	9.3	16.1	0.58
	2	2	0.06	179	359	6.0	18.4	0.33
	5	2	8	191	341	6.9	16.3	0.42
	1	40	0	91	293	6.9	15.4	0.44
	9	40	0.06	171	396	8.1	19.4	0.42
	4	10	0.2	167	422	8.3	23.0	0.36
6	7	2	0	158	388	10.3	18.1	0.57
	2	2	0.06	179	408	7.0	21.4	0.33
	5	2	8	191	417	10.9	21.1	0.51
	1	40	0	91	358	9.3	18.3	0.51
	9	40	0.06	171	440	6.3	20.0	0.31
	4	10	0.2	167	485	9.0	24.6	0.37

Table 4 (Cont'd)

Week of study	Guinea pig	Supplement Asc. A. (mg./day)	Supplement Ca Panto. (mg./day)	Initial wt. (gm.)	Wt. at end of week (gm.)	Ave. wt. gain (gm./day)	Ave. food intake (gm./day)	Wt. gain per gm. food eaten (gm./day)
7	7	2	0	158	434	6.6	18.6	0.35
	2	2	0.06	179	447	5.6	23.3	0.24
	5	2	8	191	456	5.6	21.3	0.26
	1	40	0	91	413	7.9	20.4	0.38
	9	40	0.06	171	499	8.4	22.0	0.38
	4	10	0.2	167	538	7.6	25.3	0.30
8	7	2	0	158	449	2.1	16.6	0.13
	2	2	0.06	179	481	4.9	22.7	0.21
	5	2	8	191	510	7.7	21.9	0.35
	1	40	0	91	467	7.7	21.9	0.35
	9	40	0.06	171	557	8.3	25.0	0.33
	4	10	0.2	167	594	8.0	27.3	0.29
9	7	2	0	158	468	2.7	15.6	0.17
	2	2	0.06	179	529	6.9	23.6	0.29
	5	2	8	191	547	5.3	22.1	0.24
	1	40	0	91	520	7.6	23.1	0.33
	9	40	0.06	171	612	7.9	24.4	0.32
	4	10	0.2	167	644	7.1	26.0	0.27

while consuming 12.7 gm. of food per day. The remainder of the animals gained from 3.4 to 6.6 gm. per day and consumed from 10.9 to 15.7 gm. of food per day.

At the beginning of the second week, supplements of ascorbic and pantothenic acids were fed according to the experimental design described in the Section, Method of Procedure. The two pigs (6 and 5) getting maximal amounts of calcium pantothenate received by accident a total of 180 mg. of the vitamin during the first two days of experimental feeding. Beginning with the third day and continuing until the termination of the study, they were given 8 mg. of calcium pantothenate per day.

Weight gain as well as food intake improved considerably after the first week. Average weight gain per day ranged from 4.6 (pig 3) to 8.6 grams. Food intake and efficiency went up correspondingly. By the third week the three pigs on diets deficient in ascorbic acid (pigs 8, 3 and 6) were eating less food, losing weight and showing other symptoms of scurvy. Guinea pig 3 (0 A.A., 0.06 P.A.) was sacrificed on the 16th day after ascorbic acid withdrawal from the diet. Pig 2 (2 A.A., 0.06 P.A.) was consuming about the same amount of food as during the previous week but gaining only 2.3 gm. per day. In spite of the vitamin limitations imposed on them, the rest of the animals gained from 4.6 to 7.7 gm. each day and food efficiency remained good.

During the fourth week the remaining two guinea pigs fed the ascorbic acid-deficient diet were sacrificed, both on the same day. One animal (pig 8) had been receiving a diet also deficient in pantothenic acid for 20 days, while the other (pig 6) had been receiving 8 mg. of calcium pantothenate each day. Both pigs 8 and 6 had diarrhea (pig 8 had bloody diarrhea) and the teeth of both could be removed very easily, indicating that their deficiency was more chronic than that of guinea pig 3 (0 A.A., 0.06 P.A.), whose teeth had been firm. Although 8 mg. of calcium pantothenate (pig 6) failed to alleviate scorbutic symptoms, a survival study might have furnished more information. Guinea pig 6 was probably about one week older than pig 8. It is known that the younger the animal, the more quickly deficiency symptoms are produced. Therefore, if age were a factor, one would have expected pig 8 to show evidences of deficiency a little earlier than pig 6. During the first two weeks both animals had been equally efficient in utilization of food.

Guinea pigs 7 and 5 (which were receiving 2 mg. ascorbic acid and 0 and 8 mg. pantothenic acid, respectively) both lost weight and consumed less food during the fourth week than earlier. Pig 7 had diarrhea the entire week, while pig 5 displayed signs of illness all week from unknown

causes. Guinea pig 2 (2 A.A., 0.06 P.A.) had continued from the first week of the study to be a good eater but poor gainer. Its food efficiency record did show some improvement in the fourth week over the previous week, however. The other three pigs gained from 7.4 to 8.3 gm. per day and were quite efficient in utilization of food.

During the fifth week guinea pigs 7 and 5 improved vastly over the preceding week, while the other animals remained about the same.

During the sixth week, 5 of the animals increased their food consumption and with the exception of pig 9 made considerably greater weight gains. Pig 9 (40 A.A., 0.06 P.A.) ate more food but gained less weight than the week before.

During the seventh week all 6 pigs increased their food consumption over that of the previous week but they all made smaller weight gains with the exception of pig 9.

During the eighth and ninth weeks, pig 7 (2 A.A., 0 P.A.) had a smaller food intake than earlier and increased only slightly in weight. Again the animal had occasional bouts of diarrhea during both weeks. The other five animals continued to have good appetites and to make fair to good increases in weight. While on synthetic diet, the control animal (10 A.A., 0.06 P.A.) gained an average of 7.4 gm. per day, which compared favorably with the 7 to 8 gm. per day growth rate advocated by Cannon et al., (1945).

When the animals are grouped according to intake of ascorbic acid (Figure 4), the data on the group receiving 2 mg. ascorbic acid per day indicated that the animals grew equally well on minimal and maximal amounts of calcium pantothenate. No difference in food consumption was noted.

Data on the two pigs getting 40 mg. ascorbic acid per day appear to indicate that guinea pigs do not require a dietary source of pantothenic acid, for pig 1 (40 A.A., 0 P.A.) had a growth rate superior to that of guinea pig 9 (40 A.A., 0.06 P.A.) and equal to that of the control (10 A.A., 0.2 P.A.). This animal was, no doubt, very proficient in intestinal synthesis of pantothenic acid. It neither had diarrhea nor showed any signs of lassitude.

It was noted that when no ascorbic acid was fed and when 2 mg. per day were fed, increasing calcium pantothenate from none to 0.06 mg. per day produced an increase in weight gain, but, when 40 mg. ascorbic acid were fed, the level of pantothenic acid had no effect on weight gain.

When the animals are grouped according to intake of calcium pantothenate (Figure 5), the animal getting 40 mg. ascorbic acid per day had a weight record superior to that of the pig getting only 2 mg. per day when no calcium pantothenate was included in the diet. Frequent bouts of diarrhea in guinea pig 7 (2 A.A., 0 P.A.) were certainly

Figure 4. Growth curves of guinea pigs fed 4 levels of ascorbic acid and 4 levels of calcium pantothenate by levels of ascorbic acid fed

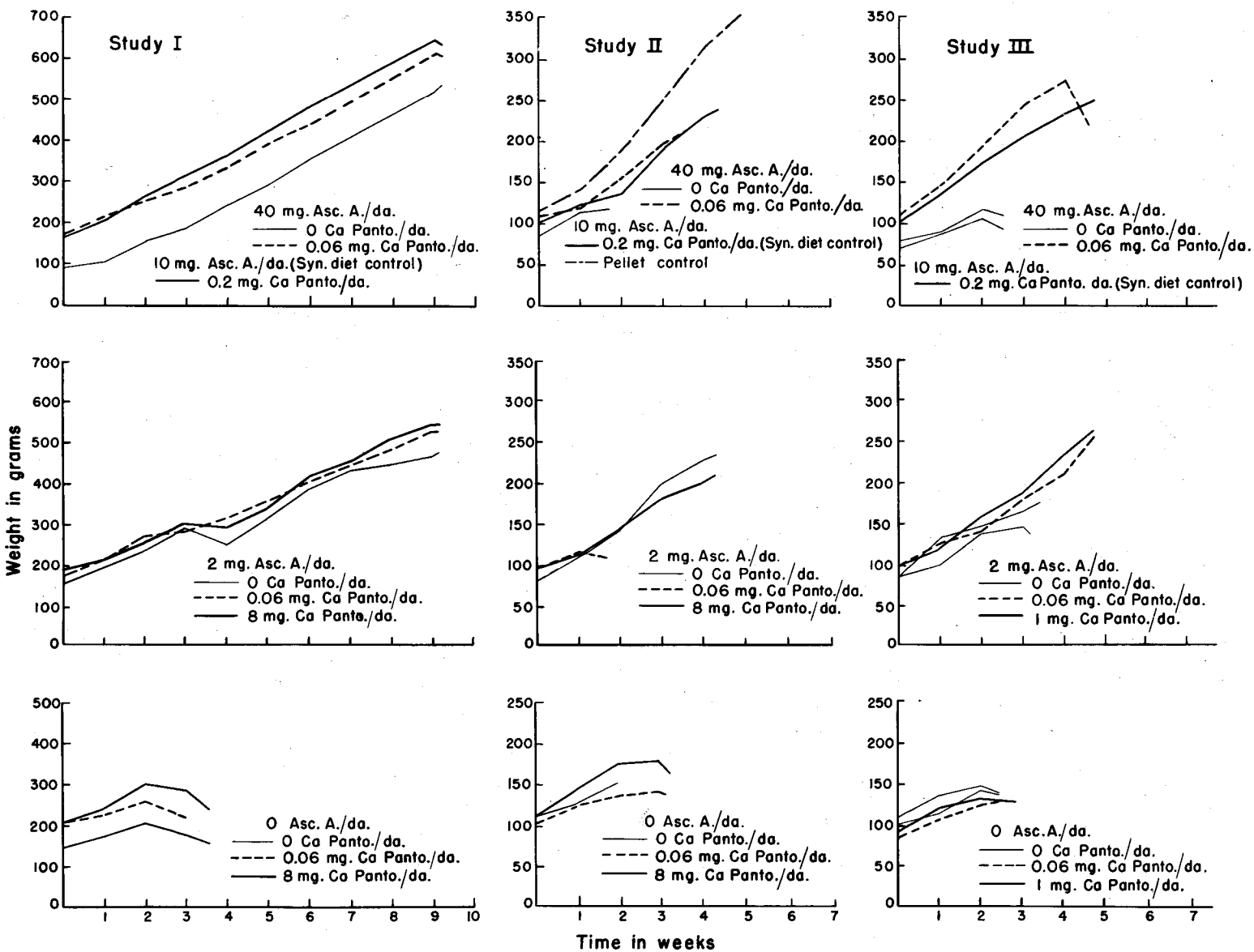
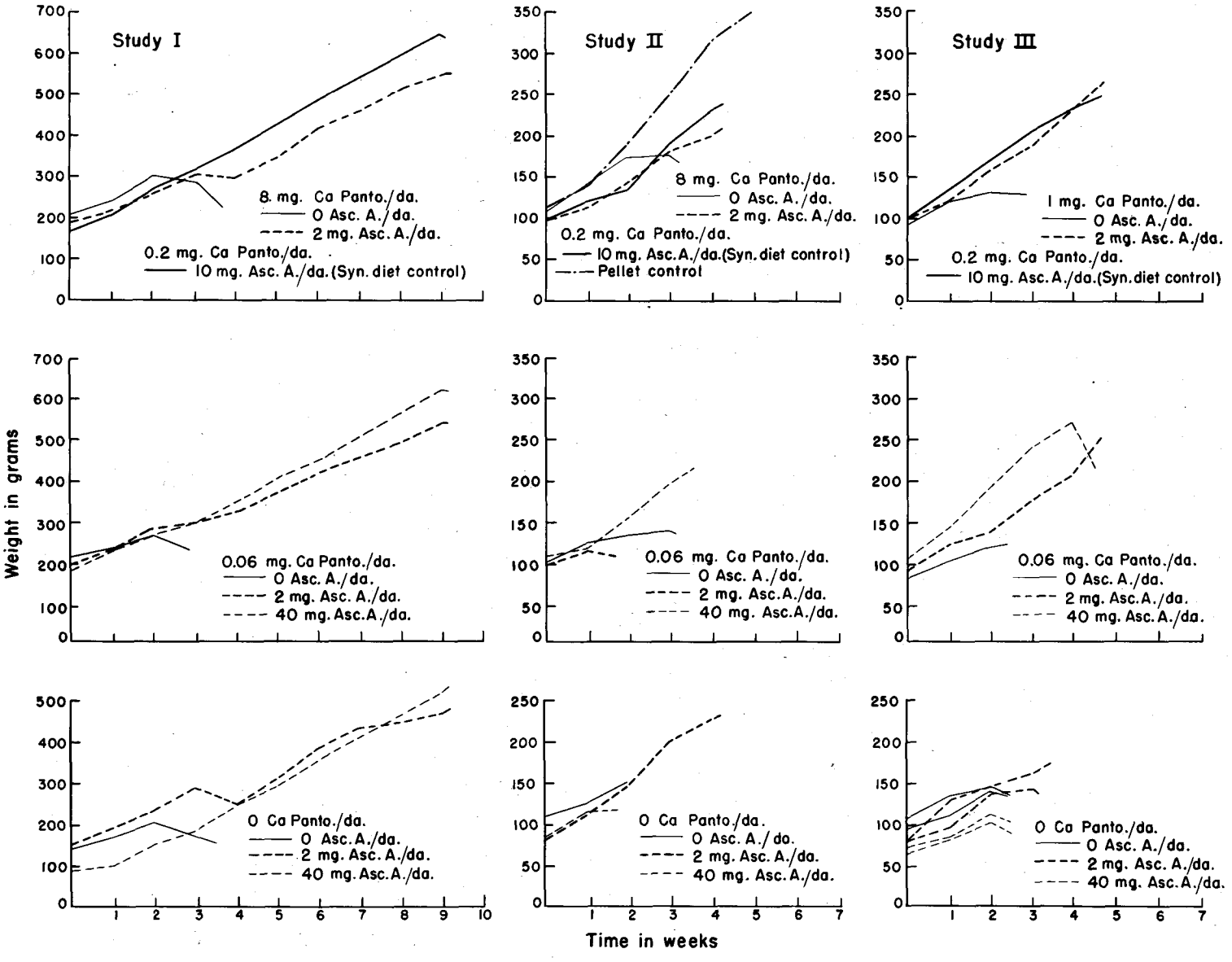

Study I
40 mg. Asc. A./da.
0 Ca Panto./da.
0.06 mg. Ca Panto./da.
10 mg. Asc. A./da.(Syn. diet control)
0.2 mg. Ca Panto./da.
Study II
40 mg. Asc. A./da.
0 Ca Panto./da.
0.06 mg. Ca Panto./da.
10 mg. Asc. A./da.
0.2 mg. Ca Panto./da.(Syn. diet control)
Pellet control
Study III
40 mg. Asc. A./da.
0 Ca Panto./da.
0.06 mg. Ca Panto./da.
10 mg. Asc. A./da.
0.2 mg. Ca Panto. da.(Syn. diet control)
Weight in grams
2 mg. Asc. A./da.
0 Ca Panto./da.
0.06 mg. Ca Panto./da.
8 mg. Ca Panto./da.
2 mg. Asc. A./da.
0 Ca Panto./da.
0.06 mg. Ca Panto./da.
8 mg. Ca Panto./da.
2 mg. Asc. A./da.
0 Ca Panto./da.
0.06 mg. Ca Panto./da.
1 mg. Ca Panto./da.
0 Asc. A./da.
0 Ca Panto./da.
0.06 mg. Ca Panto./da.
8 mg. Ca Panto./da.
0 Asc. A./da.
0 Ca Panto./da.
0.06 mg. Ca Panto./da.
8 mg. Ca Panto./da.
0 Asc.A./da.
0 Ca Panto./da.
0.06 mg. Ca Panto./da.
1 mg. Ca Panto./da.
Time in weeks

Figure 5. Growth curves of guinea pigs fed 4 levels of ascorbic acid and 4 levels of calcium pantothenate by levels of calcium pantothenate fed


Study I
8 mg. Ca Panto./da.
0 Asc. A./da.
2 mg. Asc. A./da.
0.2 mg. Ca Panto./da.
10 mg. Asc. A./da.(Syn. diet control)
Study II
8 mg. Ca Panto./da.
0 Asc. A./da.
2 mg. Asc. A./da.
0.2 mg. Ca Panto./da.
10 mg. Asc.A./da.(Syn.diet control)
Pellet control
Study III
1 mg. Ca Panto./da.
0 Asc. A./da.
2 mg. Asc. A./da.
0.2 mg. Ca Panto./da.
10 mg. Asc.A./da.(Syn.diet control)
Weight in grams
0.06 mg. Ca Panto./da.
0 Asc. A./da.
2 mg. Asc. A./da.
40 mg. Asc.A./da.
0 Ca Panto./da.
Time in weeks

detrimental to its growth and well-being. Whether the diarrhea was due to a deficiency of pantothenic acid or to an infection in the gastrointestinal tract was not determined. The diarrhea appeared not to be infectious since no other animals contracted it. At any rate, increasing ascorbic acid increased both weight gain and food efficiency in this group of animals when no pantothenic acid was fed.

