THE B VITAMIN REQUIREMENT OF THE BABY PIG

I. NIACIN
II. PANTOTHENIC ACID

by

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AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Animal Husbandry

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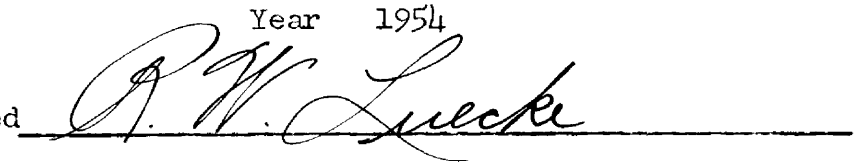
Approved 

Stephen Stothers

Problems resulting from the use of commercial milks in raising baby pigs have indicated a need for fundamental information concerning the quantitative requirement of the baby pig for nutrients. The work presented is an attempt to establish the requirement of the baby pig for both niacin and pantothenic acid, and to obtain more information regarding the symptoms and pathology when these vitamins are deficient.

Baby pigs three to four days of age were used in all experiments. The pigs were fed a synthetic milk idet consisting of casein (Labco) cerelose, lard, salts, plus added vitamins. The milk was approximately 15.2 percent solids and was homogenized at about 3,000 lbs. pressure, and then immediately cooled and stored. After homogenization and cooling, a niacin solution or calcium pantothenate solution was added at the required levels.

The one reported niacin trial failed to establish a quantitative requirement of the thrifty baby pig for niacin, due to the high tryptophan content of the regular casein milk. The isolated tests conducted, demonstrated that runt pigs did require niacin in addition to the tryptophan of the regular casein milk. Four pigs developed niacin deficiency symptoms during the isolated tests. The gross findings on necropsy were inflammation of the cecum and colon, with certain areas showing ulcers and pseudomembranes. Microscopically there was a ballooning of the glands of the cecum and colon with the lumen being distended with mucus and leucocytes.

On the basis of the three reported pantothenic acid trials, the baby pig requirement for pantothenic acid is 12.5 mg. of calcium pantothenate per kg. solids.

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Baby pigs receiving milk containing no calcium pantothenate developed a diarrhea within two to four weeks. In most cases of baby pigs receiving suboptimal amounts of calcium pantothenate intermittent scours developed within two and one-half to five and one-half weeks. Locomotor incoordination usually developed in an additional 7 to 10 days after the initial onset of scours, if the pigs did not become so weakened that they died prior to this time.

The lesions present in the large intestine and nervous system of pantothenic acid deficient pigs are essentially those described by previous workers. Microscopically there was a marked decrease of the goblet cells in the cecum, colon and rectum. Increased connective tissue was noted in the submucosa of the large intestine and especially with pigs which had scoured persistently for a long period of time. The pantothenic acid deficient pigs also showed loss of myelin, and degeneration of the dorsal root ganglion cells.

The observation that the glomerular layer of the adrenals of almost all pantothenic acid deficient pigs was decreased in thickness, has not been reported in any previous research with pigs.

Electrophoretic studies of the sera of five positive controls and four pantothenic acid deficient pigs, four to eight weeks of age, showed a gamma globulin content of 8.2 percent. No appreciable differences were noted in the gamma globulin content of normal and pantothenic acid deficient pigs.

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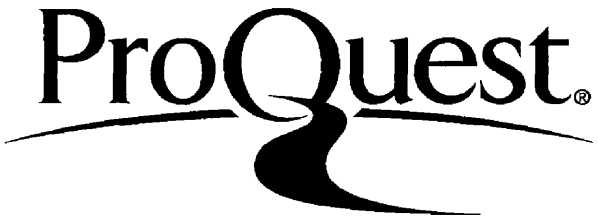

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INTRODUCTION

Synthetic milk diets have been used on an experimental basis with baby pigs since 1939 (Wintrobe). Increased research in this field after 1945 led directly to the development of the commercial synthetic milk diets.

The introduction of commercial synthetic milk diets for baby pigs three years ago, gave impetus to nutrition research in this area. As more problems developed even more emphasis was placed on fundamental research concerning the requirements of the pig for certain nutrients.

The importance of correct vitamin supplementation in weanling pig rations has been shown during the last ten to fifteen years by various research workers. McMillen et al. (1949) showed the importance of calcium pantothenate, nicotinic acid and riboflavin additions to natural rations. Luecke et al. (1949) produced pantothenic acid deficiencies experimentally on diets of natural feedstuffs. Additional evidence by these and other workers has demonstrated the importance of pantothenic acid supplementation to corn-soybean oil meal diets fed to weanling pigs. Since the B-vitamins are so essential for the weanling pig, their importance for baby pigs should not be overlooked.

No published work is available regarding niacin deficiency symptoms or the niacin requirement of baby pigs.

Wiese et al. (1951) developed pantothenic acid deficiency in baby pigs fed a synthetic milk diet. Work at the Michigan station (1952) indicated that the requirement of the baby pig for pantothenic acid was between 10 and 20 mg. of calcium pantothenate per kilogram of solids.

The large difference between what appeared marginal and optimal necessitated additional trials to establish more exactly the requirement.

Information regarding deficiency symptoms for certain vitamins with regard to the baby pig is of definite interest not only to the swine industry but to the veterinary profession which is called upon very frequently to treat nutritional disorders.

The work reported in this thesis was undertaken with the idea of establishing the requirement of the baby pig for both niacin and pantothenic acid; and to obtain more information regarding the symptoms and pathology when these vitamins are deficient.

REVIEW OF LITERATURE

Part I -- Niacin

In 1926, Goldberger et al. proved that pellagra was associated with the lack of a vitamin. He called this vitamin the P.-P. factor. In 1935 Birch et al. showed that the pellagra preventing factor was distinct from both riboflavin and vitamin B_6, previously identified. Later, this P.-P. vitamin was identified by Elvehjem et al. (1937) as nicotinic acid, now more commonly called niacin. Chemically it is pyridine-3-carboxylic acid. Niacin is a white crystalline solid, soluble in hot water and alkali, and is stable to heat, acids and alkalies.

Warburg and Christian (1935) established that the amide of niacin was a component of what was then called a coenzyme. Euler et al. (1935) developed a chemical structure for the coenzyme, which was called diphosphopyridine nucleotide (DPN) or coenzyme I. Subsequent work led to the finding of triphosphopyridine nucleotide (TPN) or coenzyme II.

Basically, these enzymes involving niacinamide, are an essential part of the enzyme system concerned with hydrogen transport (oxidation) in the living cell. These coenzymes act in series with flavoprotein enzymes and, like them, are hydrogen acceptors and hydrogen donors.

Some of the dehydrogenation reactions in which these enzymes are believed involved are lactate to pyruvate, glutamic acid to α-keto glutaric acid, and retinene to vitamin A.

No attempt will be made to give a complete summary of all the niacin research in species other than swine. Birch et al. (1937) fed pigs on a diet consisting largely of maize and obtained subnormal

growth for five or six weeks, then diarrhea developed, the appetite failed and no growth resulted. Autopsy revealed a diffuse cellular inflammation of the mucus membrane of the cecum and large intestine - a condition characteristic in pigs dying of infectious enteritis or pig paratyphoid. It was postulated that something missing from the basal diet was essential for the metabolism of the cells of the mucus lining of the intestine. Thus, when this essential factor was missing, the cell's power to resist invasion by bacteria was lowered.

Chick et al. (1938) reported on the effects of administering nicotinic acid to two pigs fed on a maize-peameal-casein diet deficient in vitamin B. Both animals were losing weight rapidly, had diarrhea and dermatitis and were refusing food. Nicotinic acid was given first intramuscularly in doses of 100 mg. and then by mouth in doses of 60 mg. a day. Within twenty-four hours of the first injection, appetite returned and there followed a steady reversal of the former condition. Similar work was reported by Hughes (1938). Madison et al. (1939) also elaborated on this.

Davis et al. (1940) following the same idea of Birch et al. (1937) concluded from their research that necrotic enteritis of swine develops primarily as a result of nutritional deficiency (niacin). More pigs were affected when fed a basal grain ration of yellow corn than when fed a barley ration.

Hughes (1943) using a basal ration of beet sugar, casein, and salt mixture established the minimum requirement of niacin to be 5 to 10 mg. per 100 pounds of live weight daily. Weanling pigs of 32.2 pounds weight

were used to start in this trial. Powick et al. (1947a) estimated the niacin requirement of the pig from three to nine weeks of age to be 0.6 to 1.0 mg. per kilogram of live weight per day, with pigs started at 12.7 pounds. Comparable requirement values of Hughes would be 0.11 to 0.22 mg. per kilogram live weight per day, but possibly part of the difference in these requirements could be explained on the basis of the lighter pigs used by Powick et al. Work by Braude et al. (1946) suggests that the niacin requirement decreases as pigs grow older and larger.