There was very little difference in food consumption between two of the animals getting 0.06 mg. calcium pantothenate (pigs 3 and 2) but the pig consuming the maximal amount of ascorbic acid (pig 9) grew slightly better and utilized food better. The animal getting minimal ascorbic acid, maximal calcium pantothenate (pig 5) had a record practically equivalent to that of guinea pig 2 which was ingesting minimal amounts of both calcium pantothenate and ascorbic acid. Thus, increasing ascorbic acid increased the weight gain as well as food efficiency in this group of guinea pigs, when 0.06 mg. calcium pantothenate were fed per day.

To summarize the observations made on weight gain, food intake and efficiency of the guinea pigs in Study I (Table 5), it would appear that increasing the ascorbic acid level increased weight gain and food efficiency when no pantothenic acid was fed as well as when 0.06 mg. were fed per day.

Table 5. Summary of weight gain, food consumption and food efficiency of guinea pigs fed synthetic diet supplemented with four levels of ascorbic acid and four levels of calcium pantothenate

Study I

Guinea pig	Supplement		Initial wt. (gm.)	No. of days on expt.	Final wt. (gm.)	Ave. wt. gain (gm./day)	Ave. food intake (gm./day)	Wt. gain per gm. food eaten (gm./day)
	Asc. A. (mg./day)	Ca Panto. (mg./day)						
8	0	0	173	18	156	-0.9	8.7	-0.11
3	0	0.06	226	14	220	-0.4	10.6	-0.04
6	0	8	241	18	233	-0.4	13.9	-0.03
7	2	0	198	56	468	4.8	15.2	0.32
2	2	0.06	213	56	529	5.6	19.2	0.29
5	2	8	215	56	547	5.9	18.3	0.32
1	40	0	103	57	535	7.6	16.4	0.46
9	40	0.06	217	57	610	6.9	19.5	0.35
4	10	0.2	207	57	638	7.6	22.6	0.33

Since the results obtained with 8 mg. calcium pantothenate were almost identical with those obtained with 0.06 mg., one could reason that the effect was derived either from intestinal synthesis of pantothenic acid or from compensation by the ascorbic acid. The former would be more likely.

Study II and Study III. For both Study II and Study III, 2 to 4-day-old animals were ordered and the pigs were placed on the experimental diets with the various supplements of ascorbic and pantothenic acids immediately after their arrival. The only difference between these two studies lies in the fact that the maximal amount of calcium pantothenate in Study II was 8 mg. and in Study III, 1 mg. per day.

Weight gain, food consumption, and food efficiency data are presented in Table 6. When the animals were grouped according to ascorbic acid intake (Figure 4), the 3 pigs getting neither ascorbic acid nor pantothenic acid (pigs 8, 8, and 8A) developed scorbutic symptoms earlier than those animals receiving only pantothenic acid. The scorbutic animals were sacrificed in order to obtain blood samples when the symptoms of scurvy were so pronounced that it was feared the animals might die within 12 hours. One of these animals, guinea pig 8 in Study II, had caught its leg in the cage and broken it on the 13th day, while the survival

Table 6. Weekly food consumption, gain in weight and food efficiency of guinea pigs fed synthetic diet supplemented with four levels of ascorbic acid and four levels of calcium pantothenate

Studies II and III

Week of study	Study and guinea pig no.	Supplement Asc. A. (mg./day)	Supplement Ca Panto. (mg./day)	Initial wt. (gm.)	Wt. at end of week (gm.)	Ave. wt. gain (gm./day)	Ave. food intake (gm./day)	Wt. gain per gm. food eaten (gm./day)
1	II-8	0	0	112	127	2.1	4.6	0.47
	III-8	0	0	110	136	3.7	8.6	0.43
	III-8A	0	0	99	113	2.0	6.4	0.12
	II-3	0	0.06	103	125	3.1	5.3	0.59
	III-3	0	0.06	85	105	2.9	5.0	0.57
	II-6	0	8	112	145	4.7	7.9	0.60
	III-6	0	1	93	120	3.9	6.9	0.56
	II-7	2	0	84	113	4.1	5.6	0.74
	III-7	2	0	83	99	2.3	7.0	0.33
	III-7A	2	0	85	132	6.7	9.1	0.73
	II-2	2	0.06	98	117	2.7	4.9	0.56
	III-2	2	0.06	95	125	4.3	8.1	0.53
	II-5	2	8	99	115	2.3	3.7	0.62
	III-5	2	1	99	120	3.0	5.9	0.51
	II-1	40	0	85	116	4.4	5.6	0.79
	III-1	40	0	68	85	2.4	5.1	0.47
	III-1A	40	0	77	86	1.3	2.6	0.50

Table 6 (Cont'd)

Week of study	Study and guinea pig no.	Supplement Asc. A. (mg./day)	Supplement Ca Panto. (mg./day)	Initial wt. (gm.)	Wt. at end of week (gm.)	Ave. wt. gain (gm./day)	Ave. food intake (gm./day)	Wt. gain per gm. food eaten (gm./day)
	II-9	40	0.06	109	120	1.6	5.3	0.30
	III-9[a]	40	0.06	107	145	5.4	6.6	0.83
	II-4	10	0.20	101	123	3.1	5.9	0.54
	III-4	10	0.20	100	135	5.0	7.0	0.71
	II-Pellet control	10	--	116	142	3.7	7.4	0.50
2	II-8	0	0	112	153[b]	3.7	7.1	0.52
	III-8	0	0	110	147	1.6	9.0	0.17
	III-8A	0	0	99	141	4.0	7.3	0.55
	II-3	0	0.06	103	136	1.6	5.9	0.27
	III-3	0	0.06	85	123	2.6	6.4	0.40
	II-6	0	8	112	175	4.3	9.4	0.45
	III-6	0	1	93	131	1.6	7.1	0.22
	II-7	2	0	84	145	4.6	8.1	0.56
	III-7	2	0	83	138	5.6	8.1	0.68
	III-7A	2	0	85	147	2.1	7.0	0.31

[a]Developed a growth in heart-lung area.

[b]Final weight (broke leg).

Table 6 (Cont'd)

Week of study	Study and guinea pig no.	Supplement: Asc. A. (mg./day)	Supplement: Ca Panto. (mg./day)	Initial wt. (gm.)	Wt. at end of week (gm.)	Ave. wt. gain (gm./day)	Ave. food intake (gm./day)	Wt. gain per gm. food eaten (gm./day)
	II-2	2	0.06	98	Died[c]	--	--	--
	III-2	2	0.06	95	139	2.0	8.0	0.25
	II-5	2	8	99	145	4.3	9.7	0.44
	III-5	2	1	99	157	5.3	9.0	0.59
	II-1	40	0	85	119[d]	0.6	7.0	0.09
	III-1	40	0	68	104	2.7	7.1	0.38
	III-1A	40	0	77	114	4.0	5.7	0.70
	II-9	40	0.06	109	155	5.0	8.9	0.56
	III-9[a]	40	0.06	107	192	6.7	9.1	0.73
	II-4	10	0.20	101	136	1.9	9.3	0.20
	III-4	10	0.20	100	171	5.1	8.4	0.61
	II-Pellet control	10	--	116	192	7.1	16.0	0.45

[c]Lung infection.

[d]Final weight; on experiment 5 days during this week.

Table 6 (Cont'd)

Week of study	Study and guinea pig no.	Supplement Asc. A. (mg./day)	Supplement Ca Panto. (mg./day)	Initial wt. (gm.)	Wt. at end of week (gm.)	Ave. wt. gain (gm./day)	Ave. food intake (gm./day)	Wt. gain per gm. food eaten (gm./day)
3	III-8	0	0	110	139[e]	-2.7	4.3	-0.62
	III-8A	0	0	99	137[f]	-1.0	5.8	-0.17
	II-3	0	0.06	103	141	0.71	7.9	0.09
	III-3	0	0.06	85	127[f]	1.0	6.0	0.17
	II-6	0	8	112	179	0.57	9.3	0.06
	III-6	0	1	93	129[g]	-0.3	7.3	-0.04
	II-7	2	0	84	202	8.1	13.9	0.59
	III-7	2	0	83	145	1.0	6.7	0.15
	III-7A	2	0	85	163	2.3	10.0	0.23
	III-2	2	0.06	95	118	5.6	13.6	0.41
	II-5	2	8	99	183	5.4	12.3	0.44
	III-5	2	1	99	186	4.1	11.6	0.36

[e]Final weight; on experiment 3 days during this week.

[f]Final weight; on experiment 4 days during this week.

[g]Final weight; on experiment 6 days during this week.

Table 6 (Cont'd)

Week of study	Study and guinea pig no.	Supplement: Asc. A. (mg./day)	Supplement: Ca Panto. (mg./day)	Initial wt. (gm.)	Wt. at end of week (gm.)	Ave. wt. gain (gm./day)	Ave. food intake (gm./day)	Wt. gain per gm. food eaten (gm./day)
	III-1	40	0	68	92[e]	-4.0	2.7	-1.5
	III-1A	40	0	77	107[e]	-2.3	3.7	-0.64
	II-9	40	0.06	109	198	6.1	11.1	0.55
	III-9[a]	40	0.06	107	241	7.0	13.3	0.53
	II-4	10	0.20	101	192	8.0	12.6	0.64
	III-4	10	0.20	100	205	4.9	11.9	0.58
	II-Pellet control	10	--	116	251	8.4	22.3	0.38
4	II-6	0	8	112	169[h]	-5.0	7.0	-0.71
	II-7	2	0	84	229[i]	3.9	14.0	0.28
	III-7	2	0	83	139[i]	1.0	2.0	0.50
	III-7A	2	0	85	175[e]	4.0	10.0	0.40
	III-2	2	0.06	95	209	4.4	12.6	0.33
	II-5	2	8	99	201	2.6	10.0	0.26
	III-5	2	1	99	233	6.7	14.6	0.58

[h]Final weight; on experiment 2 days during this week.

[i]Final weight; on experiment 1 day during this week.

Table 6 (Cont'd)

Week of study	Study and guinea pig no.	Supplement Asc. A. (mg./day)	Supplement Ca Panto. (mg./day)	Initial wt. (gm.)	Wt. at end of week (gm.)	Ave. wt. gain (gm./day)	Ave. food intake (gm./day)	Wt. gain per gm. food eaten (gm./day)
	II-9	40	0.06	109	215[f]	4.2	11.5	0.37
	III-9[a]	40	0.06	107	272	4.4	12.0	0.37
	II-4	10	0.20	101	233	5.9	12.4	0.47
	III-4	10	0.20	100	232	3.9	13.6	0.28
	II-Pellet control	10	--	116	317	9.4	25.6	0.37
5	II-7	2	0	84	235[h]	3.0	14.0	0.21
	III-2	2	0.06	95	253[d]	8.8	14.2	0.62
	II-5	2	8	99	211[h]	5.0	11.5	0.43
	III-5	2	1	99	261[d]	5.6	15.2	0.37
	III-9[a]	40	0.06	107	218[f]	-13.5	2.5	-5.40
	II-4	10	0.20	101	240[h]	3.5	11.5	0.30
	III-4	10	0.20	100	248[d]	3.2	10.8	0.30
	II-Pellet control	10	--	116	352[d]	7.0	27.5	0.25

time for the other two animals was 16 and 17 days. The two animals getting 0.06 mg. calcium pantothenate per day (pigs 3) survived 20 and 16 days. The pig getting 8 mg. calcium pantothenate lived 22 days, while the one getting 1 mg. per day lived 19 days. In the latter two groups (0 A.A., 0.06 and 8 or 1 P.A.), the smaller animals had the shorter survival times. In general, the data indicated that pantothenic acid had a slight benefit over no pantothenic acid in the diet, however, larger amounts of the vitamin did not exhibit a beneficial effect over and above that of a minimal amount.

Of the three animals receiving 2 mg. ascorbic acid but no calcium pantothenate (pigs 7, 7, and 7A), one animal (pig 7 in Study III) had severe diarrhea and had exhibited signs of illness for 2 days before it was sacrificed on the 22nd day of the study. Guinea pig 7A broke its leg and was sacrificed on the 24th day of the study. The third animal survived until the study was terminated. One of the animals (pig 2 in Study II) getting the minimal amounts of both ascorbic acid and calcium pantothenate was found dead with a severe lung infection. In this minimal ascorbic acid group (pigs 7, 2, and 5), the data indicated that increasing the amount of calcium pantothenate in the diet had no effect on weight gain or food intake.

Of the three animals getting the maximal amount of ascorbic acid but no calcium pantothenate (pigs 1, 1, and 1A), one pig was sacrificed on the 12th day (pig 1 in Study II) and the other two on the 17th day of the study. Of the two animals with maximal amounts of ascorbic acid and minimal calcium pantothenate (pigs 9), one gained weight at the same rate as the synthetic diet controls while the other (pig 9 in Study III) grew as well as the pellet control for about the first 3 weeks of the study. At autopsy a growth was found between the heart and lungs in pig 9, Study III. Undoubtedly, this growth inhibited its swallowing causing it to eat less food from the 25th day of the study, and finally to lose considerable weight.

Guinea pigs 1 and 1A (40 A.A., 0 P.A.) in Study III consumed food fairly well at the beginning but were unable to continue. Supplementation of the diet of one of these animals with calcium pantothenate might have provided interesting information. To be sure, 40 mg. of ascorbic acid did not compensate for the omission of calcium pantothenate from their diets.

The synthetic diet controls in Study II and Study III gained an average of 4.6 gm. and 4.9 gm. per day during the course of the study. Their rate of growth compares favorably with animals of similar initial weights, on a similar diet, reported by Reid and Briggs (1954). The pellet

control was superior to the controls fed synthetic diet in both food consumption and growth, but the latter ranked somewhat higher in food efficiency.

In summary one might conclude that in Studies II and III (Table 7) large amounts of either ascorbic acid or pantothenic acid produced no beneficial effects on growth or food intake when the other was omitted. Comparisons made between guinea pigs 8 (0 A.A., 0 P.A.) and 1 (40 A.A., 0 P.A.) indicated that deficiency symptoms appeared at approximately the same time in both groups of pigs, namely, 12 to 18 days. Increasing the amount of ascorbic acid in the diet produced no benefits on survival time when no pantothenic acid was fed. No trends in growth, food consumption, or food efficiency were observed with increasing amounts of either of the two vitamins.