Additional work was done by Powick et al. (1947b) to study possible nicotinic acid deficiency under conditions of practical feeding. On the basis of the reported nicotinic acid content of natural feeds (Ellis and Madsen, 1943), it seemed that an otherwise practical diet might be expected to supply at least 0.6 mg. of nicotinic acid per kilogram live weight per day. This level was the nicotinic acid requirement of the pig obtained by Powick et al. (1947a) using purified diets. In two experiments with growing pigs receiving diets containing 80 and 84 percent of corn respectively and supplying at least 0.7 and 0.8 mg. of nicotinic acid respectively per kilogram live weight per day, no evidence of nicotinic acid deficiency was noted. A third experiment with growing pigs receiving diets containing 40 and 70 percent of corn combined with purified materials and supplying 0.36 and 0.47 mg. of nicotinic acid respectively per kilogram live weight per day, demonstrated severe nicotinic acid deficiency.

Wintrobe et al. (1945) reported on experiments which were interpreted as indicating that only pigs receiving a low protein diet are in need of nicotinic acid. He suggested an amino acid or protein nicotinic acid relationship.

Although Wintrobe and his associates (1945) were the first in the case of pigs to postulate a possible deficiency of certain amino acids as being part of the problem, actually Krehl et al. (1945a, 1945b) had postulated and proven this idea with rats. Krehl and his associates made note first of all, that rats fed a diet containing 40 percent corn were subject to growth depression, but that increasing the protein content from 15 to 20 percent alleviated this condition. These workers decided that the protective action of casein could not be explained on the basis of its niacin content, but must be due to other factors. It was known that corn was low in the essential amino acids, lysine and tryptophan, so it seemed only logical to study the effect of adding these amino acids to the basal corn ration. Lysine addition had no effect, but the addition of L-tryptophan at a level of 0.4 percent to the low casein-corn grits basal, gave a dramatic response. Still later work by Krehl et al. (1946a, 1946b) verified their earlier results and also showed that similar effects could be produced with certain non-corn rations which were low in tryptophan and niacin.

Briggs (1945) reported the interchangeability of nicotinic acid and tryptophan in preventing nicotinic acid deficiency in chicks receiving a purified diet low in nicotinic acid and tryptophan, but containing

no corn. These and other contributions leave little doubt that tryptophan and nicotinic acid can function interchangeably, in the species considered.

Work by Groschke and Briggs (1946) showed that niacin is concerned in some manner with the metabolism of other amino acids, especially glycine, arginine, and alanine. Other workers studied this same idea with Rosen and Perlzweig (1949) stating that the impairment of growth when amino acids are added to a low casein diet is due to the impairment of the metabolic process involved in the tryptophan-niacin relationship.

Work by Luecke et al. (1947) with pigs substantiated the work of Krehl et al. (1945b, 1946b) conducted with rats. Using a more practical ration than that of Wintrobe (1945), Luecke et al. was able to show that when a 14 percent protein ration containing 87 percent corn was supplemented with D, L-tryptophan excellent growth was obtained. The fact that two pigs in this lot did have a milk inflammatory condition of the colon indicated that possibly a dietary source of nicotinic acid is still needed in addition to the tryptophan, or that the level of tryptophan supplementation was too low. Results of the experiment tend to emphasize the latter viewpoint. This work was in a large measure verified by Powick et al. (1948).

The actual pathway by which tryptophan is converted to nicotinic acid in rats was finally established by Heidelberger et al. (1948, 1949). They fed tryptophan containing C^{14} in the 3-carbon of the indole ring, and niacin was excreted with the isotope in the carboxyl group.

The intermediates in the synthesis were established as the result of work by Heidelberger et al. (1949) and Mitchell and Nyc (1948).

Cartwright et al. (1948) using 20 pigs reported on a niacin deficiency anemia. Ten percent protein diets were used which are considered low for pigs only 21 to 28 days of age. Significant anemia was present generally within 50 days after the start of the experiment. Maximal anemia developed between 60 to 120 days and was frequently associated with a slight reticulocytosis. With one exception the anemia in each case was normocytic and normochromic. Slight anemia was present in the controls but not comparable to those in the niacin deficient group. An increase in the level of protein intake from 10 percent to 26 percent relieved the anemia but niacin was needed as well to get a significant growth increase.

Work by Ludiovici and Axelrod (1951) with rats showed that niacin alone connot satisfy the requirement for antibody synthesis on a basal diet lacking tryptophan. A normal antibody response was obtained in the animals receiving added tryptophan and no niacin. This may be partially explained by the fact that tryptophan may serve as a direct precursor of niacin or niacin derivatives. Thus as contrasted to pantothenic acid deficiency which causes a severe impairment of antibody response (Axelrod et al. 1947), niacin-tryptophan deficiency causes only a moderate impairment of antibody response.

Part II -- Pantothenic Acid

In 1933, R. J. Williams and his associates first extracted pantothenic acid from very diverse tissues. In 1938 the same group of investigators determined the chemical nature of pantothenic acid. The chemical name of pantothenic acid is 2, 4-dihydroxy-3, 3-dimethyl butyryl-beta alanine. It is predominantly of acid character but shows also some basic properties. The vitamin is readily soluble in water and is sensitive towards acid, bases, and heat. Pantothenic acid is commercially available in the form of its crystalline synthetic calcium or sodium salt.

The part that pantothenic acid plays in the metabolism of animals has only recently been established. Work by Lipmann et al. (1947) showed pantothenic acid to be a constituent of coenzyme A. As such it is involved in acetylation processes. Decreased acetylation of aromatic amines in the pantothenic acid deficient rat has been reported in 1948 by Riggs and Hegsted and in 1949 by Shils et al.

Suppler et al. (1942) suggested an alteration in secretion of adrenal cortical hormone in the case of a pantothenic acid deficient rat. Work by Winters et al. (1952) tends to confirm this hypothesis. They observed a marked depression of adrenal cholesterol in the deficient rats. The reduction of cholesterol content in the adrenal glands may represent a decreased synthesis rather than an increased utilization of this steroid for conversion to hormone. It may be that coenzyme A is a necessary part of the enzymatic complement of the adrenal cortex which synthesizes cholesterol.

Olson and Stare (1951) established that pantothenic acid deficiency is associated with a decrease in the tissue content of coenzyme A and reduced capacity for acetylation. Thus it would be anticipated that biosynthesis of cholesterol which is dependent upon acetylation would be inhibited in pantothenic acid deficient animals. Work by Guggenheim and Olson (1952) failed to reveal differences in cholesterol content of liver, heart, and blood serum from deficient rats and pair-fed control animals. Work by Boyd (1953) however, has shown that the effect of pantothenic acid deficiency can be masked or annulled by the feeding of fat, which probably explains the failure of Guggenheim et al. (1952) and Olson et al. (1951) to adduce evidence of an effect of pantothenic acid deficiency on cholesterol synthesis.

However, the work of Guggenheim and his associates (1952) did agree with previously reported work by Winters et al. (1952) showing a marked depression of adrenal cholesterol in pantothenic acid deficient rats. Work by Hurley and Morgan (1952) with rats, seems to suggest that since pantothenic acid deficiency imposes a continuous stress on the adrenal cortex, and that the deficient animals exposed to a second stress will fail to respond as compared to normal controls. Additional work by Ershoff (1953) showed that pantothenic acid deficient animals did not survive cold environmental conditions as compared to normal controls, in agreement with the idea proposed by Hurley and Morgan.

The antibody response of an animal is considered a measure of its ability to respond to stress - in this case, infection. Work by

Axelrod et al. (1947) and Ludiovici et al. (1949) showed that in pantothenic acid deficient rats, antibody response is decreased.

Most recently work by Zucker and Zucker (1954) has shown the development of loss of natural resistance to a specific bacterial infection in pantothenic acid deficient rats.

Later work by Ludiovici et al. (1951) showed that D-L-methionine has a significant sparing action upon pantothenic acid requirement for antibody production. In view of this research, it is interesting to postulate an inter-relationship between pantothenic acid and amino acid metabolism. The work of Chantrenne (1951) suggested a relationship between pantothenic acid and peptide bond formation in that coenzyme A is involved in the synthesis of hippuric acid. The suggestion that acetyl amino acids may be involved in the biologic formation of peptide bonds invites speculation on the role of coenzyme A and the "active two carbon units" in antibody synthesis.

Korkes et al. (1951) have shown that coenzyme A functions in the condensation of pyruvate with oxaloacetate to form citrate. The formation of the active acetate compound acetyl-coenzyme A is believed to represent an essential step in the oxidative metabolism of carbohydrates as well as with fatty acids and amino acids.

The earlier work with pantothenic acid and its physiological activity was done with rats and chicks. Within the past 10 to 12 years more work and emphasis has been placed on the role of pantothenic acid in the nutrition of the pig.

In 1916, Wehrbein reported on an undiagnosed paralysis in pigs which lasted two to four months. The pigs had good appetites but anemia developed and emaciation became more and more apparent. He concluded that it was not an infectious disease, and that certain strains of pigs were more susceptible than others. Doyle (1937) reported on a paralysis he believed due to an infectious form of disease. The "disease", however, was sporadic and had been observed in suckling pigs. He noted inflammatory changes of the large nerve trunks from the hind legs of cases characterized by spastic or "springhalt" symptoms.

Chick et al. (1938) reported on pantothenic acid deficiencies in pigs and stated that the symptoms corresponded closely to the description given by Wehrbein (1916). The pigs fed a purified basal diet deficient in pantothenic acid developed a flaccid palsy in the hind quarters.

Hogan and Johnson (1940) reported on observations made on baby pigs which exhibited leg weaknesses such as goose-stepping, weaving gait, and sickle hocks, as well as other abnormalities probably attributable to vitamin deficiencies. He concluded that the rations commonly used for brood sows were deficient in some factor or factors.

Wintrobe et al. (1940) published work on the relation of diet to the occurence of ataxia and degeneration in the nervous system of pigs. Forty-four pigs averaging three weeks of age were raised on a basal diet containing casein, sugar, lard, a mineral mixture, cod liver oil, ascorbic acid and varying amounts of yeast. If the yeast content was

reduced to a low level or omitted entirely, and thiamine, riboflavin, and nicotinic acid added to the diet, a disturbed gait and extensive lesions of the nervous system developed. Wintrobe made an intensive study of the nerve damage that was attributed to pantothenic acid deficiency.

Lesions were noted in the peripheral nerves and in the spinal cord. In the nerves there was irregularity and swelling of the myelin sheath in isolated areas. Fragments of myelin in the form of small and large clumps were present. Many droplets of free fat could be seen in these areas side by side with clumps of degenerating myelin. It was noted that degeneration was more advanced in the sciatic nerves than in the brachial nerves. In many cases no lesions were seen in the latter, while early changes appeared in the former. Degeneration of the myelin sheath in the spinal ganglia and proliferation of sheath cell degeneration in posterior roots and dorsal funiculi of the spinal cord were noted.

Hughes (1942a) produced pantothenic acid deficiency in pigs started at 35 to 43 pounds. A purified ration containing fifteen percent casein was used in the trial. Symptoms of pantothenic acid deficiency were an early decrease in appetite and slow growth as well as an inability to move about in a normal manner within about one month after being placed on a deficient diet. The deficient pigs apparently lost their sense of equilibrium and coordination for they goose-stepped and often fell. After about 70 days, two of the five deficient pigs lost most of their hair and had diarrhea which was somewhat bloody. Autopsies

of the deficient pigs showed gastritis which included a reddened area on the floor of the stomach about the size of a normal hand. Scattered throughout this area were haemorrhagic spots ranging in size from one to two millimeters. Some inflammation of the large intestine occurred and in one pig many abscesses were present.

Hughes and Ittner (1942b) also published a paper establishing the minimum requirement of pantothenic acid for the growing pig to be between 7.8 and 11.8 mg. per 100 pounds live weight. The same basal diet was used as before - 81 percent sugar, 15 percent purified casein, salt mix 4 percent plus the required vitamins. The pantothenic acid deficient pigs exhibited the same symptoms as mentioned in his previous work (1942a). Extreme goose-stepping and rhythmic kicking with first one hind leg and then the other were symptoms observed.

Recently, Moustgaard (1954) reported on Danish experiments which established the pantothenic acid requirement of growing young pigs at 10 mg. per pound of feed.

Following up earlier work (1941), Ellis et al. (1943) showed that the addition of calcium pantothenate to a heated diet greatly reduced the incidence and severity of locomotion incoordination, but that the further addition of pyridoxine appeared necessary for the full prevention of this disturbance.

Wintrobe (1943) made another intensive study of pantothenic acid deficiency in swine with more reference to symptoms other than the neurological ones that he reported in 1940. His report was more detailed than Hughes (1942) but the gross symptoms were much the same

in each case. The pigs used were started at three to five weeks of age. Deficiency symptoms developed within 11 days. Diarrhea, loss of appetite, rough hair coats, abnormal gait, and a failure to gain weight were observed. The pantothenic acid deficient pigs gained only 20 to 92 grams per day and in one case a loss of weight occurred. The controls gained 433 grams per day. The impairment of growth was far more severe than was obtained in deficiencies of vitamins B_1, B_2, nicotinic acid or B_6. The onset of the gait abnormality took 32 to 52 days in pigs started at three weeks of age, and 93 to 107 days for pigs started at five weeks of age. Diarrhea began about 32 to 52 days after the start of the experiment. A patchy alopecia developed over the rump within three weeks after the start of the experiment. The bowel was congested and edematous. In the colon, injury to cells lining the glands was observed. The mucus vacuoles had disappeared and the cells became atrophic. Wintrobe stated that the absence of mucus secretion is due to the lack of some enzyme or enzymes normally secreted by the colonic epithelium; thus, the mucus vacuoles disappeared. He attributed the poor growth to diarrhea and loss of appetite, although a specific effect as well was possibly involved. The diarrhea which developed soon became constant and sometimes considerable mucus was present. In many instances a bloody discharge appeared.

Wintrobe observed as in his earlier work (1940) the degeneration of sensory neurons. A moderate normocytic anemia was observed in 13 out of 18 pigs. Treatment with pantothenic acid alleviated the anemia. Administration of five hundred or more milligrams of panto-

thenic acid per kilogram of body weight was accompanied by cessation of diarrhea. After treatment there was improved growth, and autopsy showed an improved condition of the intestinal tract. The abnormal gait improved but complete restoration of function did not occur. Wintrobe noted no changes in the adrenal glands in his report (1943).

The work by Chick (1938), Hughes (1942) and Wintrobe (1940, 1943) with pantothenic acid deficient pigs was with purified diets rather than natural feedstuffs, and this work has been stressed in some detail to give an accurate picture of the pantothenic acid deficiency symptoms and its implications. With pigs fed natural feedstuffs, deficiencies appearing may not be as severe or extensive, but where nutritional abnormalities occur one must know all of the possibilities so that accurate diagnosis can be made. Some work has been mentioned, however, where natural diets were used and symptoms of pantothenic acid deficiency appeared. Hanson (1943) mentioned various degrees of unthriftiness and locomotor incoordination of the rear limbs in pigs fed rations including corn, tankage, and soybean oil meal. The addition of various dried brewer's yeasts to the ration resulted in marked improvement of appetite, rate of gain, and some improvement of coordination. Ellis (1943), previously mentioned, stated that the rather frequent occurrence of locomotor incoordination in growing swine fed on normal diets of corn with supplements appears to be due to the borderline level of pantothenic acid present. Various nutrition reviews since 1943 have reiterated the same idea and stressed the importance of correct B-vitamin supplementation of pig rations.

Work by McMillen et al. (1949) showed that the addition of pantothenic acid, nicotinic acid and riboflavin to a ration of corn, oats, soybean oil meal, meat scraps, alfalfa meal, and complex mineral mixture, gave significant increases in daily gains and reduced feed consumption per unit of gain by 22 to 25 percent. Luecke et al. (1949, 1950) reported that a basal ration of corn, casein, soybean oil meal, and minerals did not contain enough pantothenic acid to prevent symptoms of locomotor incoordination and myelin degeneration from appearing. Symptoms of incoordination did not appear until the seventh week of the experiment. In a second trial pantothenic acid deficiency occurred on a low protein corn-soybean meal ration. The deficiency symptoms noted were most severe when thiamine, riboflavin, nicotinic acid, and pyridoxine were added to the basal ration. No incoordination was observed when the unsupplemented corn-soybean ration was fed, but growth was very poor. It was concluded that the addition of nicotinic acid and riboflavin stimulated growth to such an extent that the levels of pantothenic acid in the ration were insufficient to prevent deficiency. It was noted that two pigs in the pantothenic acid deficient lot were completely paralyzed in the hind quarters, which is quite similar to the symptoms described in the early work by Wehrbein (1916) and Doyle (1937).