Hematology

Experiment A

After 15 days on a diet of natural foods containing 0.15 per cent omega-methylpantothenic acid, there was no appreciable difference in red blood cell counts, red blood cell volumes or hemoglobin values between three experimental pigs and their litter mate controls (Table 8). Litter

Table 7. Summary of weight gain, food intake and food efficiency of guinea pigs fed synthetic diet supplemented with four levels of ascorbic acid and four levels of calcium pantothenate

Study II and Study III

Study and guinea pig no.	Supplement		Initial wt. (gm.)	No. of days on expt.	Final wt. (gm.)	Ave. wt. gain (gm./day)	Ave. food intake (gm./day)	Wt. gain per gm. food eaten (gm./day)
	Asc. A. (mg./day)	Ca Panto. (mg./day)						
II-8	0	0	112	14	153	2.9	5.9	0.50
III-8	0	0	110	17	139	1.7	8.0	0.21
III-8A	0	0	99	18	137	2.1	6.6	0.32
II-3	0	0.06	103	22	139	1.6	6.3	0.26
III-3	0	0.06	85	18	127	2.3	5.8	0.40
II-6	0	8	112	23	169	2.5	8.7	0.28
III-6	0	1	93	20	129	1.8	7.1	0.25
II-7	2	0	84	30	235	5.0	10.1	0.50
III-7	2	0	83	22	139	2.5	7.0	0.36
III-7A	2	0	85	24	175	3.8	8.9	0.42
II-2	2	0.06	98	Died[a]	--	--	--	--
III-2	2	0.06	95	33	253	4.8	10.7	0.45
II-5	2	8	99	30	211	3.7	9.1	0.41
III-5	2	1	99	33	261	4.9	10.5	0.47

[a]Lung infection.

Table 7 (Cont'd)

Study and guinea pig no.	Supplement Asc. A. (mg./day)	Supplement Ca Panto. (mg./day)	Initial wt. (gm.)	No. of days on expt.	Final wt. (gm.)	Ave. wt. gain (gm./day)	Ave. food intake (gm./day)	Wt. gain per gm. food eaten (gm./day)
II-1	40	0	85	12	119	2.8	6.2	0.46
III-1	40	0	68	17	92	1.4	5.5	0.26
III-1A	40	0	77	17	107	1.8	4.1	0.43
II-9	40	0.06	109	25	215	4.2	8.0	0.53
III-9	40	0.06	107	32	218	3.5	9.3	0.37
II-4	10	0.20	101	30	240	4.6	10.1	0.46
III-4	10	0.20	100	33	248	4.5	10.3	0.44
II-pellet control	10	--	116	33	352	7.2	18.4	0.39

Table 8. Hematological data at autopsy of guinea pigs fed omega-methylpantothenic acid and their litter mate controls

	Erythrocytes millions/mm.[3]		Hemoglobin gm./100 ml.		Packed red cell volume per cent	
Guinea pigs	Analogue	Control	Analogue	Control	Analogue	Control
After 15 days						
7	4.29	4.70	10.29	11.12	32.8	34.5
8	5.22	5.02	12.76	11.99	39.8	36.8
9	5.36	5.64	13.15	12.12	40.8	38.8
Ave.	4.96	5.12	12.07	11.74	37.8	36.7
After 47 days						
1[a]	5.04	5.46	10.48	13.28	34.8	41.2
2	3.17	5.55	7.58	14.20	24.2	42.8
3[b]	4.03	5.77	9.63	13.76	29.0	42.5
4	--	5.74	--	13.83	--	43.0
5	4.77	5.88	11.14	13.98	34.5	43.2
6	3.42	5.50	7.84	14.02	24.2	43.0
Ave.	4.09	5.65	9.33	13.84	29.3	42.6

[a]Sacrificed on 35th day of study.

[b]Sacrificed on 45th day of study.

mates 7E and 7C both were slightly anemic as evidenced by the values for all three determinations. At the termination of the study when analogue had been fed for 35 to 45 days, large differences were observed between the experimental pigs and their controls, with the experimental group showing signs of anemia. The red cell counts, hemoglobin concentrations, and red cell volumes were all markedly decreased. When hemoglobin concentrations were plotted against red blood cell counts, a correlation of 0.96 was obtained with a linear regression which was highly significant at the 1 per cent level, indicating that as the red cell count changed the hemoglobin concentration changed in the same direction.

Hemoglobin values of the six control pigs at the end of the study compare favorably with the values reported by Woodruff et al., (1953) for their controls fed Rockland feed. For 27 animals an average of 13.95 ± 0.14 gm. per 100 ml. was obtained using the oxyhemoglobin method. The average for six pigs in this study was 13.84 gm. per 100 ml. of blood. Woodruff reported an average red blood cell count of 4.42 ± 0.10 millions per $mm.^3$ which is considerably lower than the average cell count of 5.65 in the present investigation. Cannon et al., (1945) found an average hemoglobin concentration of 14.00 gm./100 cc. of blood in their guinea pigs which had been maintained on a ration of crude natural

foodstuffs. Unfortunately they did not cite their method for hemoglobin determination, so it is difficult to make comparisons with the data obtained in the present experiment.

Experiment B

Study I. The red cell volumes and hemoglobin values of the animals increased with increased ascorbic acid intake (Table 9). An exception to this generalization was guinea pig 5, which was fed a minimal amount of ascorbic acid and 8 mg. calcium pantothenate. Its hemoglobin concentration and red cell volume were the highest in Study I. When comparisons were made within groups based on intake of calcium pantothenate, hemoglobin concentration and packed red cell volume increased with increases in ascorbic acid in Studies I and III when no calcium pantothenate was fed. On 0.06 mg. per day of calcium pantothenate there were increases in both hemoglobin concentration and in packed red cell volume with increases in ascorbic acid in all three studies.

The three pigs in Study I receiving no ascorbic acid showed an increase in hemoglobin values and packed red cell volume with an increase in calcium pantothenate. This trend was not confirmed in Studies II and III.

Studies II and III. In these studies the animals were considerably younger than in Study I. When the blood data

Table 9. Hematological data of guinea pigs fed synthetic diet supplemented with four levels of ascorbic acid and four levels of calcium pantothenate

Guinea pig no.	Supplement		Study I		Study II		Study III	
	Asc. A. (mg./day)	Ca Panto. (mg./day)	Hb (gm./100 ml.)	Red blood cell vol. (per cent)	Hb (gm./100 ml.)	Red blood cell vol. (per cent)	Hb (gm./100 ml.)	Red blood cell vol. (per cent)
8	0	0	9.74	29.9	12.00	34.0	10.06	30.0
8A	0	0	--	--	--	--	11.37	--
3	0	0.06	10.06	31.5	10.41	31.6	10.71	--
6	0	8 or 1[a]	11.48	34.6	10.40	32.5	11.02	35.0
7	2	0	12.43	37.3	11.67	32.0	12.54	41.2
7A	2	0	--	--	--	--	10.52	34.6
2	2	0.06	12.91	39.5	Died[b]	--	11.02	34.1
5	2	8 or 1[a]	14.57	44.3	12.09	34.1	12.22	40.6
1	40	0	13.10	39.0	11.72	33.2	10.37	32.6
1A	40	0	--	--	--	--	17.73	--
9	40	0.06	13.44	40.0	11.04	31.8	13.34	43.5
4	10	0.20	13.28	40.0	12.14	33.8	13.03	42.0
Pellet control	10	--	--	--	10.98	32.0	--	--

[a]8 mg. in Studies I and II and 1 mg. in Study III.

[b]Lung infection.

of the animals were compared, there was a tendency for hemoglobin concentration and red cell volume to increase with ascorbic acid intake in Study III but not in Study II. In Study II, the hematological values of the entire group of pigs were relatively low, including those of the pellet control. In Study III, the values tended to run a little higher. Guinea pig 1A was observed to consume only small amounts of water, hence its hemoglobin concentration of 17.73 gm. per 100 ml. may be the result of hemoconcentration. Although anemia was characteristic of animals fed the analogue diet, it was not found in animals whose diets lacked pantothenic acid. This may indicate that anemia was produced only when the pantothenic acid deficiency was developed over a longer period of time.

Reid and Briggs (1953) reported an average hemoglobin concentration of 14.7 ± 0.20 gm. per 100 ml. of blood, and packed red cell volume of 43.4 ± 0.37 per cent for guinea pigs maintained on their semi-synthetic diet for 62 days. In Experiment B of the present investigation only the control animal of Study I (10 A.A., 0.2 P.A.) is suitable for comparison with their animals on the basis of age and diet. The control animal (pig 4) had a hemoglobin value of 13.28 gm. per 100 ml. of blood and a packed red cell volume of 40.0 per cent. Reid and Briggs did not state their methods for hemoglobin and hematocrit determinations, therefore it

is difficult to determine the significance of the difference between their value and that of the present investigation.

When blood serum or plasma ascorbic acid was plotted against hemoglobin concentration, a correlation of 0.42 was obtained with a regression which was highly significant at the 1 per cent level (Figure 6). Changes in the quantity of formed elements in the blood are believed to begin in the guinea pig deprived of ascorbic acid before any of the usual gross symptoms of scurvy appear. Presnell (1934) reported that the blood of scorbutic guinea pigs had a longer coagulation time, a lower hemoglobin concentration and red cell volume, and fewer red blood cells than the blood from a healthy guinea pig.

An interesting study showing the relationship between ascorbic acid intake and erythrocyte count and hemoglobin values in human subjects has been reported in the Chinese Journal of Nutrition. Mei and Shen (1947) administered 500 mg. of ascorbic acid to 9 male subjects for a period of 20 days and found that the erythrocyte count and hemoglobin increased significantly by 6.3 per cent and 4.8 per cent over initial values during the first 10 days of saturation. These values dropped gradually in the next 10 days.

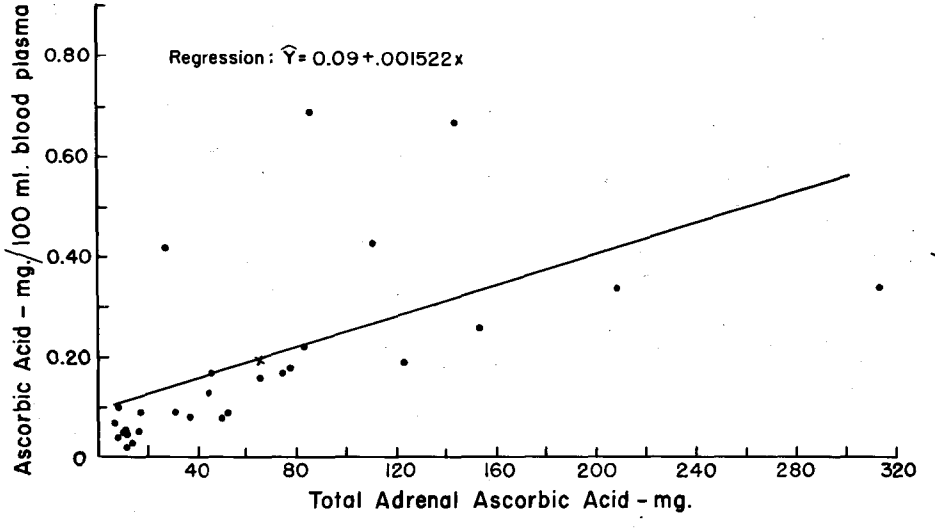
Figure 6. Regressions of:

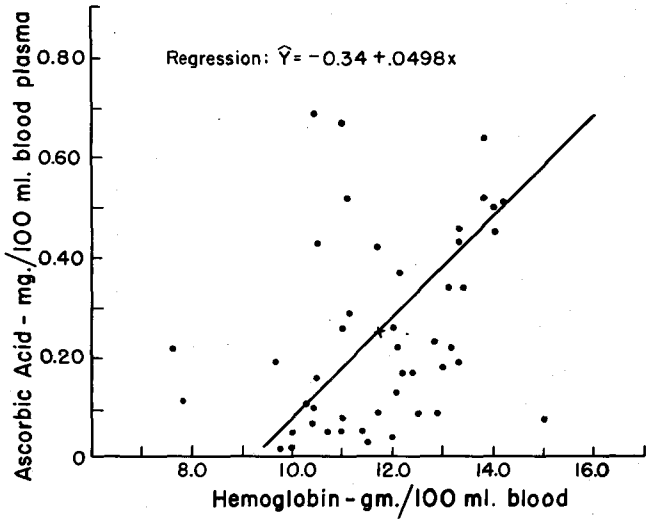
Blood plasma ascorbic acid on total adrenal ascorbic acid,

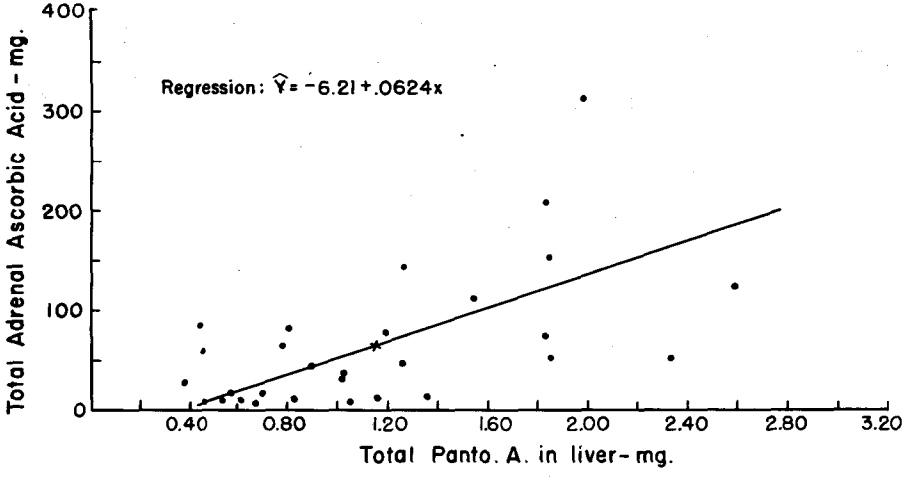
Blood plasma or serum ascorbic acid on hemoglobin,

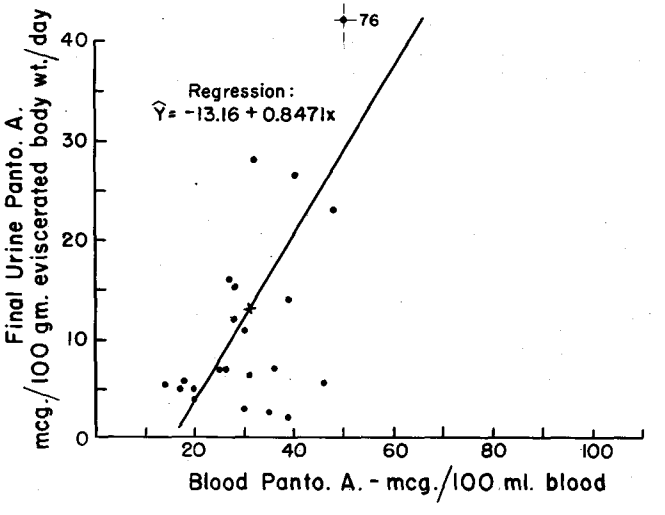
Total adrenal ascorbic acid on total liver pantothenic acid,

Final urine pantothenic acid on blood pantothenic acid


Regression: $\hat{Y}$ = 0.09 + .001522x
Ascorbic Acid - mg./100 ml. blood plasma
0.80
0.60
0.40
0.20
0
40
80
120
160
200
240
280
320
Total Adrenal Ascorbic Acid - mg.