Sharma et al. (1952) reported on the pathology of the intestine and other organs of weanling pigs when fed a ration of natural feedstuffs low in pantothenic acid. The large intestine first showed degenerative changes and a few ecchymotic haemorrhages and, in the

later stages, small superficial discrete ulcers. The columnar epithelium showed degenerative changes and there was a marked hyperemia of the lamina propria. The cellular reaction was mainly lymphocytic. The crypts of Lieberkühn showed cystic dilations and hyperplasia of the lymph nodules.

All of the work mentioned has been carried out with weanling pigs, but since only one paper has been published on the pantothenic acid deficiency in baby pigs, it has been necessary to present the material to serve as a basis for comparison with work presented in this thesis.

Weise et al. (1951) reported on pantothenic acid deficiency in baby pigs fed a synthetic milk diet. The symptoms reported were poor growth, loss of appetite, scours, coughing, loss of sucking reflex, a dark brown exudate around the eye, spastic gait, goose-stepping, alopecia and low urinary excretion of pantothenic acid. In general, these symptoms agree with those observed in older pigs. However, post mortem examination failed to reveal any internal gross lesions. The deficient animals had little subcutaneous fat and internal fat was lacking. The pigs used in the trials reported were started at two days of age and at 56 days the control pigs averaged 39 pounds in weight. The weights of the deficient pigs after 56 days on trial did not represent the weights of pigs pantothenic acid deficient for the entire period since they all received calcium pantothenate after 28 to 33 days on trial when they were in advanced stages of pantothenic acid deficiency. The daily supplementation of 10 to 20 mg. of calcium pantothenate resulted in complete recovery and great improvement of appetite and growth.

EXPERIMENTAL PROCEDURE

The niacin experiment was conducted in the fall of 1953 with the isolated tests being conducted in the fall of 1953, winter and spring of 1954.

Pure bred Chester Whites and pigs from a Yorkshire-Chester White cross were used in the one reported niacin experiment, involving a total of 20 pigs. Duroc Jerseys, Yorkshire-Chester Whites cross breds, and Yorkshire-Berkshire cross breds were used in the isolated tests, involving a total of 12 pigs.

Five experimental trials were conducted to establish the pantothenic acid requirement of the baby pig. The first two of these were conducted in the fall of 1952, but were complicated by factors believed unrelated to pantothenic acid. These early pantothenic acid trials led to modifications of experimental procedure in an attempt to eliminate the chance of such complicating factors introducing too much error. The first pantothenic acid experiment reported in this thesis was conducted in the spring of 1953, with the last two reported being conducted in the spring and summer of 1954.

A total of 102 baby pigs were used in all five pantothenic acid experiments, 66 of these pigs being in the reported trials. In Experiment I, the pigs were of Chester White breeding or from a Yorkshire-Chester cross. Pigs of Yorkshire breeding or from a Yorkshire-Berkshire cross were used in Experiment II. Duroc Jersey pigs were used in Experiment III.

In the reported experiments the pigs were removed from the sow after 72 to 96 hours depending on the vigor and size of the pigs at birth.

The pigs were placed in metal cages with screen floors for a depletion period of four days. During this period they received a standard basal milk minus the vitamin to be studied. This depletion period was carried out so that at the end of four days, the pigs would be accustomed to drinking milk and adjusted to the new environment, and then only the more vigorous and healthy pigs of a litter would be selected for the actual experimental period.

All pigs were lotted as fairly as possible according to weight, sex, and litter.

All of the pigs used in the actual trial were individually fed. This technique allowed the collection of accurate feed consumption records.

During the depletion period the pigs were fed at three hour intervals, six times a day. After this period, they were fed at four hour intervals, five times a day according to their appetites, at 8:00 A.M., 12:00 noon, 4:00 P.M., 8:00 P.M., and 12:00 midnight.

The baby pigs were starved for the first 8 to 10 hours of the depletion period after which, with a little encouragement, they readily learned to drink warm milk placed in a trough. For the first week to ten days the milk was warmed to approximately body temperature and then gradually decreased to the temperature of the refrigerated milk (3°-4°C.). Heat lamps were provided for the first week or two, while room temperature was thermostatically controlled at approxi-

mately 70°F. This temperature was exceeded, however, in the last pantothenic acid experiment since it was conducted during a portion of the summer months when daytime temperatures averaged between 80°and 90°F. It is not believed that this affected the experiment to any great extent.

In all experiments the pigs were weighed at four day intervals.

The basal synthetic milk had the following composition (in percent):

Vitamin-free Casein	-	30
Salts	-	6
Lard	-	10
Cerelose	-	54
Added vitamins		

The lard content was increased to 20 percent and the cerelose content decreased to 44 percent in pantothenic acid experiments II and III, and in all the niacin experiments.

The salt mixture was a modified mixture of Phillips and Hart (1935):

NaCl	-	118.8 grams
K_2HPO_4	-	257.6 "
$CaHPO_4$	-	338.0 "
Ca Lactate	-	228.8 "
$MgSO_4 \cdot 7H_2O$	-	35.4 "
$FeSO_4 \cdot 7H_2O$	-	19.3 "
KI	-	0.6 "
$MnSO_4 \cdot H_2O$	-	1.0 "
$ZnCl_2$	-	0.2 "
$CuSO_4 \cdot 5H_2O$	-	0.2 "
$CoCl_2 \cdot 6H_2O$	-	0.1 "
		1,000.0 grams

All of the ingredients in the salt mixture were weighed on a gram balance and then mixed in a small cement mixer.

The milk was prepared in ten gallon milk cans. Twenty-eight liters of hot water (about 70°C.) were placed in each can. The casein

was dissolved according to the method of Bird et al. (1935). Sodium bicarbonate equal to 4.75 percent of the weight of the casein was added to the heated water and then the casein was added slowly. The mixture was stirred constantly by use of an electric stirrer. When the casein was in suspension, the cerelose was added. This was followed by the addition of the lard containing the fat soluble vitamins A, D, E, and K. Portions of the salt mixture were placed in a Waring blender and then some of the hot solution from the milk cans was added and mixed with the salts to cause a suspension which was then added to the milk mixture in the cans. The final mixture was homogenized at approximately 3,000 pounds pressure. The homogenized milk was placed in a dairy water cooler for two hours before placing in a walk-in cooler where the milk was stored until feeding time. The B-vitamin solution previously made was added at the required levels after homogenization and cooling of the milk mixture. A separate calcium pantothenate or niacin solution was also added at the same time at the required levels.

The vitamins added and the concentrations were as follows:

Vitamin	Mg./Kg. milk
Thiamine HCl	0.65
Riboflavin	0.65
Inositol	26.00
Choline HCl	260.00
p-Aminobenzoic Acid	2.60
Pteroylglutamic Acid	0.052
Biotin	0.01
Pyridoxine HCl	0.65
Alpha-tocopherol acetate	1.00
2-methyl-1, 4-naphthoquinone	0.28
Vitamin A	2,000 I.U. per kg.
Vitamin D	200 I.U. per kg.

In the niacin experiments, calcium pantothenate was included in the B-vitamin solution at the level of 3.00 mg. per kg. milk. In the pantothenic acid experiments niacin was included in the B-vitamin solution at the level of 2.50 mg. per kg. milk.

A total of 5,000 grams of solids made up of casein, cerelose, lard, and salt mix, was added to each milk can. Thus each can contained approximately 33 kg. of milk.

The B-vitamins were weighed on an analytical balance and put into 20 percent alcohol solution. It was necessary to grind the vitamins inositol and p-aminobenzoic acid to a fine powder using a mortar and pestle before adding them to the solution. A fortified cod liver oil containing known amounts of vitamins A and D was used. Vitamins E and K were added to the cod liver oil.

Vitamin K (Menadione, Merck) in the powder form, was added to some heated cod liver oil to facilitate getting it into solution.

Niacin Experiment

Lot 1 - Basal (negative control)
Lot 2 - Basal plus 10 mg. niacin per kg. solids
Lot 3 - " " 15 mg. " " " "
Lot 4 - " " 20 mg. " " " "
Lot 5 - " " 25 mg. " " " "
The length of the experimental period was 30 days.

In the isolated tests carried out, no niacin was added to any of the diet except during the recovery studies when injections were also given. The isolated tests were conducted over variable periods depending upon the appearance of deficiency symptoms.

Niacin assays were carried out according to the microbiological method of Krehl et al. (1943).