Regression: $\hat{Y}$ = -0.34 + .0498x
Ascorbic Acid - mg./100 ml. blood plasma
0.80
0.60
0.40
0.20
0
8.0
10.0
12.0
14.0
16.0
Hemoglobin - gm./100 ml. blood


Regression: $\hat{Y}$ = -6.21 + .0624x
Total Adrenal Ascorbic Acid - mg.
400
300
200
100
0
0.40
0.80
1.20
1.60
2.00
2.40
2.80
3.20
Total Panto. A. in liver - mg.


76
Regression:
$\hat{Y}$ = -13.16 + 0.8471x
Final Urine Panto. A.
mcg./100 gm. eviscerated body wt./day
40
30
20
10
0
20
40
60
80
100
Blood Panto. A. - mcg./100 ml. blood

A few laboratories (Daft et al., 1945, Ashburn et al., 1947, and Carter et al., 1945) have reported a fatal aplasia of the bone marrow with anemia, leucopenia, and granulocytopenia in pantothenic acid-deficient rats. Daft et al. reported that the administration of pantothenic acid was usually unsuccessful therapy unless folic acid was administered also. However, only adequate amounts of pantothenic acid, not folic acid, could prevent the blood disorder. Carter et al. reported that pantothenic acid therapy was successful in only 25 per cent of their rats. It is interesting that the anemia in scurvy responds to folic acid therapy.

Ascorbic Acid Findings

Blood serum or plasma ascorbic acid concentration

Experiment A. After 15 days of 0.15 per cent intake of omega-methylpantothenic acid, the serum of three experimental animals average 0.19 mg. of ascorbic acid per 100 ml. while that of their litter mates averaged 0.38 mg. per cent. At the termination of the study, the serum of the experimental group averaged 0.23 mg. and of the controls 0.51 mg. per cent. The values for the two groups did not overlap at either time (Table 10).

Table 10. Concentration of ascorbic acid, pyruvic acid and pantothenic acid in the blood of guinea pigs fed omega-methylpantothenic acid and their litter mate controls

Guinea pigs	Ascorbic acid mg./100 ml. serum		Pyruvic acid mg./100 ml. blood		Pantothenic acid mcg./100 ml. blood
	Analogue	Control	Analogue	Control	Control
After 15 days					
7	0.11	0.52	2.01	1.56	
8	0.23	0.26	0.86	1.65	
9	0.22	0.37	1.57	1.30	
Ave.	0.19	0.38	1.48	1.50	
After 47 days					
1[a]	0.43	0.46	4.93	1.55	--
2	0.22	0.51	1.73	1.53	45
3[b]	0.19	0.52	4.36	2.00	58
4	0.12	0.64	8.14	--	25
5	0.29	0.50	3.43	2.71	35
6	0.11	0.45	2.78	2.26	30
Ave.	0.23	0.51	4.23	2.01	39

[a]Sacrificed on 35th day of study.

[b]Sacrificed on 45th day of study.

Vestling and Rebstock (1945) gave the normal range of ascorbic acid in guinea pig blood as 0.19 to 0.31 mg. per cent; during scurvy, 0.02 to 0.09 mg. per cent. Within the normal range, as defined by these two workers, falls the concentration which Penny and Zilva (1946) report as representative of the "saturated state" in the guinea pig, namely, 0.25 mg. per cent. Todhunter and Brewer (1940) gave 0.54 mg. per 100 ml. of plasma as the normal amount, while Munsell et al., (1944) gave 0.56 mg. per cent. Very large variations may be found in the literature as to what constitutes the normal range of ascorbic acid in the blood or plasma of the guinea pig.

Karel and Chapman (1944) showed that in addition to wide variation among animals, large variations occurred due to the time elapsing between the last intake of the vitamin and the withdrawal of the blood sample. They obtained the highest concentration (0.72 mg. per cent) 4 to 6 hours after feeding. From 15 to 44 hours after feeding, the concentration remained constant at about 0.16 mg. per cent. They gave a mean value of 0.30 mg. per 100 ml. of plasma as the normal concentration for the guinea pig.

In Experiment A, all pigs were fed ascorbic acid at the same time, usually about the middle of the morning. Fortunately the two animals displaying the deficiency

syndrome did so early in the morning, so the ascorbic acid for that day was withheld for both pairs of litter mates. All animals were given 10 mg. of the vitamin once each day throughout the entire study. This amount was more than sufficient throughout the study in terms of the 0.7 mg. per 100 gm. of body weight, which Pfander and Mitchell (1952) consider the minimum requirement for male guinea pigs, weighing 200 to 300 grams.

Guinea pig 1E was the first animal to show symptoms of a deficiency. When it was sacrificed on the 35th day of the study, it had a serum ascorbic acid concentration of 0.43 mg. while its litter mate control had 0.46 mg. per cent. The next highest serum concentration in the experimental group was 0.29 mg. per cent for pig 5E which, from outward appearances, seemed to be in the best physical condition, in comparison with the other experimental animals which also survived until the study was terminated.

In the study of pantothenic acid deficiency, very few investigators have made determinations for serum ascorbic acid at the same time. Ralli (1952), in studying the importance of pantothenic acid in stress conditions imposed on young men, found, among other changes observed after the stress, a significant elevation of ascorbic acid in whole blood. What the significance of this finding may be

in light of the present investigation, it is difficult to say.

Experiment B. In Experiment B, blood plasma ascorbic acid determinations were made once each week immediately before the animals were set up on funnels for weekly urine collections. The blood sample was obtained from an ear vein. Plasma concentrations seemed to be related to the intake of the vitamin in all three studies (Tables 11 and 12). Some of the animals getting 2 mg. ascorbic acid per day had plasma values which bordered on scurvy. Large amounts of calcium pantothenate in the diet did not have any effect upon the blood plasma concentration of ascorbic acid.

Weight of the adrenal glands

Experiment B. When the weights of the adrenal glands (Table 13) were compared for the animals within each study, no trend in size is evident with either increasing or decreasing amounts of either of the two vitamins. Since the animals varied considerably in age and size when placed on experiment, and since they were sacrificed at different times during the course of the experiment, it seemed advisable, for the sake of comparison, to calculate the weights of the adrenals with respect to body size. When comparisons of weight of adrenals per 100 gm. of body weight were made,

Table 11. Ascorbic acid concentration in blood plasma of guinea pigs fed synthetic diet supplemented with four levels of ascorbic acid and four levels of calcium pantothenate

Study I

Guinea pig	Ascorbic acid (mg./day)	Ca panto. (mg./day)	Ascorbic acid in mg./100 ml. plasma — Week of study									
			1	2	3	4	5	6	7	8	9	10
8	0	0	--	0.12	0.06	0.02[a]						
3	0	0.06	--	--	0.02[b]							
6	0	8	--	0.19	--	0.03[a]						
7	2	0	--	0.11	0.13	0.08	0.10	0.16	0.19	0.10	0.20	0.17[a]
2	2	0.06	--	0.12	0.18	0.09	0.20	0.24	0.13	0.05	0.17	0.09[a]
5	2	8	--	0.14	--	0.15	--	0.10	0.16	0.13	0.24	0.08[a]
1	40	0	--	1.03	--	0.51	--	0.66	0.78	0.57	0.32	0.34[a]
9	40	0.06	--	0.28	--	0.37	--	0.46	0.64	0.58	0.35	0.34[a]
4	10	0.2	--	0.16	--	0.23	--	0.19	0.31	--	0.18	0.19[a]

[a]Final values, blood obtained from portal vein; all other determinations made on blood from ear vein.

[b]Value obtained 5 days previously was 0.03 mg.

Table 12. Ascorbic acid concentration in blood plasma of guinea pigs fed synthetic diet supplemented with four levels of ascorbic acid and four levels of calcium pantothenate

Studies II and III

Guinea pig	Supplement		Ascorbic acid values in mg./100 ml. plasma									
			Study II					Study III				
			Week of study					Week of study				
	Asc. A. (mg./day)	Ca Panto. (mg./day)	1	2	3	4	5	1	2	3	4	5
8	0	0	--	0.04[a]	--	--	--	--	0.14	0.05[a]	--	--
8A	0	0	--	--	--	--	--	--	--	0.05[a]	--	--
3	0	0.06	0.26	--	0.07[a]	--	--	--	--	0.05[a]		
6	0	8 or 1[b]	--	0.06	0.10	0.10[a]	--	--	--	0.05[a]	--	--
7	2	0	--	0.08	0.09	0.05	0.09[a]	--	--	--	0.09[a]	--
7A	2	0	--	--	--	--	--	--	0.11	0.29	0.16[a]	--
2	2	0.06	0.26	Died[c]	--	--	--	--	0.12	0.15	0.24	0.08[a]
5	2	8 or 1[b]	--	0.12	0.25	0.12	0.13[a]	--	0.15	--	0.20	0.17[a]
1	40	0	--	0.42[a]	--	--	--	--	--	0.69[a]	--	--
1A	40	0	--	--	--	--	--	--	--	--	--	--
9	40	0.06	0.29	0.41	0.71	0.67[a]	--	--	--	--	0.35	0.43[a]
4	10	0.20	0.41	0.42	0.19	0.26	0.22[a]	--	0.15	--	--	0.18[a]
Pellet control	10	--	--	--	0.26	0.36	0.26[a]	--	--	--	--	--

[a]Final values (blood obtained from portal vein; all other determinations made on blood from ear vein).

[b]8 mg. in Study II; 1 mg. in Study III.

[c]Lung infection.

Table 13. Adrenal weights and ascorbic acid concentrations of adrenal glands of guinea pigs fed synthetic diet supplemented with four levels of ascorbic acid and four levels of calcium pantothenate

Study and guinea pig no.	Supplement		Wt. of adrenals (mg.)	Wt. of adrenals per 100 gm. body wt. (mg.)	Wt. of adrenals per 100 gm. evis. body wt. (mg.)	Asc. A. per 100 gm. adrenals (mg.)	Total Asc. A. in adrenals (mcg.)
	Asc. A. (mg./day)	Ca Panto. (mg./day)					
I-8	0	0	158.6	101.7	133.3	--	--
II-8[a]	0	0	86.0	56.6	81.9	9.08	7.81
III-8	0	0	87.8	63.2	82.8	18.51	16.25
III-8A	0	0	76.1	55.5	74.6	13.75	10.46
I-3	0	0.06	141.6	64.4	89.1	8.09	11.46
II-3	0	0.06	90.2	64.4	94.0	6.58	5.94
III-3	0	0.06	74.0	58.3	84.1	13.09	9.69
I-6	0	8	200.4	86.0	98.2	6.86	13.75
II-6	0	8	95.7	56.6	68.4	8.82	8.44
III-6	0	1	80.1	62.1	89.0	11.86	9.50
I-7	2	0	240.0	50.2	62.2	31.26	75.02
II-7	2	0	99.6	42.4	56.6	30.96	30.84
III-7	2	0	121.8	88.9	148.5	13.80	16.81
III-7A	2	0	135.2	77.3	104.8	49.16	66.46
I-2	2	0.06	256.1	48.4	57.4	20.66	52.91
II-2	2	0.06	Died[b]	--	--	--	--
III-2	2	0.06	104.0	41.1	56.2	36.05	37.49

[a]Broke leg before end of study.

[b]Lung infection.

Table 13 (Cont'd)

Study and guinea pig no.	Supplement Asc. A. (mg./day)	Supplement Ca Panto. (mg./day)	Wt. of adrenals (mg.)	Wt. of adrenals per 100 gm. body wt. (mg.)	Wt. of adrenals per 100 gm. evis. body wt. (mg.)	Asc. A. per 100 gm. adrenals (mg.)	Total Asc. A. in adrenals (mcg.)
I-5	2	8	184.9	33.8	40.1	27.48	50.81
II-5	2	8	98.0	46.4	62.8	45.66	44.75
III-5	2	1	94.0	36.0	48.0	48.76	45.83
I-1	40	0	154.8	28.7	33.2	135.25	209.37
II-1	40	0	72.0	60.5	82.8	38.00	27.36
III-1	40	0	106.3	115.5	158.7	80.56	85.64
III-1A	40	0	70.0	65.4	109.4	84.38	59.07
I-9	40	0.06	272.9	44.6	51.6	115.00	313.84
II-9	40	0.06	94.0	43.7	58.0	152.92	143.74
III-9	40	0.06	129.1	59.2	70.9	85.93	110.94
I-4	10	0.20	228.0	35.3	40.8	54.00	123.12
II-4	10	0.20	107.0	44.6	67.7	78.08	83.55
III-4	10	0.20	94.1	37.9	52.0	83.02	78.12
II-Pellet control	10	--	116.3	33.0	39.8	132.57	154.18

it was evident that in Study I the three scorbutic animals had the largest glands, the adrenals of the two pigs with chronic scurvy being considerably larger than those of the animal with acute scurvy. In Study II, the three scorbutic animals also had the largest adrenals, however, those of guinea pig 1 (40 A.A., 0 P.A.) were equally large. The size of the adrenals from animals receiving 2 mg. of ascorbic acid were the same size as those from animals receiving 40 mg. of ascorbic acid.

In Study III, a large range of adrenal weights was found within each group. The four animals getting no calcium pantothenate had the largest adrenals, some of which were considerably larger than those of the scorbutic animals. The adrenals of young animals are known to be larger than those of older animals, but certainly a factor or factors other than age must have been responsible for the considerable difference in size between the adrenals of guinea pigs 1 and 1A (115.5 versus 65.4 mg.). Both pigs were sacrificed on the same day and differed only slightly in body weight. When the adrenal weights were calculated per 100 gm. eviscerated body weight, they were found to be related to intake of calcium pantothenate, so long as there was some ascorbic acid in the diet.

To summarize the above findings, it appeared that there was an increase in size of the adrenal glands in scurvy,

the glands becoming particularly large when the condition was chronic. On a pantothenic acid-deficient diet, the adrenals also became enlarged, especially if symptoms of pantothenic acid deficiency were also observed. It is difficult to say whether the increase in size is due to an outright deficiency of the two vitamins involved, or whether it is more directly associated with the inanition of relatively short duration which accompanied these deficiencies.

Guinea pigs, deficient in ascorbic acid, are known to show a marked loss in fat and cholesterol from the adrenal gland (Bessey et al., 1934). There is a similar loss in rats deficient in pantothenic acid (Deane and McKibbin, 1946). The roles played by these 2 vitamins in the functioning of the adrenal gland is a very popular topic for research at the present time. Unfortunately there is a great deal of conflicting information in the literature probably due to differences in experimental conditions. Certainly the primary function of ascorbic acid in the adrenal gland has not been elucidated as yet. There seems to be general agreement, however, that a pantothenic acid deficiency produces an impairment in the function of the adrenal gland. This can probably be explained by the depletion of Coenzyme A in the gland (McHenry, 1955).

Ascorbic acid concentration in the adrenals

All three studies show that as dietary ascorbic acid was increased, the concentration of ascorbic acid per 100 gm. of adrenal was also increased. When total ascorbic acid for the adrenals was calculated, several interesting observations were made. In Study II, both animals getting no calcium pantothenate showed very small but similar amounts of ascorbic acid regardless of the fact that guinea pig 1 received a large amount of ascorbic acid with its diet. This animal (pig 1) had convulsions shortly before it was sacrificed and when its adrenal glands were examined it was difficult to distinguish between the cortex and the medulla.

In Study III, guinea pig 7 (2 A.A., 0 P.A.) developed woolly fur, diarrhea, and hemorrhagic adrenals and had almost a scorbutic concentration of ascorbic acid in its adrenals. Guinea pig 1 (40 A.A., 0 P.A.) with much enlarged adrenals had a higher concentration of total ascorbic acid in the adrenals than guinea pig 1A (40 A.A., 0 P.A.) with adrenals of normal size. Hence, considerable individual variation was apparent.