Pantothenic Acid Experiments

Experiment I

Lot 1 - Basal (negative control)
Lot 2 - Basal plus 5 mg. calcium pantothenate per kg. solids
Lot 3 - " " 10 mg. " " " " "
Lot 4 - " " 15 mg. " " " " "
Lot 5 - " " 20 mg. " " " " "

Experiment II

Lot 1 - Basal (negative control)
Lot 2 - Basal plus 7.5 mg. calcium pantothenate per kg. solids
Lot 3 - " " 10.0 mg. " " " " "
Lot 4 - " " 12.5 mg. " " " " "
Lot 5 - " " 15.0 mg. " " " " "

Experiment III

Lots were the same as for Experiment II

Experiments I and II were conducted for 40 days and Experiment III for 48 days. Some of the pigs were kept longer than this length of time, if recovery studies were being carried out and in Experiment III all pigs in Lots 1, 2, and 3 were kept on test for 56 days.

Pantothenic acid assays, according to the microbiological method of Skeggs and Wright (1944), were carried out on the prepared milk for all experiments. The samples were digested using the enzyme Mylase P as outlined by Buskirk et al. (1948). Some pantothenic acid assays on blood were carried out on Experiment I and on urine in Experiment III.

Blood electrophoretic studies were carried out using one pig from Experiment II and six from Experiment III, as well as two weanling pigs from the swine barn to serve as controls. These studies were conducted using Longsworth's technique (1942).

The blood for assay and electrophoresis studies was obtained by the use of a hypodermic needle inserted slightly ahead of the sternum of the pig to obtain the blood from the anterior venous sinus.

Pathological Studies

In both niacin and pantothenic acid experiments blood samples were collected from the ear veins of all pigs for hemoglobin determination and white blood cell counts. These samples were usually taken every two weeks as a check upon the pigs' general health and condition. When an anemia developed in one of the isolated niacin tests, weekly blood samples were taken to follow its progress and to study the effect of certain treatments.

All animals that died were necropsied shortly after death or in some cases were killed in extremis and blocks of tissues were taken and fixed in formal-saline. Hematoxylin-eosin was used on all sections and Weil's (1945) stain for myelin sheaths. Nissl bodies were stained by a thionin technique described by Fletcher (1947). All tissues of deficient pigs were compared with those taken from control pigs which served as a standard.

RESULTS

Part I -- Niacin Studies

Table 1. Results of Niacin Experiment with Baby Pigs[1]

Lot	Niacin per kg. solids mg.	Initial weight lbs.	Final weight lbs.	Average daily gain lbs.	Average solids consumed per lb. gain lbs.
1	0	5.06 ±.48	14.85 ± 1.38	.33 ±.04	1.23 ±.07
2	10	5.08 ±.36	16.88 ± 1.03	.39 ±.03	1.16 ±.001
3	15	4.99 ±.32	15.69 ± 1.56	.36 ±.04	1.18 ±.05
4	20	4.97 ±.33	16.62 ± 1.05	.39 ±.03	1.18 ±.03
5	25	4.78 ±.26	15.77 ± 1.49	.37 ±.04	1.17 ±.07

[1] - Four pigs per lot on experiment 30 days

The results of this trial show no significant differences between the lots. Although the pigs in the lots receiving different amounts of niacin did consistently better than the pigs in the lot receiving no niacin, this difference can be adequately accounted for by the fact that one pig in the lot receiving no niacin developed a torticollic condition about ten days after the start of the experiment, and gained poorly during the rest of the trial. This same pig also developed scours intermittently which were harder to control than was the case with any of the other pigs. In all lots one or two of the pigs did develop scours usually early in the experimental period. This condition corrected itself in 12 to 24 hours after the inclusion of sulfaguanidine in the milk, which was then fed in smaller quantities compared to the period just previous to the onset of scouring. An occasional case of vomiting occurred and was attributed to overfeeding.

The main contributing factor to a lack of significant differences was believed to be the high tryptophan content of the synthetic milk. Considerable research by Krehl et al. (1945b), Luecke et al. (1947), and Heidelberger et al. (1949) has shown that tryptophan may be converted to niacin where a lack of the vitamin occurs and adequate amounts of tryptophan are present. The casein used in this experiment was analyzed for tryptophan and found to contain 0.99 percent.

The assumption that the high tryptophan content of the diet was a deterrent to the development of a niacin deficiency and any estimation of the requirement for this vitamin, led to some "isolated" tests to develop a diet that would result in the development of a niacin deficiency. The results of these tests are given in Table 2.

Acid hydrolysis of a protein destroys tryptophan so it was decided to test a 1:1 mixture of casein and acid hydrolyzed casein (Test 1) as the source of protein (30 percent of the basal diet) on two pigs. These two pigs were fed for the first eight days after removal from the sow on the regular casein milk containing no niacin before being tested on the casein-acid hydrolyzed casein mixture. After two and one-half weeks on this diet both pigs started to scour and the feces appeared stringy with mucus. With one pig the scouring persisted until autopsied after being five weeks on the diet, and too weak and emaciated to exist much longer. The other pig scoured intermittently for one week after the initial onset and then after three and one-half weeks on the diet the diarrhea was continuous. A prolapse developed in this pig after four and one-half weeks.

Table 2. Results of Tests to Develop Niacin Deficiency Symptoms in Baby Pigs

Test no.	Experimental period	Source of protein[1]	No. pigs	Condition of pigs	Initial weight	Final weight	Average daily gain	Solids consumed per lb. gain
	days				lbs.	lbs.	lbs.	lbs.
1	33	AHC:C(1:1)	2	Thrifty	4.63±.09	7.11±1.04	.08±.03	4.82±1.94
2	31	G:C(1:1)	2[2]	Thrifty	10.35±.12	12.37 ±.96	.07±.04	5.32±2.07
3	19	C	2[2]	Runts	4.73±.16	7.14 ±.38	.13±.01	2.08 ±.24
4	49	C	3	Thrifty	4.42±.12	26.26 ±.74	.45±.02	1.22 ±.08
			3	Runts	2.14±.13	11.93 ±.16	.20±.002	1.64 ±.03

[1] - AHC = acid hydrolyzed casein
C = regular vitamin-free casein
G = gelatin

[2] - Littermates removed from the sow at 24 days of age

The pig had been injected with 100 mg. niacin, one and one-half days before the prolapse occurred in an attempt to prevent its occurrence. The prolapse was amputated and niacin treatment continued resulting in a marked improvement in the pig's appearance, appetite, and ability to gain. It was noted that during all of the period that the pigs received the milk containing acid hydrolyzed casein the milk appeared unpalatable as the pigs drank only small quantities of it at a time and developed no increased appetite for it. Actual taste tests revealed that the milk had a very bitter and acrid taste as contrasted to the sweet and palatable taste of the casein milk.

Because of the lack of palatability of the acid hydrolyzed casein milk, it was decided to try a gelatin, casein mixture as the source of protein (1:1). Also, gelatin has no tryptophan. This complete dry mixture was tested on two Duroc pigs, (Test 2), which were taken from the sow at twenty-four days of age. These pigs weighed 10.35 pounds at the start of the trial and averaged 12.39 pounds after 31 days on experiment. At this time, both pigs were showing anorexia to some extent and diarrhea. One pig was autopsied and the other was injected with niacin. An almost immediate improvement was noted in appetite and gain, and a decrease of diarrhea within 4 to 5 days. This pig was kept for a total of 34 days after the first injection of niacin during which the pig continued to gain although brief intermittent periods of diarrhea did occur.

Two other Duroc pigs, runts at birth and littermates of the other two Durocs used, and brought in at the same time, were started

on the standard casein milk complete except for niacin (Test 3). Although it was intended to eventually feed them the gelatin-casein dry feed mix as with the other two pigs, niacin deficiency symptoms appeared before this change could be carried out. In the case of one pig, the deficiency became so severe within 15 days, it was necessary to treat it with niacin injections which resulted in an immediate reversal of weight loss and diarrhea. The other pig developed severe symptoms within 16 days on trial and started to lose weight rapidly after an additional four to five days and was autopsied after 23 days on trial.

The apparent difference between runt pigs and thrifty pigs, as shown by the results obtained in Test 3, led to a study in which three runt pigs (average weight 2.14 pounds) and three thrifty pigs (average weight 4.42 pounds) were fed the standard casein milk (dry mix) without niacin for 49 days. Results are shown in Test 4 of Table 2. Two of the three runt pigs developed slight scours in two and one-half weeks, which cleared up and then reappeared in 9 to 10 days, and continued intermittently with all three pigs until the 49th day. Weekly hemoglobin determinations had revealed a gradual lowering of hemoglobin blood values attributed to niacin deficiency. To prove this theory, one pig was injected with niacin after 49 days on experiment, and within a week the hemoglobin level of the blood had increased 4.3 gm. percent. The hemoglobin levels of the other two pigs continued suboptimal for the three weeks that they were continued on trial, despite iron supplementation during this period. Vitamin B_{12} injections had also failed prior to the 49th day to cause an increase in hemoglobin values.