The amount of calcium pantothenate in the diet was reflected in the liver. When total adrenal ascorbic acid was plotted against total liver pantothenic acid (Figure 6), a correlation of 0.54 was obtained. The linear regression

was highly significant at the 1 per cent level, suggesting that a change in the amount of one vitamin in one organ was accompanied by a change in the amount of the other vitamin in the other organ.

When serum or plasma ascorbic acid concentrations at the end of the experiment were plotted against total adrenal ascorbic acid, the fairly high correlation of 0.60 was obtained. The linear regression (Figure 6) was highly significant at the 1 per cent level, indicating that as the amount of ascorbic acid in the adrenal gland changed there was a corresponding change in the concentration of this vitamin in the blood plasma or serum.

To summarize these observations, it has been shown that as dietary ascorbic acid was increased, the ascorbic acid per 100 grams of adrenals was also increased. There was considerable variation with respect to both the size of the adrenals and the concentration of ascorbic acid in the adrenals of the animals within each group, suggesting that factors other than ascorbic acid were involved. A highly significant relationship was observed between the total amount of ascorbic acid in the adrenals and the total amount of pantothenic acid in the liver.

Pantothenic Acid Findings

Blood pantothenic and pyruvic acids

Experiment A. Blood pantothenic acid levels for five control animals at the conclusion of the experiment ranged from 25 to 58 mcg. per 100 ml. of blood (Table 10). Higher blood pantothenic acid values corresponded with lower pyruvic acid levels and vice versa indicating an inverse relationship.

Blood pyruvic acid values were essentially the same for both experimental and control animals after the initial 15 days of the experiment. By the end of the study, the group receiving omega-methylpantothenic acid had a mean value twice as high as the control group, indicating some accumulation of pyruvic acid in the blood. The range of values for five control animals was 1.53 to 2.71 mg., while that for six experimental pigs was 1.73 to 8.14 mg. per 100 ml. of blood.

It has been shown that the pyruvic acid content of the blood is increased following exercise (Friedemann and Barborka, 1941), the ingestion of sugar (Bueding et al., 1941) and during infections (Davis and Bauer, 1944). Some workers have fasted their animals overnight and restricted their movements for several hours before sacrificing them.

In neither Experiment A nor B was this possible because of the suddenness of the onset of symptoms. In Experiment A, the litter mate control was sacrificed at the same time as the experimental animal so the pyruvic acid values have a comparable basis.

Experiment B. Blood pantothenic acid values ranged from 13 to 50 mcg. per 100 ml. of blood and the level in the blood reflected the dietary intake of the vitamin (Table 14). Guinea pig 1 of Study I (40 A.A., 0 P.A.), with the high blood concentration of 46 mcg. per cent, surely was very proficient in intestinal synthesis of the vitamin. The guinea pigs numbered 7 in all three studies (2 A.A., 0 P.A.) had extremely low blood concentrations of pantothenic acid, ranging from 13 to 18 mcg. per cent. When the blood levels of the guinea pigs which showed symptoms associated with a pantothenic acid deficiency were examined, guinea pig 7 (2 A.A., 0 P.A.) of Study I (diarrhea) had a blood value of 14 mcg. per cent, pig 1 (40 A.A., 0 P.A.) of Study II (convulsions) had a value of 25 mcg. per cent, and guinea pig 7 of Study III (woolly fur, diarrhea, and hemorrhagic adrenals) had a value of 18 mcg. per cent. Unfortunately data for guinea pigs 1 and 1A (both 40 A.A., 0 P.A.) of Study III are missing. When blood pantothenic acid was plotted against plasma ascorbic acid or adrenal ascorbic acid, no relationship was observed.

Table 14. Pantothenic acid in blood and liver and pyruvic acid in blood of guinea pigs fed synthetic diet supplemented with four levels of ascorbic acid and four levels of calcium pantothenate

Study and guinea pig no.	Supplement		Blood		Liver			
	Asc. A. (mg./day)	Ca Panto. (mg./day)	Panto A. (mcg./100 ml.)	Pyruvic acid (mg./100 ml.)	Panto A. per gm. wet wt. (mcg.)	Panto A. per 100 gm. body wt. (mcg.)	Panto A. per 100 gm. evis. body wt. (mcg.)	Total panto A. in liver (mcg.)
I-8	0	0	30	1.39	128.1	571.3	748.1	890.3
II-8[a]	0	0	28	3.22	84.1	304.4	440.7	462.6
III-8	0	0	20	1.86	100.6	507.0	664.0	704.2
III-8A	0	0	--	3.57	106.4	442.6	594.8	606.5
I-3	0	0.06	31	2.10	95.2	525.5	727.3	1156.7
II-3	0	0.06	48	3.24	127.0	481.3	701.0	673.1
III-3	0	0.06	--	2.26	103.6	423.7	612.3	538.7
I-6	0	8	40	1.75	145.8	581.7	664.8	1355.9
II-6	0	8	50	2.87	124.6	619.3	747.6	1046.6
III-6	0	1	--	3.11	140.0	639.8	918.4	826.0
I-7	2	0	14	2.77	96.7	382.0	473.8	1827.6
II-7	2	0	18	2.51	89.0	435.2	581.2	1023.5
III-7	2	0	13	2.24	111.7	420.0	701.5	575.3
III-7A[a]	2	0	17	2.68	89.1	448.2	607.7	784.1
I-2	2	0.06	35	4.78	96.0	350.4	415.7	1852.8
II-2	2	0.06	Died[b]	--	--	--	--	--
III-2	2	0.06	20	2.10	91.5	409.0	559.1	1034.0

[a]Broke leg before end of study.

[b]Lung infection.

Table 14 (Cont'd)

Study and guinea pig no.	Supplement		Blood		Liver			
	Asc. A. (mg./day)	Ca Panto. (mg./day)	Panto A. (mcg./100 ml.)	Pyruvic acid (mg./100 ml.)	Panto A. per gm. wet wt. (mcg.)	Panto A. per 100 gm. body wt. (mcg.)	Panto A. per 100 gm. evis. body wt. (mcg.)	Total panto A. in liver (mcg.)
I-5	2	8	--	2.40	111.2	427.0	507.1	2335.2
II-5	2	8	32	3.01	78.7	428.9	580.0	905.0
III-5	2	1	39	3.18	122.7	484.7	645.4	1263.8
I-1	40	0	46	1.82	85.9	342.7	396.0	1846.8
II-1	40	0	25	2.09	117.6	316.3	432.8	376.3
III-1	40	0	--	2.52	112.8	490.7	673.4	451.2
III-1A	40	0	--	--	113.9	426.0	711.9	455.6
I-9	40	0.06	39	2.74	82.6	326.3	377.5	1994.8
II-9	40	0.06	26	2.43	149.0	588.6	782.2	1266.5
III-9	40	0.06	--	4.54	138.8	713.4	853.6	1554.6
I-4	10	0.2	30	2.19	109.9	401.1	463.8	2593.6
II-4	10	0.2	28	2.54	85.7	335.9	510.0	805.6
III-4	10	0.2	36	3.12	119.0	479.6	656.9	1190.0
II-Pellet control	10	--	27	2.80	115.5	527.8	636.4	1859.6

As far as is known, no data for the concentration of pantothenic acid in the blood of the guinea pig has been reported. However, in the chick (Snell et al., 1940), in the duck (Trager, 1943), in the dog (Silber, 1944), and in swine (Luecke et al., 1949) a lowered pantothenic acid content of the blood and tissues has been reported when pantothenic acid is removed from the diet.

Blood pyruvic acid in Studies I, II and III ranged from 1.39 to 4.78 mg. per 100 ml. and showed no correlation with the concentration of pantothenic acid in the blood. In Studies I and III there seemed to be some tendency for high blood pyruvic acid values to be associated with high total pantothenic acid in the liver. Pyruvic acid in Study I ranged from 1.39 to 4.78 mg. per 100 ml. of blood, in Study II, from 2.09 to 3.24, and in Study III from 1.86 to 4.54 mg. per cent. It is difficult to explain the occurrence of some of these high values. In Study I, guinea pig 2 (2 A.A., 0.06 P.A.) with the highest pyruvic acid level of 4.78 mg. per cent, had an average concentration of pantothenic acid in the blood (35 mcg. per 100 ml.), a relatively high amount of total pantothenic acid in the liver (1853 mcg.), no signs of an infection, but quite a few small white areas on the liver. An investigation into a possible relationship between the condition of the liver

at autopsy and the blood pyruvic acid concentration failed to show that a definite relationship existed. For example, in Study I, guinea pig 5 (2 A.A., 8 P.A.), which had necrosis in one entire lobe of the liver, had 2.40 mg. of pyruvic acid per 100 ml. of blood. In Study III, pig 9 (40 A.A., 0.06 P.A.) with a blood pyruvic acid of 4.54 mg. per cent, had a severe lung infection, a fatty liver, and a growth in the heart-lung area which was probably responsible for the animal's drastic decrease in food intake. It is possible that a failure to remove feed cups a few hours before sacrificing the animals may be partially responsible for some of the high blood pyruvic acid values. The synthetic diet contained a high concentration of both sucrose and dextrose.

Pantothenic acid in the liver

Experiment B. Because of variations in the age of the animals in Study I compared with Studies II and III, comparisons must be made by studies (Table 14). With only one or two exceptions total pantothenic acid in the liver reflected intake of the vitamin. Those animals which displayed symptoms associated with pantothenic acid deficiency, namely, pig 7 (2 A.A., 0 P.A.) in Study I, pig 1 (40 A.A., 0 P.A.) in Study II, and pigs 7, 1, and 1A in Study III,

all had decreased amounts of pantothenic acid in the liver. Pigs 1 and 1A of Study III had almost identical amounts of pantothenic acid in the liver and yet showed considerable variation in the size of the adrenals and concentration of ascorbic acid in the adrenals. Large amounts of ascorbic acid did not compensate for sub-optimal amounts of pantothenic acid in the diet insofar as pantothenic acid in the liver is concerned.

Pantothenic acid in the urine

Experiment B. The excretion of pantothenic acid in the urine was influenced by the intake of the vitamin in all three studies, and the amount of ascorbic acid in the diet had no influence on the amount of pantothenic acid excreted. When the excretion of the vitamin was calculated per 100 gm. body weight, the data once again reflected intake of calcium pantothenate (Tables 15 and 16).

In Study I, when eight collections were made from each of six animals, it was noted that the two animals getting no calcium pantothenate excreted an average of 19 and 20 mcg. per day, while the control pig getting 0.2 mg. excreted an average of 22 mcg. per day.

When the pantothenic acid excreted in the final urine collection was calculated per 100 gram eviscerated body

Table 15. Pantothenic acid in urine of guinea pigs fed synthetic diet supplemented with four levels of ascorbic acid and four levels of calcium pantothenate

Study I

Pantothenic acid in urine per day	Week							
	1	2	3	4	5	6	7	8
Pig 8 (0 A.A., 0 P.A.)								
mcg.	29.7	46.6	19.9					
mcg./100 gm. body wt.	15.9	21.7	12.1					
mcg./100 gm. evis. body wt.			16.7					
Pig 3 (0 A.A., 0.06 P.A.)								
mcg.	42.0	10.2						
mcg./100 gm. body wt.	16.9	4.7						
mcg./100 gm. evis. body wt.		6.4						
Pig 6 (0 A.A., 8 P.A.)								
mcg.	2920.8	158.0	81.8					
mcg./100 gm. body wt.	1070.0	50.2	33.4					
mcg./100 gm. evis. body wt.			40.1					
Pig 7 (2 A.A., 0 P.A.)								
mcg.	21.6	10.0	17.2	21.8	12.8	9.2	7.7	21.1
mcg./100 gm. body wt.	9.4	3.7	6.1	7.7	3.6	2.2	1.7	4.9
mcg./100 gm. evis. body wt.								5.5
Pig 2 (2 A.A., 0.06 P.A.)								
mcg.	20.4	19.5	26.0	18.4	36.0	9.4	10.0	11.0
mcg./100 gm. body wt.	7.9	7.2	8.8	5.4	9.4	2.1	2.1	2.2
mcg./100 gm. evis. body wt.								2.5

Table 15 (Cont'd)

Pantothenic acid in urine per day	Week 1	2	3	4	5	6	7	8
Pig 5 (2 A.A., 8 P.A.)								
mcg.	341.2	113.0	--	86.7	55.7	36.0	34.0	53.6
mcg./100 gm. body wt.	149.0	40.8	--	26.7	14.4	8.1	7.1	10.0
mcg./100 gm. evis. body wt.								11.6
Pig 1 (40 A.A., 0 P.A.)								
mcg.	9.4	22.2	12.4	11.2	7.0	11.4	15.0	25.5
mcg./100 gm. body wt.	6.7	12.9	5.5	4.1	2.1	2.8	3.4	5.0
mcg./100 gm. evis. body wt.								5.5
Pig 9 (40 A.A., 0.06 P.A.)								
mcg.	42.8	40.1	22.8	21.6	8.9	9.4	6.4	11.1
mcg./100 gm. body wt.	18.1	14.3	7.0	5.8	2.1	1.9	1.2	1.9
mcg./100 gm. evis. body wt.								2.1
Pig 4 (10 A.A., 0.2 P.A.)								
mcg.	43.4	25.6	35.2	21.6	9.3	10.4	11.5	16.0
mcg./100 gm. body wt.	17.2	8.2	10.1	5.3	2.0	2.0	2.0	2.5
mcg./100 gm. evis. body wt.								2.9

Table 16. Pantothenic acid in urine of guinea pigs fed synthetic diet supplemented with four levels of ascorbic acid and four levels of calcium pantothenate

Studies II and III

Pantothenic acid in urine per day	Study II				Study III			
	Week				Week			
	1	2	3	4	1	2	3	4
Pig 8 (0 A.A., 0 P.A.)								
mcg.	9.2	16.2	--	--	6.5	5.8	--	--
mcg./100 gm. body wt.	7.2	10.6	--	--	4.7	3.7	--	--
mcg./100 gm. evis. body wt.		15.4				5.5		
Pig 3 (0 A.A., 0.06 P.A.)								
mcg.	10.2	33.8	21.8	--	6.6	5.8	--	--
mcg./100 gm. body wt.	8.6	26.2	15.7	--	6.8	4.8	--	--
mcg./100 gm. evis. body wt.			22.7			6.6		
Pig 6 (0 A.A., 0 P.A.; 8 mg. in in Study II, 1 in Study III)								
mcg.	116.0	1162.8	283.2	--	28.6	31.5	34.1	--
mcg./100 gm. body wt.	82.3	688.0	167.5	--	24.9	24.2	26.4	--
mcg./100 gm. evis. body wt.			202.3				37.8	
Pig 7 (2 A.A., 0 P.A.)								
mcg.	7.8	8.2	10.0	10.4	6.7	4.8	7.0	--
mcg./100 gm. body wt.	6.9	5.7	5.0	4.7	5.1	3.3	4.3	--
mcg./100 gm. evis. body wt.				5.9			5.4	
Pig 2 (2 A.A., 0.06 P.A.)								
mcg.	13.6	--	--	--	8.0	5.8	7.9	7.2
mcg./100 gm. body wt.	11.6	--	--	--	6.4	4.2	4.4	3.4
mcg./100 gm. evis. body wt.	Died	--	--	--				3.9

Table 16 (Cont'd)

Pantothenic acid in urine per day	Study II Week 1	2	3	4	Study III Week 1	2	3	4
Pig 5 (2 A.A., P.A.; 8 mg. in Study II, 1 in Study III)								
mcg.	211.4	123.9	79.2	43.5	44.8	27.5	16.7	26.8
mcg./100 gm. body wt.	184.0	85.4	43.3	21.2	37.3	17.5	9.0	11.5
mcg./100 gm. evis. body wt.				27.9				13.7
Pig 1 (40 A.A., 0 P.A.)								
mcg.	6.4	--	--	--	3.7	6.3	--	--
mcg./100 gm. body wt.	5.4	--	--	--	4.3	6.2	--	--
mcg./100 gm. evis. body wt.	7.4					9.4		
Pig 9 (40 A.A., 0.06 P.A.)								
mcg.	9.2	7.2	13.8	12.0	3.0	24.9	55.9	82.8
mcg./100 gm. body wt.	8.2	5.5	7.8	5.0	2.0	12.7	22.5	30.4
mcg./100 gm. evis. body wt.				7.4				45.5
Pig 4 (10 A.A., 2 P.A.)								
mcg.	14.4	13.6	24.2	19.0	6.3	7.1	16.9	13.2
mcg./100 gm. body wt.	12.1	9.6	11.7	8.4	4.5	4.1	8.6	5.7
mcg./100 gm. evis. body wt.				12.0				7.3
Pellet control								
mcg.				46.2				
mcg./100 gm. body wt.				15.2				
mcg./100 gm. evis. body wt.				15.8				

weight and plotted against the concentration of pantothenic acid in the blood, a correlation of 0.54 was obtained and the linear regression (Figure 6) was significant at the 1 per cent level.