Table 3. Hemoglobin Averages in gm. percent for Baby Pigs in Test 4

Days on experiment	7	21	35	42	49	56	63	70
Runt pigs	11.1	9.5	7.4	5.8	6.2	7.1	6.1	6.2
Thrifty pigs	12.8	11.3	10.6	12.5				

Examination of the blood cells from the runt pigs classified the anemia as being microcytic in nature. Cartwright et al. (1948) using older pigs and low protein diets without niacin showed that a normocytic anemia developed. Since in Test 4 both the runt pigs and thrifty pigs were on high protein diets (30 percent casein) and the thrifty pigs did not develop anemia it may be concluded that apparently runt pigs are inefficient in converting tryptophan into niacin. Extending this idea further, perhaps runt pigs do not grow as well as thrifty pigs because of inefficient metabolism of most nutrients. Heredity, nutritional status at birth and during the suckling period undoubtedly play a role in the development of a pig into a runt or a healthy fast growing pig.

It was noted that a moderate leukocytosis accompanied the diarrhea in the niacin deficient pigs.

Pathology of Niacin Deficiency in the Baby Pig

Gross Pathology

Only four animals showing symptoms of niacin deficiency were necropsied. The gross findings on necropsy were inflammation of the cecum and colon with certain areas showing ulcers or pseudomembranes

which, when removed, showed an inflamed and ulcerated point of attachment. The pseudomembrane was not tightly attached but enough so that it was not removed when washed with a gentle stream of water. In one animal the jejunum was congested and the ileum appeared somewhat thickened. Hemorrhage into the fundic portion of the stomach was seen in one animal. In another animal ascites and hydropericardium were observed.

Microscopic Pathology

Microscopically there was ballooning of the glands in the cecum and colon with the lumen being distended with mucus and leukocytes. The cells lining the glands were thinned and flattened. There appeared to be a deficiency of glands in the mucosa. Ulcerative areas with loss of mucosa and infiltration with leukocytes were seen. Two of the animals showed a mild fatty change in the adrenal cortex. No changes were noted in the central nervous system or in any other organ.

One animal after exhibiting typical symptoms was treated with nicotinamide by intraperitoneal injection. The feces became solid in five days and except for several mild exacerbations remained so, and the animal made an apparent complete recovery. Necropsy on this animal showed no abnormality, grossly and microscopically, only a small focus of gliosis was seen in the brain stem and another small focus in the cerebrum. The significance of these foci of gliosis is not known. The animal exhibited no symptoms attributable to the central nervous system at any time.

Part II -- Pantothenic Acid

Experiment I

Table 4. Results of Pantothenic Acid Experiment with Baby Pigs[1]

Lot no.	Calcium pantothenate per kg. solids	Initial weight	Final weight	Average daily gain	Average solids consumed per lb. gain
	mg.	lbs.	lbs.	lbs.	lbs.
1	0	4.40±.33	7.92[2]±.88	.10±.01	2.64 ±.42
2	5	4.28±.25	11.15[3]±.47	.17±.01*	1.91 ±.21
3	10	4.33±.16	19.94 ±.34	.39±.01**	1.19 ±.03**
4	15	4.42±.18	21.78±1.25	.44±.03**	1.15 ±.04**
5	20	4.33±.17	21.41 ±.66	.43±.01**	1.14 ±.02**

1 - Four pigs per lot on trial lasting 40 days.
2 - The final weight for pigs in lot 1 includes one animal that survived only 28 days and the remaining three which were removed after 35 days due to severe deficiency.
3 - One pig in lot 2 removed after 38 days due to severe deficiency.
* - Significant at 5 percent level.
** - Significant at 1 percent level.

From the data given in Table 4, it is shown that the lots receiving 10, 15 and 20 mg. of calcium pantothenate per kilogram of solids gained significantly better than the lot receiving 5 mg. of calcium pantothenate. Statistical analysis of feed efficiency shows that the lots receiving 10, 15, and 20 mg. of calcium pantothenate per kilogram of solids were very significantly more efficent than either the basal lot or the lot receiving 5 mg. of calcium pantothenate per kilogram of solids.

In Experiment I, scours appeared at three to four weeks and "goose-stepping" in four and one-half to five and one-half weeks. All of the pigs receiving 0 and 5 mg. of calcium pantothenate per kilogram of dry matter developed severe deficiency symptoms. The

deficiency symptoms in lot 2 required three to four days longer before detection as compared to lot 1 receiving no calcium pantothenate. One pig in the lot receiving 10 mg. of calcium pantothenate per kilogram of dry matter started to scour at four weeks of age and this persisted until the end of the trial. Post mortem studies of this pig showed typical pantothenic acid deficiency symptoms, with edema and some hemorrhage of the gut.

Two pigs, one in the negative control lot and the other in the lot receiving 5 mg. of calcium pantothenate per kilogram of dry matter, developed a prolapse within four to four and one-half weeks on trial. This was believed due to the severity of the scouring and constant irritation. Also, two pigs, one in the negative control lot and the other the pig with the prolapse from the lot receiving 5 mg. of calcium pantothenate per kilogram of solids, not only "goose-stepped" but periodically exhibited "running attacks". During these attacks of short duration, the hind legs kicked furiously and apparently quite involuntarily.

None of the pigs in the lots receiving 15 and 20 mg. of calcium pantothenate per kilogram of dry matter showed symptoms of pantothenic acid deficiency.

Two pigs in the lot receiving 5 mg. of calcium pantothenate per kilogram of dry matter developed severe deficiency symptoms and were then injected with 100 mg. of calcium pantothenate solution. Within 48 hours these pigs showed a marked improvement including cessation of scours. Continued treatment resulted in improved appetites and

increased gains and the pigs lost most of their rough general appearance.

Pantothenic acid assays of the blood demonstrated no significant differences between lots.

Experiment II

Growth results and feed consumption figures are given in Table 5. The results of Experiment I had shown 15 mg. of calcium pantothenate per kg. solids to be adequate and the 5 mg. of calcium pantothenate per kg. of solids definitely inadequate, with the 10 mg. of calcium pantothenate per kg. solids apparently being marginal. In order to establish the requirement still more exactly, the levels of 7.5, 10, 12.5, and 15 mg. of calcium pantothenate per kg. of solids were tested.

Table 5. Results of Pantothenic Acid Experiment with Baby Pigs[1]

Lot no.	Calcium pantothenate per kg. solids	Initial weight	Final weight	Average daily gain	Average solids consumed per lb. gain
	mg.	lbs.	lbs.	lbs.	lbs.
1	0	3.88±.36	7.31[2] ±.87	.08±.03	2.35 ±.27
2	7.5	3.90±.32	17.95[3]±2.40	.35±.05**	1.29 ±.09**
3	10.0	3.93±.04	21.04 ± .81	.44±.01**	1.14 ±.02**
4	12.5	3.93±.23	22.41 ± .95	.46±.01**	1.10 ±.02**
5	15.0	3.76±.23	22.57 ± .93	.47±.01**	1.07 ±.02**

1 - Five pigs per lot except for lot 1 where six pigs were used. Trial lasted 40 days.
2 - Final weight for pigs in lot 1 included four that were removed after 15 to 22 days on trial due to severe deficiency.
3 - One pig in lot 2 was removed after 32 days on trial due to severe deficiency.
** - Significant at the 1 percent level.

All of the lots receiving calcium pantothenate gained significantly better at the 1 percent level than the lot receiving no calcium pantothenate. This relationship also held true in regard to efficiency of gain. Lots 4 and 5 receiving 12.5 and 15.0 mg. of calcium pantothenate respectively gained significantly better at the 5 percent level than lot 2 receiving 7.5 mg. of calcium pantothenate per kg. of solids.

Five pigs were in each lot except for lot 1 where six pigs were used. Of these six pigs, four began to scour within two to two and one-half weeks after being placed on the pantothenic acid deficient diet. The other two pigs (numbers 2-7 and 5-2) compared to the four mentioned, were much larger at birth and when removed from the sow, and did not begin to scour until about four weeks after being placed on the pantothenic acid deficient diet. This difference can, no doubt, be accounted for by the greater pantothenic acid body storage of these two larger pigs, thus a longer time being necessary for depletion of the vitamin. Pig 5-2 exhibited a slight goose-step four days after the onset of scouring but within another four days the scouring ceased for a day or so and the goose-step disappeared. Solid feces were noted for a day or two and then scouring was noticed again. Coincident with the cessation of scours and goose-stepping was a gain in weight, and a slightly improved appetite. Apparently the pig was receiving some source of pantothenic acid as a result of bacterial synthesis in the gut or some other process. It is possible that coprophagy may have been a partial source. Apparently this source of pantothenic acid was not sufficient or sustained over a long enough period of time as noted by the recurrence of scours, decreased appetite, and loss in weight once more.