When the pantothenic acid excreted was calculated as per cent of dietary intake (Tables 17 and 18), the results varied for individual animals from week to week as well as among the animals on the various intakes of the vitamin. Those animals getting no calcium pantothenate excreted relatively large amounts of the vitamin, and showed considerable fluctuation from week to week.

Guinea pig 9 in Study III (40 A.A., 0.06 P.A.) had an interesting and unusual record. This animal excreted 5 per cent of its calcium pantothenate intake the first week, 42 per cent the second week, 93 per cent the third week, and 96 per cent the fourth week. When the animal was placed on experiment, it had an excellent appetite and a very good growth rate, then for no apparent reason the animal ate only small amounts of the ration and lost considerable weight. At autopsy a growth which probably inhibited swallowing was discovered between the heart and lungs. A lung infection was also found. The infection may have been responsible for the increased excretion of pantothenic acid. However, there was a very high concentration of pantothenic acid in the liver.

Table 17. Intake and excretion of pantothenic acid of guinea pigs fed synthetic diet supplemented with four levels of ascorbic acid and four levels of calcium pantothenate

Study I

Guinea pig		Week 1	2	3	4	5	6	7	8
8	Intake[a]	0	0	0	--				
	Excretion[a]	118.8	139.8	39.0[b]					
	Per cent excreted								
3	Intake[a]	240	180	--	--				
	Excretion[a]	168.0	30.6	--	--				
	Per cent excreted	70.0	17.0	--	--				
6	Intake[a]	32000	24000	18000	--				
	Excretion[a]	11683.2	474.0	162.0[c]	--				
	Per cent excreted	36.5	2.0	0.9	--				
7	Intake[a]	0	0	0	0	0	0	0	0
	Excretion[a]	86.4	30.0	51.6	65.4	38.4	27.6	23.1	63.3
	Per cent excreted								

[a]In micrograms.

[b]46-3/4 hr. sample.

[c]47-1/2 hr. sample.

Table 17 (Cont'd)

Guinea pig		Week 1	2	3	4	5	6	7	8
2	Intake[a]	240	180	180	180	180	180	180	180
	Excretion[a]	81.6	58.5	78.0	55.2	108.0	28.2	30.0	33.0
	Per cent excreted	34.0	32.5	43.3	30.7	60.0	15.7	16.7	18.3
5	Intake[a]	32000	24000	--	24000	24000	24000	24000	24000
	Excretion[a]	1364.8	339.0	--	260.1	167.1	108.0	102.0	160.8
	Per cent excreted	4.3	1.4	--	1.1	0.7	0.4	0.4	0.7
1	Intake[a]	0	0	0	0	0	0	0	0
	Excretion[a]	37.6	66.6	37.2	33.6	21.0	34.2	45.0	76.5
	Per cent excreted								
9	Intake[a]	240	180	180	180	180	180	180	180
	Excretion[a]	171.2	120.3	68.4	64.8	26.7	28.2	19.2	33.3
	Per cent excreted	71.3	66.8	38.0	36.0	14.8	15.7	10.7	18.5
4	Intake[a]	800	600	600	600	600	600	600	600
	Excretion[a]	173.6	76.8	105.6	64.8	27.9	31.2	34.5	48.0
	Per cent excreted	21.7	12.8	17.6	10.8	4.6	5.2	5.8	8.0

Table 18. Intake and excretion of pantothenic acid of guinea pigs fed synthetic diet supplemented with four levels of ascorbic acid and four levels of calcium pantothenate

Studies II and III

Guinea pig		Study II Week 1	Study II Week 2	Study II Week 3	Study II Week 4	Study III Week 1	Study III Week 2	Study III Week 3	Study III Week 4
8	Intake[a]	0	0	--	--	0	0	--	--
	Excretion[a]	27.6	34.3[b]	--	--	19.5	17.4	--	--
	Per cent excreted								
3	Intake[a]	180	180	180	--	180	180	--	--
	Excretion[a]	30.6	101.4	65.4	--	19.8	17.4	--	--
	Per cent excreted	17.0	56.3	36.3	--	11.0	9.7	--	--
6	Intake[a]	3000	3000	1000		3000	3000	1000	
	Excretion[a]	348.0	3488.4	318.0[c]	--	85.8	94.5	38.3[c]	--
	Per cent excreted	11.6	116.3	31.8		2.9	3.2	3.8	
7	Intake[a]	0	0	0	0	0	0	0	--
	Excretion[a]	23.4	24.6	30.0	31.2	20.1	14.4	21.0	--
	Per cent excreted								

[a]In micrograms.

[b]51 hr. sample (broke leg).

[c]27 hr. samples.

Table 18 (Cont'd)

Guinea pig		Study II Week 1	2	3	4	Study III Week 1	2	3	4
2	Intake[a]	180	--	--	--	180	180	180	180
	Excretion[a]	40.8	Died	--	--	24.0	17.4	23.7	21.6
	Per cent excreted	22.7	--	--	--	13.3	9.7	13.2	12.0
5	Intake[a]	3000	3000	3000	3000	3000	3000	3000	3000
	Excretion[a]	634.2	371.7	237.6	130.5	134.4	82.5	50.1	80.4
	Per cent excreted	21.1	12.4	7.9	4.4	4.5	2.8	1.7	2.7
1	Intake[a]	0	--	--	--	0	0	--	--
	Excretion[a]	19.2	--	--	--	11.1	18.9	--	--
	Per cent excreted								
9	Intake[a]	180	180	180	180	180	180	180	260
	Excretion[a]	27.6	21.6	41.4	36.0	9.0	74.7	167.7	248.4
	Per cent excreted	15.3	12.0	23.0	20.0	5.0	41.5	93.2	95.5
4	Intake[a]	600	600	600	600	600	600	600	600
	Excretion[a]	43.2	40.8	72.6	57.0	18.9	21.3	50.7	39.6
	Per cent excreted	7.2	6.8	12.1	9.5	3.2	3.6	8.4	6.6

Silber (1944) found that the amount of pantothenic acid which dogs excreted in the urine was proportional to the amount in the diet. The fecal excretion remained fairly constant but only a small fraction of the intake appeared in the urine (Silber, 1945).

Findings at Autopsy

Experiment A

Observations made during autopsy may be found in Tables 19 and 20. The appearance of the animals immediately before autopsy was also noted and recorded. The animals were rated as good in appearance if they were active, had pink color of ears, nose, and paws, and smooth, unruffled fur. The three experimental and three control animals, which were sacrificed after 15 days of 0.15 per cent omega-methylpantothenic acid consumption, were all rated good. The only abnormality recorded during autopsy for these animals was the presence of atelectasis in guinea pig 7C. One-half of one lobe was involved.

By the time Experiment A was terminated, all six control pigs were rated good in appearance. Since the control animals in this experiment had an excellent rate of growth

Table 19. Observations made immediately before and at autopsy of guinea pigs fed omega-methylpantothenic acid and their litter mate controls

Guinea pig	Appearance before autopsy	Autopsy findings		
		Adrenals	Liver	Kidneys
1C[a]	Good	Gray	Dark brown	Red-brown
1E[a]	Salivation, watering eyes, lying on side, immobile hind legs, seemed paralyzed	Pink	Light with lighter streaks, looks fatty	Look fatty, cortex seems spongy
2C[a]	Good	Cream-colored	Dark brown with several white areas	Red-brown
2E[a]	Very soft fur, pale, listless, hunched; had looked ill for past week	Tan	Light brown	Light in color
3C	Good	Gray	Dark brown	Red-brown
3E	Head retraction, convulsions, pale, salivation, unable to right itself	Pink	Light brown	Very light in color
4C	Good	Cream-colored	Dark brown with small white areas	Red-brown
4E	Watering eyes, pale, hunched position, hind legs seem to go out	Right is red, left is brown	Mottled	Cortex looks hemorrhagic

[a]Histological examination of testes and adrenals made.

Table 19 (Cont'd)

Guinea pig	Appearance before autopsy	Autopsy findings		
		Adrenals	Liver	Kidneys
5C	Good	Cream-colored	Dark brown with white areas	Red-brown
5E	Pale but wiry and active, soft fur	Tan	Brown	Left is light brown, right is red-brown
6C	Good	Cream-colored	Dark brown with white area on one lobe	Red-brown
6E	Pale, acts sick	Tan	Light brown with white areas	Light in color, cortex looks hemorrhagic

Table 20. Observations made immediately before and at autopsy of guinea pigs fed omega-methylpantothenic acid and their litter mate controls

Guinea pig	Appearance before autopsy	Autopsy findings		
		Visceral fat	Spleen	G. I. tract
1C[a]	Good	Large amount	2.8 x 1.6 mm.	Cecum-full, intestines not distended as in 1E
1E[a]	Salivation, watering eyes, lying on side, immobile hund legs, seemed paralyzed	Some	--	Cecum-empty, intestines full of fluid and distended, air in stomach
2C[a]	Good	Large amount	3.2 x 1.5 mm.	Some air in stomach
2E[a]	Very soft fur, pale, listless, hunched; had looked ill for past week	Some	4.0 x 2.4	Cecum and stomach empty, some air in intestines
3C	Good	Very large amount	3.0 x 1.6	Some air in stomach
3E	Head retraction, convulsions, pale, salivation, unable to right itself	Some	3.1 x 1.6	Cecum and stomach empty, yellow fluid in intestines
4C	Good	Large amount	2.4 x 1.1 mm.	Cecum and stomach look good

[a]Histological examination of testes and adrenals made.

Table 20 (Cont'd)

Guinea pig	Appearance before autopsy	Autopsy findings		
		Visceral fat	Spleen	G. I. tract
4E	Watering eyes, pale, hunched position, hind legs seem to go out	Some	2.4 x 1.8	Cecum empty, intestines collapsed
5C	Good	Large amount	2.6 x 1.3	Some air in stomach
5E	Pale but wiry and active, soft fur	Some	2.4 x 1.35	Stomach and cecum empty, intestines collapsed
6C	Good	Very large amount	2.6 x 1.3	Cecum and stomach look good
6E	Pale, acts sick	Some	3.2 x 1.9	Liquid and air in stomach

and gave the appearance both externally and internally of a normal healthy guinea pig, the condition of the various organs at autopsy was considered a standard which the other animals, in both Experiments A and B, must attain in order to be considered healthy guinea pigs. For example, the adrenals of the controls were consistently either gray or cream-colored. Any color other than this was interpreted as showing various degrees of abnormality, the tan color observed in several animals being considered a borderline color, while pink, red or brown adrenals were considered to be hemorrhagic. The same procedure pertained to the liver. The controls consistently had dark brown livers. Four of the six controls exhibited small white areas on the surface of the liver. Since these areas occurred only on the very surface and did not penetrate to the interior, they were not considered serious abnormalities. Livers which were a lighter color than dark brown were regarded as showing a possibility of fatty infiltration. Those which were mottled and streaked with still lighter colors of brown were interpreted as being fatty. The kidneys of the control animals were a red-brown color and the cortex and medulla were clearly differentiated. Kidneys which did not meet these qualifications were considered to be abnormal. The experimental animals showed various abnormalities.

Guinea pig 1E was found lying on its side and completely immobile early on the morning of the 35th day of the study. When placed on the balance, the pig lay motionless on its side as if in coma. About one hour later, it was able to get up and move about, but the hind legs seemed stiff and useless. After another hour, watery eyes and excessive salivation were observed. The animal dragged itself to the feed cup as if hungry, but seemed to lack the energy to eat. At autopsy, a careful examination for signs of infections revealed that none were present.

Guinea pig 3E, very suddenly, had convulsions with head retraction one morning on the 45th day of the study. After a convulsive attack the animal would lie flat on its back with all four legs extended very rigidly. The attacks with intermittent rest periods occurred for about 1 to 1-1/2 hours. Muscular weakness was apparent in the hind legs. Finally the animal sat hunched in a corner of the cage. A small amount of salivation was noticed.

By the last day of the study, three of the remaining four animals were in very poor physical condition. They were pale and listless and sat hunched in a corner of the cage. One had soft woolly fur while another had watering eyes which were partially closed. Only guinea pig 5E remained active, but even it was pale and had soft woolly fur.

The adrenal glands displayed a large variety of colors. Those of the control animals were either gray or cream-colored. The adrenals of three of the experimental animals were tan, those of guinea pigs 1E and 3E were pink, and pig 4E had one red gland and one brown one.

There was considerable difference between the two groups of litter mates in the color of their livers. All six of the controls had dark brown livers. Four of these animals had a few white areas about the size of pinpoints on the surface. All of the experimental pigs had livers which were light brown in color. The liver of guinea pig 4E was mottled, that of 1E had lighter colored streaks coursing through the organ. Pig 6E had small white areas on the surface similar to those found in some of the control pigs. The color of the liver of guinea pig 5E was intermediate, it was not as dark as that of the controls but darker than that of the other experimental animals.

The contrast in the color of the kidneys in the two groups of animals was striking also. The kidneys of the control pigs were without exception a red-brown color; those of the experimental animals were a much lighter brown color. The kidneys of pig 1E actually looked fatty and the cortex seemed spongy and swollen. The cortex of the kidney of guinea pigs 4E and 6E had hemorrhagic areas. Strangely,

guinea pig 5E had one kidney which was a red-brown color and one that was lighter brown.

The largest dimensions lengthwise and crosswise of the spleens were measured with a ruler and recorded in millimeters. In the three pairs of pigs sacrificed after the first 15 days of the study, the spleens of the two groups were practically the same size. When the study was terminated, the experimental animals (with the exception of guinea pig 5) had the larger spleens. Those of pigs 2E, 4E, and 6E were considerably larger than the spleens of their litter mates. The spleens of these animals were no doubt hypertrophied in an effort to produce more blood. Pigs 2E and 6E were extremely anemic, while blood data for pig 4E are missing. Carter et al., (1945) reported that splenomegaly occurred along with a severe anemia in about 60 per cent of their pantothenic acid-deficient rats.

Large stores of visceral fat were found in both groups of animals, however, those found in the controls were more abundant. The gastro-intestinal tracts of the experimental animals showed intestines which were either distended, collapsed, or filled with fluid, while their cecums were relatively empty. The cecums of the controls were fuller because of their larger food intake. No sizes of an outright infection were observed in either group of animals. Atelectasis was observed in the lungs of guinea pig 4E.

Many of the symptoms observed in the guinea pigs of the present investigation have been reported in pantothenic acid-deficient dogs: lowered growth rate, decreased appetite, sudden prostration or coma, convulsions and gastrointestinal symptoms. Spasticity of the hind quarters occurred in some dogs during the last week of deficiency. Excessive salivation was also noted. Gross examination of tissues showed fatty livers and signs of hemorrhagic degeneration in the cortex and medulla of the kidneys (Schaefer et al., 1942 and Silber, 1944). Hemorrhagic adrenals were not reported in dogs by these workers, but have been found in the rat (Daft and Sebrell, 1939). Excessive lacrimation, diarrhea and incoordinated movements of the hind legs have been reported in young swine (Luecke et al., 1949).

Experiment B

The various symptoms which may be found in a scorbutic guinea pig were carefully evaluated and a quantitative system of scoring adopted in presenting the data (Table 21). The method of scoring was a modification of that used by Sherman et al., (1922). If the animal had severe hemorrhages, very fragile bones and loose teeth, its rating was +++. The presence of severe hemorrhages, less fragility of bones and less looseness of teeth was rated ++. The

Table 21. Findings at autopsy of guinea pigs fed synthetic diet with four levels of ascorbic acid and four levels of calcium pantothenate

Guinea pig	Supplement Asc. A. (mg./day)	Supplement Ca Panto. (mg./day)	No. of days on expt'l. diet	Scorbutic symptoms	Liver	Kidneys	Fat	Adrenals
				Study I				
8	0	0	19	+++	Light brown	Light areas on surface continuous to interior, interior very pale	No subcutaneous fat	No abnormalities noted
3	0	0.06	15	++	Light brown	--	--	No abnormalities noted
6	0	8	19	+++	Dark brown with several white areas on surface	Very pale	Some subcutaneous fat	No abnormalities noted
7	2	0	58	+	Medium brown with several white areas on surface	Light brown	Some subcutaneous fat, quite a lot visceral fat	No abnormalities noted
2	2	0.06	58	+	Medium brown with several white areas	Light brown	More subcutaneous and visceral fat than pig 7	No abnormalities noted

Table 21 (Cont'd)

Guinea pig	Supplement Asc. A. (mg./day)	Supplement Ca Panto. (mg./day)	No. of days on expt'l. diet	Scorbutic symptoms	Liver	Kidneys	Fat	Adrenals
5	2	8	57	tr?	One large lobe necrotic	Red-brown	More visceral fat than pigs 2 or 7, some subcutaneous fat	No abnormalities noted
1	40	0	58	tr?	Medium brown, with fairly large white area penetrating surface and several pinpoint areas	Light brown	Abundance of visceral fat, small to fair amount subcutaneous	No abnormalities noted
9	40	0.06	58	tr	Medium to dark brown with more white areas than pig 1	Red-brown	Very large amount visceral fat, fair amount subcutaneous fat	No abnormalities noted
4	10	0.2	58	tr?	Light and dark areas, several small white areas	--	Visceral fat abundant, most subcutaneous fat	No abnormalities noted

Table 21 (Cont'd)

Guinea pig	Supplement Asc. (mg./day)	Supplement A. Ca Panto. (mg./day)	No. of days on expt'l. diet	Scorbutic symptoms	Liver	Kidneys	Fat	Adrenals
				Study II				
8[a]	0	0	13	++	Brown	Light brown	No subcutaneous fat	No abnormalities noted
3	0	0.06	21	++	Brown	Light brown	--	No abnormalities noted
6	0	8	22	++	Brown with yellow tinge (fatty)	Mottled, look fatty	Some of each	No abnormalities noted
7	2	0	30	--	Red-brown	Brown	Small amount of each	No abnormalities noted
2	2	0.06	12--Died of lung infection					
5	2	8	30	--	Brown with yellow color throughout liver (fatty)	Brown	Some of each	No abnormalities noted

[a]Broke leg.

Table 21 (Cont'd)

Guinea pig	Supplement Asc. A. (mg./day)	Ca Panto. (mg./day)	No. of days on expt'l. diet	Scorbutic symptoms	Liver	Kidneys	Fat	Adrenals
1	40	0	12	--	Red-brown	Seem light	None of each	Very soft, cortex and medulla not distinct
9	40	0.06	25	--	Red-brown	Red-brown	More visceral fat than pig 3, small amount of subcutaneous	No abnormalities noted
4[b]	10	0.2	30	tr	Red-brown with a small area near edge showing calcification	Red-brown	Very little visceral fat	No abnormalities noted
PC	10	--	33	--	Dark brown	Brown	Lots of visceral fat, some subcutaneous fat	No abnormalities noted

[b]Lung infection.

Table 21 (Cont'd)

Guinea pig	Supplement Asc. A. (mg./day)	Ca Panto. (mg./day)	No. of days on expt'l. diet	Scorbutic symptoms	Liver	Kidneys	Fat	Adrenals
				Study III				
8	0	0	16	+++	Light brown mottled with yellow areas	Very light brown	None	White color
8A	0	0	17	++	Red-brown	Red-brown	None	Tan
3	0	0.06	17	+++	Red-brown with deep yellow fringe	Red-brown	None	Lighter tan than pig 8A
6	0	1	19	+++	Red-brown	Red-brown	None	Gray-cream
7	2	0	22	--	Red-brown	Brown	--	Hemorrhagic
7A[c]	2	0	24	--	Two largest lobes had several small tan areas	Brown	--	Gray-cream
2	2	0.06	33	tr	Red-brown with some mottling	Considerable fatty infiltration extending through cortex	Some visceral fat	Gray-cream

[c]Broke leg.

Table 21 (Cont'd)

Guinea pig	Supplement Asc. (mg./day)	A. Ca Panto. (mg./day)	No. of days on expt'l. diet	Scorbutic symptoms	Liver	Kidneys	Fat	Adrenals
5[d]	2	1	33	+	Red-brown with mottling, seems fatty	Brown with some mottling	Some visceral fat	Gray-cream color
1[d]	40	0	17	--	Red-brown	Light brown	No visceral fat	Hemorrhagic
1A	40	0	17	--	Red-brown	Red-brown	No visceral fat	Hemorrhagic
9[e]	40	0.06	32	--	Light brown with ill-defined yellow areas, seems fatty	Cortex and medulla not clearly differentiated, some fatty infiltration noted	--	Gray-cream
4	10	0.2	33	tr	Red-brown	No right kidney, left kidney large	Some visceral fat	Right adrenal is round

[d]Lung infection.

[e]Growth in heart-lung area.

presence of less severe hemorrhages, with teeth and bones normal, was rated +. If an occasional small hemorrhage was noted, tr, designating trace was used. The symbol, tr?, designated doubt as to whether or not the condition observed was hemorrhagic, while a dash, --, indicated no difference from the normal.

Study I. In considering the scorbutic group of animals, it is noted that the earliest pig to be sacrificed showed the least severe symptoms of scurvy. This seems entirely reasonable. The group of three animals receiving 2 mg. of ascorbic acid daily exhibited borderline scurvy. Beginning with the fourth week, pigs 2 and 5 were getting less ascorbic acid than 0.7 mg. per 100 gm. body weight, while pig 7 was getting less than this amount beginning with the sixth week of the study. It may be possible that the large amount of calcium pantothenate protected pig 5 against scurvy to some extent. For the group of three animals receiving 40 mg. of ascorbic acid daily it is difficult to find an explanation for their scorbutic ratings. It may be that the area recorded as possibly hemorrhagic may have been just a tiny vein in pigs 1 and 4. Guinea pig 9 had two small hemorrhages over the right knee. There was no apparent explanation for this.

The livers of all nine animals varied in color and appearance but all nine had a light fringe which may have been due to the lessened thickness of the organ around the periphery. In the group of three scorbutic animals, pigs 8 (0 A.A., 0 P.A.) and 3 (0 A.A., 0.06 P.A.) had fatty livers, while pig 6 (0 A.A., 8 P.A.) had small white areas on the surface of a dark brown liver. Of the group getting 2 mg. ascorbic acid pigs 7 (0 P.A.) and 2 (0.06 P.A.) had livers which were medium brown in color with some white areas on the surface. One entire lobe of the liver of guinea pig 5 (8 P.A.) was almost completely necrotic with scarcely any hepatic tissue left. The third group of three animals displayed the small white areas on the surface of the organ which have been mentioned previously. The liver of pig 1 (40 A.A., 0 P.A.) was a medium brown color, that of pig 9 (40 A.A., 0.06 P.A.) medium to dark brown, while that of the control (10 A.A., 0.2 P.A.) had light and dark areas which were interpreted as being fatty.

Considering the animals in groups, according to their ascorbic acid intake, it was noted that pigs 8 (0 A.A., 0 P.A.) and 6 (0 A.A., 0.06 P.A.) both had fatty kidneys, pig 8 having fatter kidneys than pig 6. In the group receiving 2 mg. of ascorbic acid, the kidneys of pig 5 (8 P.A.) were considered normal while those of pigs 7

(0 P.A.) and 2 (0.06 P.A.) were lighter than normal. Unfortunately, the condition of the kidneys of the control animal was not recorded. Guinea pig 9 (40 A.A., 0.06 P.A.) had normal appearing kidneys while those of pig 1 (40 A.A., 0 P.A.) were lighter than normal in color.

All animals except those exhibiting scurvy had large stores of visceral fat; the amount in the group of three animals receiving 2 mg. of ascorbic acid increased with increasing amounts of calcium pantothenate intake. No abnormalities were observed in the adrenal glands of these animals.

Studies II and III. Approximately 2 to 4-day-old animals were used in these studies. The scorbutic group displayed symptoms which were rated either ++ or +++. In Study II, the group receiving 2 mg. of ascorbic acid had no signs of scurvy while in Study III, guinea pig 2 (0.06 P.A.) showed a trace and pig 5 (1 P.A.) exhibited a borderline case of scurvy. The latter animal had a lung infection which probably increased its requirement for the vitamin. Evidently a large amount of calcium pantothenate did not compensate for the insufficient amount of ascorbic acid. In the third group (40 mg. or 10 mg. ascorbic acid), the control pig had a rating of tr because of the presence of one small hemorrhage in the cecum. This animal had a

lung infection. In Study III, the control pig was also rated tr because of a few tiny hemorrhages in the cecum. The other animals in this group of both studies were rated --, indicating no difference from the normal animal.

In Experiment B, the livers were weighed at autopsy and the weights ranged from 3.2 to 24.2 grams (Table 22). When they were calculated per 100 gm. body weight, these organs showed a striking similarity in size. All ranged between approximately 4 and 5.5 grams. Only the liver of pig 1 of Study II deviated from this range. Its weight (2.7 gm. per 100 gm. body weight) can be explained, in part, by the fact that this liver was partially dehydrated before it was weighed.

With some exceptions, the livers of the animals in Study II and Study III were rated as normal in appearance by gross inspection. In Study II the livers of pigs 6 and 5 (both getting maximal amounts of calcium pantothenate) were fatty. One small area near the edge of the liver of the control pig had some calcification. In Study III, pigs 8 (0 A.A., 0 P.A.), 2 (2 A.A., 0.06 P.A.), 5 (2 A.A., 1 P.A.) and 9 (40 A.A., 0.06 P.A.) all had fatty livers.

Many of the animals with fatty livers also had fatty kidneys. These included in Study II, pig 6 (0 A.A., 8 P.A.); in Study III, pigs 8 (0 A.A., 0 P.A.), 2 (2 A.A., 0.06 P.A.),

Table 22. Weight of livers of guinea pigs fed synthetic diet supplemented with four levels of ascorbic acid and four levels of calcium pantothenate

Study and guinea pig no.	Supplement		Wt. of liver (gm.)	Wt. per 100 gm. body wt. (gm.)	Wt. per 100 gm. evis. body wt. (gm.)
	Asc. A. (mg./day)	Ca Panto. (mg./day)			
I-8	0	0	6.95	4.46	5.84
II-8[a]	0	0	5.5	3.62	5.24
III-8	0	0	7.0	5.04	6.60
III-8A	0	0	5.7	4.16	5.59
I-3	0	0.06	12.15	5.52	7.64
II-3	0	0.06	5.3	3.79	5.52
III-3	0	0.06	5.2	4.09	5.91
I-6	0	8	9.3	3.99	4.56
II-6	0	8	8.4	4.97	6.00
III-6	0	1	5.9	4.57	6.56
I-7	2	0	18.9	3.95	4.90
II-7	2	0	11.15	4.89	6.53
III-7	2	0	5.15	3.76	6.28
III-7A[a]	2	0	8.8	5.03	6.82
I-2	2	0.06	19.3	3.65	4.33
II-2	2	0.06	Died[b]	--	--
III-2	2	0.06	11.3	4.47	6.11
I-5	2	8	21.0	3.84	4.56
II-5	2	8	11.5	5.45	7.37
III-5	2	1	10.3	3.95	5.26

[a]Broke leg before end of study.

[b]Lung infection.

Table 22 (Cont'd)

Study and guinea pig no.	Supplement Asc. A. (mg./day)	Supplement Ca Panto. (mg./day)	Wt. of liver (gm.)	Wt. per 100 gm. body wt. (gm.)	Wt. per 100 gm. evis. body wt. (gm.)
I-1	40	0	21.5	3.99	4.61
II-1	40	0	3.2[c]	2.69	3.68
III-1	40	0	4.0	4.35	5.97
III-1A	40	0	4.0	3.74	6.25
I-9	40	0.06	24.15	3.95	4.57
II-9	40	0.06	8.5	3.95	5.25
III-9	40	0.06	11.2	5.14	6.15
I-4	10	0.20	23.6	3.65	4.22
II-4	10	0.20	9.4	3.92	5.95
III-4	10	0.20	10.0	4.03	5.52
II-Pellet control	10	--	16.1	4.57	5.51

[c]Not weighed immediately, partially dehydrated.

5 (2 A.A., 1 P.A.) and 9 (40 A.A., 0.06 P.A.). Pig 1 (40 A.A., 0 P.A.) showed fatty infiltration probably to a lesser degree than the animals just listed. The occurrence of fatty livers and kidneys seemed to be unrelated to either of the two vitamins being studied.

In Study II, the adrenals of only guinea pig 1 (40 A.A., 0 P.A.) appeared abnormal. The cortex and medulla were not distinct. In Study III, there were many abnormalities noted in the adrenal glands. Pigs 8A (0 A.A., 0 P.A.) and

3 (0 A.A., 0.06 P.A.) had tan-colored glands while those of pigs 7 (2 A.A., 0 P.A.), 1 (40 A.A., 0 P.A.), and 1A (40 A.A., 0 P.A.) were hemorrhagic. Since it is possible to produce hemorrhagic adrenals in young animals by inanition, this fact must not be overlooked in the case of the last two animals mentioned.

An unusual phenomenon was observed in the control pig of Study III. It had only one very large kidney which was situated on the left side, but there were two adrenal glands. The adrenal which ordinarily would have occupied the position above the kidney on the right was firmly attached to the middle of the back and was round in shape.

In Study II, the scorbutic animal getting maximal amounts of calcium pantothenate seemed to have larger fat stores than pig 8 (0 A.A., 0 P.A.). This may be accounted for by the fact that pig 6 (0 A.A., 8 P.A.) lived nine days longer. In the next group, pig 5 (2 A.A., 8 P.A.) had a little more visceral fat than pig 7 (2 A.A., 0 P.A.). In the third group of animals in Study II, the pellet control had the most abundant fat stores while the synthetic diet control, which had a lung infection, had only very little visceral fat. Pig 1 (40 A.A., 0 P.A.) had neither visceral nor subcutaneous fat. In Study III, all four scorbutic pigs, as well as pigs 1 and 1A (40 A.A., 0 P.A.), were

lacking in visceral fat. The amount of visceral fat may possibly be related to the intake of calcium pantothenate. Loss of subcutaneous and internal fat has been reported in swine as the result of a pantothenic acid deficiency (Wiese et al., 1951).