As pig 5-2 became weaker it was decided to inject him with pantothenic acid after 40 days on trial for recovery studies. Initially the results were quite positive - a cessation of scouring, gain in weight, as well as an improved appetite were noticed. However, after eight days of this treatment it appeared that the pig was not gaining weight nor developing a more improved appetite so it was sacrificed and autopsied.

Pig 2-7 developed a prolapse 12 days after the onset of scours (39 days of receiving the lot 1 milk). This prolapse was amputated but the pig did not defecate until 12 days later although the lumen appeared patent. Examination did show a slight stricture at the point of amputation. Within two or three days after the pig began to defecate again, scours were observed and then a slight goose-step. The pig became progressively weaker and died after 60 days of receiving lot 1 milk. Death occurred due to a pantothenic acid deficiency although peritonitis was a complicating factor. Like pig 5-2, pig 2-7 also exhibited a period in which there was an improved appetite, gain in weight, although the scouring did not cease. This was just prior to the onset of the prolapse.

No persistent scours developed in any of the lots receiving calcium pantothenate. It might be noted that in all lots including lot 1 after 8 to 16 days on trial, some trouble was experienced with vomiting and poor appetites which may or may not have been of an infectious nature. Eventually the heaviest pig in lot 3 (10 mg. of calcium pantothenate per kg. solids) was autopsied as he appeared to be getting progressively weaker

and unable to stand properly because of a lack of control of the rear quarters. At the time of autopsy just prior to death the pig's body temperature was 96°F. All of this was believed connected in some way with the animal's failure to recover from the period of vomiting and lack of appetite. The action of the pig was not typical of a pantothenic acid deficiency.

Four out of the five pigs receiving 7.5 mg. of calcium pantothenate per kg. of solids developed instability of the rear quarters. Two littermates developed a definite goose-step after four to four and one-half weeks on trial, eventually lost control of their rear quarters, and finally developed some incoordination of the front quarters as well. One of the two died and the other was used for recovery studies. One hundred milligrams of pantothenic acid was injected for two successive days and then every other day for a week. Within 24 hours this pig was able to walk and within 48 hours showed remarkable improvement in appetite and had started to gain in weight. As was the case with previous work at the Michigan station (1952) the recovered pig's gait never returned to completely normal.

In the case of the other two pigs in lot 2 which showed instability in the rear quarters, one pig was autopsied after 49 days on trial, while the other pig was injected with 100 mg. of pantothenic acid on three alternate days before being returned to the swine barn. It was observed that this pig became quite weak in his rear quarters very shortly after being returned but eventually almost completely recovered from the tendency to goose-step.

Experiment III

Since the results of Experiment II were not too conclusive, it was decided to repeat the experiment using Duroc Jersey pigs.

Table 6. Results of Pantothenic Acid Experiment with Baby Pigs[1]

Lot no.	Calcium pantothenate per kg. solids	Initial weight	Final weight	Average daily gain	Average solids consumed per lb. gain
	mg.	lbs.	lbs.	lbs.	lbs.
1	0	4.60±.25	7.09[2] ±.55 (6.79)[3]	.05 ±.01 (.04)	2.65 ±.10 (2.03)
2	7.5	4.59±.17	23.77 ±1.00 (22.96)	.40 ±.02** (.33)	1.19 ±.06** (1.54)
3	10.0	4.51±.27	26.17 ± .94 (28.48)	.45 ±.01** (.42)	1.06 ±.03** (1.20)
4	12.5	4.56±.32	30.54 ±1.12	.54 ±.02**	1.00 ±.02**
5	15.0	4.51±.07	29.29 ± .62	.52 ±.01**	.99 ±.01**

1 - Four pigs per lot on trial lasting 48 days.
2 - Three pigs removed from lot 1 after 23 to 26 days due to severe deficiency.
3 - Final weights, average daily gains, average solids consumed per lb. gain, recalculated on a 56 day basis for lots 1, 2, and 3.
** - Significant at the 1 percent level.

All of the lots receiving calcium pantothenate gained significantly better at the 1 percent level than lot 1 receiving no calcium pantothenate. Lots 4 and 5 receiving 12.5 and 15.0 mg. of calcium pantothenate per kg. solids respectively gained significantly better at the 1 percent level than lot 2 receiving 7.5 mg. of calcium pantothenate per kg. of solids. Lot 4 gained significantly better at the 5 percent level than lot 3 receiving 10 mg. of calcium pantothenate per kg. of solids. Lot 5 gained significantly better at the 10 percent level than lot 3.

With regard to efficiency of gain, all of the lots receiving calcium pantothenate were significantly better at the 1 percent level than lot 1. Lot 4 and lot 5 were significantly better at the 5 percent level than lot 2 receiving 7.5 mg. of calcium pantothenate per kg. of solids.

After 48 days the one remaining pig in lot 1 and all of the pigs in lots 2 and 3 were kept on trial for an additional period of eight days. It was hoped that during this period of time these pigs would develop more severe deficiency symptoms than those exhibited after 48 days on trial, because all of these pigs except one pig in lot 3, were scouring persistently and some exhibited weaknesses of the rear quarters. The pigs in lots 4 and 5 after 48 days on trial were of such a size that it seemed inadviseable to keep them any longer. If they had been kept on experiment so that their weights and gains could be calculated for 56 days, it appears that statistically the differences would have been much more significant since the pigs in lots 2 and 3 showed considerably decreased gains in the additional eight days. The weights gains, and feed efficiencies are given in parenthesis for each of these lots recalculated for 56 days.

The pigs in lot 1 began to scour in two and one-half to four weeks after being put on experiment. One pig developed a rectal hemorrhage 10 days after the onset of scours. A 100 mg. calcium pantothenate injection was given but after a blood sample was taken for electrophoretic studies, the pig was in such a weakened condition that it died two days later. One pig in this lot was used for recovery studies

when it became very weak after 23 days on experiment. Four injections of calcium pantothenate solution were given on successive days followed by six additional injections on alternate days. Within 24 hours the pig scoured less and had an improved appetite. Within three days the feces were quite firm and there was a marked improvement in the appearance and appetite of the pig. The pig gained 18.55 pounds within a period of 25 days on a recovery basis as compared to the 2.20 pounds the pig gained during the first 23 days on trial.

As in Experiment II, a pantothenic acid deficient pig in lot 1 began to scour, lost weight, and then went into a recovery period of 10 to 12 days in which it gained weight. Because of this gain in weight, two urine samples were collected on a 24 hour basis to determine pantothenic acid excretion. Since the pig was scouring at the time, the samples were actually composite urine and fecal samples as it was not possible to separate these in an ordinary metabolism cage. Analysis revealed quite a high content of pantothenic acid in these samples -- 493.5 ug. per day and 1975 ug. per day, as calculated. Theoretically the milk contained no pantothenic acid. In spite of possible corrections for error in the assay itself plus the small amount in the casein it appeared that some synthesis must have been occurring in order for the pig to gain weight and have periods of improved appetite, as well as having some pantothenic acid excreted in urine. Eventually the pig became weakened, lost weight and developed a secondary pneumonia and died. No goose-stepping was

observed in lot 1, although the one which lived for the longest period of time (53 days) appeared somewhat weak and unstable in the rear quarters during its last week.

In the lot receiving 7.5 mg. of calcium pantothenate per kg. of solids, eventually all of the pigs began to scour within $2\frac{1}{2}$ to $5\frac{1}{2}$ weeks with an average of 4 weeks after being put on trial. Weakness in the hind quarters was observed in all pigs within 6 to 7 weeks after being placed on trial. One pig in lot 3 receiving 10 mg. of calcium pantothenate per kg. solids died of peritonitis after 8 days on trial. This condition was believed due to an anatomical malformation and not the diet itself. Two of the remaining three pigs in lot 3 began to scour in 3 to $4\frac{1}{2}$ weeks after being placed on trial and after 7 weeks one of these two exhibited definite instability in its rear quarters.

None of the pigs in lots 4 or 5 exhibited any abnormal scouring or goose-stepping at any time during the trial.