Histological Examination of Testes and Adrenals

Experiment A

The testes and adrenals of guinea pigs 1E and 1C and 2E and 2C were examined histologically. Guinea pig 1E had developed an acute deficiency syndrome while guinea pig 2E had a chronic deficiency which was evident for more than a week before the animal was sacrificed. No spermatogenesis was noted in the testes of either the experimental or the control animals. The adrenal glands of both experimental animals showed changes in the medulla, which was lacking contrast cells, making it difficult to distinguish the medulla from the cortex. Grossly, the adrenals of guinea pig 1E were observed to be hemorrhagic while those of guinea pig 2E were a tan color which was interpreted as possibly showing a border line condition of the adrenals, being neither normal nor definitely abnormal. More specimens

prepared with different staining techniques are needed in order to either substantiate or nullify the observations made on the adrenal glands in this investigation.

SUMMARY AND CONCLUSIONS

Two objectives were proposed for the present investigation regarding the need for pantothenic acid and its relation to ascorbic acid in the nutrition of the guinea pig. The objectives were (1) to produce a pantothenic acid deficiency in the guinea pig in two ways, namely, by feeding an antimetabolite, omega-methylpantothenic acid, and by omitting the vitamin from the diet, and (2) to determine whether an interrelationship between pantothenic acid and ascorbic acid existed in this species similar to that reported in the rat. Daft reported that when weanling rats were fed a pantothenic acid-deficient diet supplemented with 2 per cent ascorbic acid, they showed either no pantothenic acid deficiency symptoms, or the symptoms were greatly modified. Growth was better, porphyrin accumulation on fur and whiskers less, and length of life increased.

In Experiment A, nine pairs of weanling male guinea pigs were fed a complete basal diet consisting of rabbit pellets, supplemented with 10 mg. ascorbic acid per day and one drop of oleum percomorphum per week. One pig of each pair was fed the complete basal diet with supplements and designated the control. The other pig of each pair was fed the basal diet and supplements with the addition of the antimetabolite,

omega-methylpantothenic acid, as 0.15 per cent of the diet for 15 days, followed by 0.30 per cent for 18 days, and then 0.40 per cent for the remaining 14 days of the study.

After 15 days on 0.15 per cent analogue, three experimental animals and their litter mate controls were sacrificed. Up to this time, the experimental group was eating an average of one gram more food per day but gaining 0.9 gram less weight each day than the controls. No differences were observed between the two groups in red blood cell counts, packed red cell volumes, hemoglobin levels or blood pyruvic acid levels. However, the concentration of ascorbic acid in the blood serum of the experimental pigs was only one-half that of the controls.

When the experiment was terminated, after 47 days, the experimental animals were anemic, had serum ascorbic acid levels averaging one-half that of the controls, and showed an accumulation of pyruvic acid in the blood. As the level of analogue was increased, the difference in food intake, weight gain, and food efficiency between the two groups increased also. After 18 days on 0.30 per cent intake of analogue, the experimental pigs were gaining 3.4 gram per day less weight, eating two gram per day less food and utilizing their food less efficiently than the control group. After 14 days on 0.40 per cent intake of analogue, the difference between the two groups increased to 7.1 gram per day

in weight gain and 11 gram per day in food intake.

Except for the decreased concentrations of ascorbic acid in the blood serum, the symptoms which were produced by feeding the analogue in this study, have been reported for one species or another as pantothenic acid deficiency. Physical symptoms observed in this experiment included soft woolly fur, pallor, lassitude, salivation, watering of the eyes, muscular weakness of the hind legs, convulsions, and coma. Biochemical changes were characterized by anemia, accumulation of pyruvic acid in the blood, and lowered serum ascorbic acid levels. Fatty livers and kidneys, hemorrhagic adrenals, and splenomegaly were observed upon autopsy.

In Experiment B, young male guinea pigs were fed a complete semi-synthetic ration along with four levels of ascorbic acid (0, 2, 10, and 40 mg. per day) and four levels of calcium pantothenate (0, 0.06, 0.2, and 8 or 1 mg. per day) in order to produce a pantothenic acid deficiency and to find whether or not an interrelationship existed between the two vitamins in the guinea pig.

The experiment was carried out in three separate studies. In Study I the animals ranged in weight from 91 to 210 grams and were maintained on the experimental regimen for eight weeks; in Studies II and III, two to four-day-old guinea pigs, ranging in weight from 68 to 112 grams, were maintained for four weeks.

The concentration of ascorbic acid in both the blood plasma and the adrenal glands reflected the dietary intake of the vitamin. Hypertrophy of the adrenal glands occurred in guinea pigs with scurvy, especially when the condition was chronic, and in pigs fed the pantothenic acid deficient diet especially when symptoms of pantothenic acid deficiency also were observed. Levels of pantothenic acid in the blood, in the liver, and in the urine seemed to reflect the dietary intake of calcium pantothenate. However, relatively large amounts of pantothenic acid were excreted in the urine of animals getting no dietary calcium pantothenate. The vitamin undoubtedly became available to the pigs through intestinal synthesis and coprophagy. Blood pyruvic acid levels showed no correlation with concentration of pantothenic acid in the blood.

It is believed that an acute deficiency of pantothenic acid was produced in two animals, while inanition probably complicated the symptoms suggestive of a pantothenic acid deficiency which were observed in two others. Symptoms included a soft woolly fur, lassitude, diarrhea, convulsions, and hemorrhagic adrenals. Anemia and a decrease in plasma ascorbic acid level were not found. Fatty livers occurred in pigs fed excessive amounts of calcium pantothenate.

Large amounts of calcium pantothenate failed to alleviate the symptoms of scurvy and large amounts of

ascorbic acid did not prove beneficial in pantothenic acid deficiency; neither did large amounts of either of the two vitamins have any effect on weight gain, food intake, or food efficiency.

In conclusion, a pantothenic acid deficiency was produced in guinea pigs by feeding an antimetabolite with a natural ration, and by omitting pantothenic acid from a semi-synthetic diet. The deficiency, in the first case, was believed to have been chronic, in the latter case, acute. No clear-cut interrelationships between ascorbic acid and pantothenic acid were observed.

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APPENDIX

Table A. Weights of stock colony males during first eight weeks of life

Guinea pig no.	Birth wt. (gm.)	Week 1 (gm.)	2 (gm.)	3 (gm.)	4 (gm.)	5 (gm.)	6 (gm.)	7 (gm.)	8 (gm.)
111	138	161	233	308	352	404	477	553	610
113	100	130	175	246	287	340	408	466	512
142	107	139	165	204	275	319	376	387	441
143	121	156	190	245	302	350	402	430	491
144	93	125	149	182	237	284	322	348	404
201	120	156	219	287	353	421	510	548	594
323	118	139	198	266	332	397	458	514	566
324	94	117	174	231	278	336	387	438	487
423	64	105	164	231	286	340	408	460	524
424	69	115	177	246	298	360	438	488	550
57a4	97	155	185	231	290	335	388	413	471
631	100	166	255	317	377	472	530	616	680
6 4	61	73	98	143	181	238	289	322	375
6 5	74	98	132	171	227	287	345	375	--
701	116	161	212	278	332	376	443	522	564
741	105	133	197	268	338	378	443	523	582
742	114	146	206	267	301	354	414	468	539
743	92	130	189	249	319	365	428	495	549
744	82	115	174	231	288	336	395	455	528
841	120	154	228	319	400	466	534	604	658
821	75	119	188	250	295	338	382	405	412
822	70	112	174	247	265	307	354	383	407
901	86	93	133	195	251	315	383	450	520
902	92	101	139	191	238	296	347	400	456
903	93	108	154	209	268	316	391	442	487
904	80	95	132	188	248	308	377	436	498
943	85	113	159	221	274	353	414	489	549

Table A (Cont'd)

Guinea pig no.	Birth wt. (gm.)	Week 1 (gm.)	2 (gm.)	3 (gm.)	4 (gm.)	5 (gm.)	6 (gm.)	7 (gm.)	8 (gm.)
945	117	161	--	--	--	--	--	--	--
946	116	152	--	--	--	--	--	--	--
1001	70	79	121	180	239	290	339	382	461
1002	80	81	113	168	228	282	337	388	467
1004	84	87	116	166	225	273	324	367	438
1041	126	141	200	266	332	394	461	527	592
1042	133	143	211	279	338	379	458	525	595
Ave. wt.	97	125	174	234	289	344	405	457	516
Ave. wt. gain per pig per day		4.1	7.2	8.6	7.9	7.8	8.7	7.4	8.1

Table B. Weights of stock colony females during first eight weeks of life

Guinea pig no.	Birth wt. (gm.)	Week 1 (gm.)	2 (gm.)	3 (gm.)	4 (gm.)	5 (gm.)	6 (gm.)	7 (gm.)	8 (gm.)
112	114	143	199	260	296	332	375	413	432
202	110	145	206	266	307	356	426	456	510
211	100	116	166	224	273	306	339	388	421
321	102	104	145	211	260	314	355	406	439
322	86	113	167	228	282	338	381	484	515
6 3	72	88	112	161	205	275	317	333	335
702	106	161	222	283	333	373	421	468	524
842	118	153	220	293	362	420	472	543	591
941	86	104	145	201	239	283	314	375	412
942	81	104	146	200	245	304	342	413	449
944	100	131	184	247	296	352	401	482	519
1003	70	79	120	177	240	302	359	415	479
1043	121	181	272	321	355	401	454	495	550
Ave. wt.	97	125	177	236	284	335	381	436	475
Ave. wt. gain per pig per day		3.9	7.5	8.4	6.8	7.3	6.6	7.9	5.5

Table C. Weight gain, food consumption, and food efficiency of guinea pigs fed omega-methylpantothenic acid and of their litter mate controls

Week of study	Guinea pig	Initial wt. (gm.)	Wt. at end of week (gm.)	Ave. wt. gain (gm./day)	Ave. food intake (gm./day)	Wt. gain per gm. food eaten (gm./day)
1	1C	191	238	6.7	19	0.35
	1E	195	240	6.4	13	0.49
	2C	153	213	8.6	14	0.62
	2E	139	199	8.6	14	0.61
	3C	146	224	11.1	16	0.69
	3E	171	214	6.1	17	0.37
	4C	196	234	5.4	17	0.32
	4E	160	214	7.7	13	0.59
	5C	172	210	5.4	14	0.40
	5E	146	190	6.3	14	0.45
	6C	232	271	5.6	19	0.29
	6E	206	248	6.0	19	0.32
	7C	149	190	5.9	13	0.45
	7E	165	208	6.1	16	0.39
	8C	148	181	4.7	10	0.48
	8E	147	187	5.7	17	0.34
	9C	138	181	6.1	12	0.50
	9E	145	183	5.4	14	0.38
2	1C	191	264	3.7	18	0.21
	1E	195	260	2.9	18	0.16
	2C	153	267	7.7	19	0.41
	2E	139	241	6.0	21	0.29
	3C	146	302	11.1	24	0.46
	3E	171	236	3.1	21	0.15
	4C	196	278	6.3	21	0.30
	4E	160	264	7.1	23	0.30
	5C	172	235	3.6	14	0.26
	5E	146	222	4.6	19	0.24
	6C	232	321	7.1	24	0.29
	6E	206	285	5.3	24	0.22
	7C	149	233	6.1	16	0.38
	7E	165	247	5.6	23	0.25
	8C	148	230	7.0	19	0.37
	8E	147	223	5.1	21	0.24

Table C (Cont'd)

Week of study	Guinea pig	Initial wt. (gm.)	Wt. at end of week (gm.)	Ave. wt. gain (gm./day)	Ave. food intake (gm./day)	Wt. gain per gm. food eaten (gm./day)
	9C	138	242	8.7	20	0.44
	9E	145	208	3.6	21	0.17
3	1C	191	315	7.3	22	0.33
	1E	195	308	6.9	28	0.24
	2C	153	327	8.6	27	0.32
	2E	139	282	5.9	28	0.21
	3C	146	397	13.6	33	0.42
	3E	171	265	4.1	25	0.16
	4C	196	315	5.3	23	0.23
	4E	160	306	6.0	28	0.21
	5C	172	275	5.7	21	0.27
	5E	146	259	5.3	27	0.20
	6C	232	369	6.9	28	0.24
	6E	206	342	8.1	31	0.26
	7C	149	240[a]			
	7E	165	252[a]			
	8C	148	232[a]			
	8E	147	237[a]			
	9C	138	247[a]			
	9E	145	222[a]			
4	1C	191	363	6.9	25	0.28
	1E	195	333	3.6	26	0.14
	2C	153	393	9.4	31	0.30
	2E	139	333	7.3	30	0.24
	3C	146	478	11.6	38	0.30
	3E	171	301	5.1	30	0.17
	4C	196	375	8.6	28	0.30
	4E	160	347	5.9	31	0.19
	5C	172	333	8.3	28	0.29
	5E	146	296	5.3	28	0.19
	6C	232	448	11.3	33	0.34
	6E	206	379	5.3	30	0.18

[a]On experiment first day of week only.

Table C (Cont'd)

Week of study	Guinea pig	Initial wt. (gm.)	Wt. at end of week (gm.)	Ave. wt. gain (gm./day)	Ave. food intake (gm./day)	Wt. gain per gm. food eaten (gm./day)
5	1C	191	414[b]	7.3	27	0.27
	1E	195	353[b]	2.9	26	0.11
	2C	153	493	14.3	38	0.38
	2E	139	379	6.6	30	0.22
	3C	146	564	12.3	42	0.29
	3E	171	330	4.1	29	0.14
	4C	196	442	9.6	30	0.32
	4E	160	377	4.3	29	0.15
	5C	172	402	9.9	31	0.32
	5E	146	321	3.6	23	0.16
	6C	232	531	11.9	39	0.31
	6E	206	411	4.6	32	0.14
6	2C	153	583	12.9	43	0.30
	2E	139	367	-1.7	25	-0.07
	3C	146	642	11.1	49	0.23
	3E	171	382	7.4	32	0.23
	4C	196	495	7.6	35	0.21
	4E	160	409	4.6	29	0.16
	5C	172	461	8.4	34	0.25
	5E	146	371	7.1	29	0.25
	6C	232	607	10.9	45	0.24
	6E	206	413	0.3	30	0.01
7	2C	153	591[c]	1.6	37	0.04
	2E	139	375[c]	1.6	24	0.07
	3C	146	668[d]	8.7	54	0.16
	3E	171	379[d]	-1.0	28	-0.04
	4C	196	521[c]	5.2	25	0.20
	4E	160	389[c]	-4.0	23	-0.17
	5C	172	507[c]	9.2	36	0.26
	5E	146	388[c]	3.4	27	0.12
	6C	232	653[c]	9.2	46	0.20
	6E	206	399[c]	-2.8	24	-0.12

[b]Final weights.

[c]Five days on experiment this week.

[d]Three days on experiment this week.

Table D. Final body weights and eviscerated body weights of guinea pigs fed synthetic diet supplemented with four levels of ascorbic acid and four levels of calcium pantothenate

Guinea pig	Supplement: Asc. A. (mg./day)	Supplement: Ca Panto. (mg./day)	Study I: Final body wt. (gm.)	Study I: Evis. body wt. (gm.)	Study II: Final body wt. (gm.)	Study II: Evis. body wt. (gm.)	Study III: Final body wt. (gm.)	Study III: Evis. body wt. (gm.)
8	0	0	156	119	152	105	139	106
8A	0	0	--	--	--	--	137	102
3	0	0.06	220	159	140	96	127	88
6	0	8 or 1[a]	233	204	169	140	129	90
7	2	0	478	386	235	176	137	82
7A	2	0	--	--	--	--	175	129
2	2	0.06	529	446	Died[b]	--	253	185
5	2	8 or 1[a]	547	461	211	156	261	196
1	40	0	539	466	119	87	92	67
1A	40	0	--	--	--	--	107	64
9	40	0.06	612	529	215	162	218	182
4	10	0.20	646	559	240	158	248	181
Pellet control	10	--			352	292		

[a]8 mg. in Study II; 1 mg. in Study III.

[b]Lung infection.