In the urine assays for pantothenic acid four control assays were carried out, and excretion values for pantothenic acid ranged from 3 to 6 percent of the calcium pantothenate intake. Composite urine and fecal samples were collected from one pig in lot 2. This pig was scouring continuously at the time the collections were made and eventually he became weak in the rear quarters. The collections were carried out over a period of 19 days. Excretion values initially were 40 percent of the intake, then increased to 67.8 percent, to 71 percent and to a peak of 204.4 percent of the intake the last 4 days.

Until the last four or five days this pig had been gaining slowly but consistently, then during the latter period lost three pounds at which time he was autopsied. A comparison of these excretion values with those obtained with the control animals leads to the theory that the feces probably contributed the greatest part of the pantothenic acid found in the samples. Certainly the calculated values for the last four days would seem to indicate synthesis of pantothenic acid in the gut.

Blood studies revealed that during the last one to two weeks on trial the pigs in lots 2 and 3 that had been scouring consistently had abnormally high white cell counts ranging from 30,000 to 70,000 compared to 10,000 to 20,000 as a normal count. The high white cell counts were undoubtedly a result of the inflammation of the large intestine caused by a lack of pantothenic acid.

From the electrophoretic studies, one certain observation was that the gamma globulin portion of the sera of three to eight week old pigs was much lower than that reported by Deutsch and Goodloe (1945), Moore (1945) and Foster et al. (1950) for older pigs. These low values were in agreement with the work of Foster et al. (1951) in which determinations were conducted on blood plasma from pigs one day to fifty-six days of age as well as from new-born pigs.

The average value of the gamma globulin portion for the nine pigs tested, which included five positive controls, and four pantothenic acid deficient animals, was 8.2 percent of total serum protein.

There was no appreciable difference between the values of gamma globulin for the five control animals and the four pantothenic acid deficient pigs.

Careful study of all three trials showed that the 12.5 mg. of calcium pantothenate per kg. solids was the lowest level fed in the trials in which no positive signs of pantothenic acid deficiency occurred. It then appears to be the requirement for growth of the baby pig.

Pathology of Pantothenic Acid Deficiency in Baby Pigs

Gross Pathology

The characteristic gross pathology of pigs showing a pantothenic acid deficiency was confined to the intestinal tract and particularly to the colon and cecum. In the pigs receiving no pantothenic acid there was a reddening of the ileum. In the instances where the ileum was involved there was injection of the serosal vessels and of those in the mesentery. In the case of pigs receiving suboptimal amounts of pantothenic acid and exhibiting deficiency symptoms which lasted over a longer period of time before death or removal for autopsy, the inflammation of the cecal and colonic mucosa was even more marked. The gut walls also appeared somewhat thickened. One pig had a severe ulcerative gastritis in the fundic portion of the stomach.

Fatty infiltration of the liver and kidney was seen in a few instances but congestion of the kidney was often seen.

A few of the animals showed pneumonia which was limited to the apical lobes. Most of the animals showed a rough hair coat and a scaly skin.

It was noted in Experiment III, that the weights of the adrenals from three pantothenic acid deficient pigs averaging 26.4 pounds, was 1.97 grams. These three pigs had been scouring persistently for three to four weeks and near the end of the experiment white cell counts as high as 50,000 to 70,000 had been recorded. The average adrenal weight of two positive control pigs averaging 32.7 pounds was 1.39 grams.

Microscopic Pathology

In the histopathologic studies, lesions were present in the nervous system as well as in the digestive system.

In the case of pigs receiving no pantothenic acid, histopathologic study revealed a congestion of the mucosa of the cecum and colon in most cases and in many cases, this congestion also involved the mucosa of the jejunum and ileum. A very prominent feature of the deficiency was a marked decrease or a complete disappearance of the goblet cells in the cecum, colon and rectum. The gland cells were atrophied to a more or less degree and a slight increase in connective tissue in the submucosa was noted. With the animals receiving suboptimal amounts of pantothenic acid at a level low enough so that diarrhea occurred, but not a rapid death, there was a marked increase in connective tissue in the submucosa. The connective tissue appeared scar-like with the mucosa completely absent in some areas and only the wide band of compact connective tissue seen which in some instances was wider than the muscle layers. A serosal edema was noted. There was also a marked decrease to complete disappearance of the goblet cells, a congestion of the mucosal vessels and marked atrophy of the glands with sloughing in some areas.

Microscopically in the nervous system of pigs receiving no pantothenic acid and those receiving suboptimal amounts of pantothenic acid, demyelinization of fibers with clumping of myelin was noted in the sciatic nerve. Chromatolysis of the cell bodies of the dorsal root ganglion was also demonstrated.

None of the pigs in the lot receiving 12.5 mg. of calcium pantothenate per kg. solids or any excess of this amount showed any pantothenic acid deficiency symptoms nor did they show lesions on necropsy. Histopathological studies were also negative. All of the pigs which received 5 mg. of calcium pantothenate per kg. solids, eight of nine pigs tested which received 7.5 mg. of calcium pantothenate per kg. solids, and three out of eleven which received 10 mg. of calcium pantothenate per kg. solids upon autopsy and histopathological studies showed pantothenic acid deficiency lesions.

It was noted that the glomerular layer of the adrenal was decreased in thickness in almost all pantothenic acid deficient pigs and in the pigs that were treated the layer again returned to normal thickness. No previous research with pigs has reported adrenal change with a pantothenic acid deficiency.

An interesting finding in the examination of the central nervous system was noted, the significance of which is not entirely clear. In all pigs examined, in the normal as well as in the pantothenic acid deficient animals, hemorrhages were noted in the gray matter of the spinal cord at both the lumbar and thoracic levels. In a few instances the hemorrhages were also found in the medulla, brain stem,

cerebrum and cerebellum. Wintrobe (1940) also reported hemorrhages in the nervous system of control pigs as well as those pantothenic acid deficient. No symptoms indicative of central nervous system damage were seen.

Deficient animals that were treated with injections of pantothenic acid recovered in a clinical sense rather promptly as mentioned previously. Microscopically, myelin degeneration could still be found in the sciatic nerve even though the animal had been receiving pantothenic acid for some time. The mucosa of the colon and cecum returned to normal by gross inspection and no abnormality could be detected microscopically in the mucosa itself. In the submucosa, however, fibrous tissue was still present in large amounts, and it is doubtful if the fibrosed submucosa would ever again become really normal.

CONCLUSIONS

Due to the high tryptophan content of the regular casein milk, it was not possible to estimate a niacin requirement for the thrifty baby pig. The results of the isolated tests indicated that runt baby pigs apparently did require niacin in addition to the tryptophan of the regular casein milk.

The niacin deficiency symptoms noted were in fairly close agreement with those observed by Birch *et al.* (1937), Wintrobe *et al.* (1945), and Cartwright *et al.* (1948). The gross findings on necropsy were inflammation of the cecum and colon with certain areas showing ulcers or pseudomembranes. Microscopically there was a ballooning of the glands in the cecum and colon with the lumen being distended with mucus and leucocytes. The development of a microcytic anemia in test 4 differed from the observations of Wintrobe *et al.* (1945) and Cartwright *et al.* (1948) that a normocytic anemia developed in niacin deficiency.

The pantothenic acid requirement of the baby pig appears to be 12.5 mg. of calcium pantothenate per kg. solids. Thus, the baby pig requirement is slightly higher than the National Research Council's recommendation for fifty pound pigs of 4.5 mg. per pound of feed.

The baby pigs receiving the milk devoid of calcium pantothenate developed a severe diarrhea usually within two to four weeks. Locomotor incoordination developed in an additional seven to ten days if the pigs did not become so weakened that they died prior to this time.

Almost all of the baby pigs receiving 5 mg. or 7.5 mg. of calcium pantothenate per kg. solids developed a severe diarrhea within two and one-half to five and one-half weeks. Locomotor incoordination developed in most cases within four to seven weeks.

Three out of eleven baby pigs receiving 10 mg. of calcium pantothenate per kg. of solids developed a severe diarrhea within three to five weeks. In only one of these cases, locomotor incoordination developed within seven weeks.

The lesions present in the large intestine and nervous system of pantothenic acid deficient pigs are essentially those described by Wintrobe (1940, 1943), Luecke et al. (1949) and Sharma et al. (1952). Microscopically there was a marked decrease or complete disappearance of the goblet cells in the cecum, colon, and rectum. There was an increase in the connective tissue of the submucosa and this feature was even more marked in the case of pigs which scoured persistently for a long period of time. The pantothenic acid deficient pigs also showed loss of myelin, and degeneration of the dorsal root ganglion cells.

The observation that the glomerular layer of the adrenals of almost all pantothenic acid deficient pigs was decreased in thickness has not been reported in any previous research with pigs.

The length of time in which most of these lesions appeared is a considerably shorter period of time than is reported with older pigs.

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