Research Bulletin 21 October 1952

Some Vitamin Deficiencies

of the

Suckling Pig

W. P. Lehrer, Jr. and A. C. Wiese

Agricultural Experiment Station

of the

UNIVERSITY OF IDAHO

Moscow, Idaho

Some Vitamin Deficiencies of the Suckling Pig[1]

by

W. P. Lehrer, Jr., and A. C. Wiese[2]

The production of pork and pork products is an integral part of one of the major agricultural industries in the United States. The need to increase both volume and efficiency of livestock production has been accentuated in recent years by the increased demand for meat products.

One of the limiting factors in pork production is the high pig-mortality rate during the suckling period. Such losses constitute a problem which warranted the attention of the Livestock Advisory Committee (16) of the United States Department of Agriculture. The committee reported that an estimated 33 percent of the annual pig crop dies between the farrowing and weaning dates, with an additional 10 percent loss by the time marketing weight is attained. A part of these losses, the committee stated, is attributed to infectious diseases or parasitism aggravated, in all probability, by faulty nutrition.

Recognition of the magnitude of the pig-mortality problem resulted in a recommendation, by the Livestock Advisory Committee (16), for investigation relative to the causes of death in young pigs. It was specifically proposed that such research should include nutrition projects directed toward the artificial production of deficiency symptoms in both sow and litter in an effort to simulate the conditions found in stricken animals.

The project reported in this paper was designed to produce various specific vitamin deficiencies in suckling pigs by the use of a synthetic liquid diet formulated by Lehrer, Moore, Wiese, and Pahnish (14), at the Idaho Agricultural Experiment Station, and to observe the similarity between these deficiencies and those found in the field under normal management practices. It is hoped this information will aid materially in correcting similar deficiencies in the field and in this way reduce the high pig mortality rate to a minimum.

Purified synthetic diets have been previously employed by research workers who have used the pig as an experimental animal (3, 4, 7, 8, 9, 10, 11, 13, 18, 21, 25, 27, 28, 29, 30). The response of the animals has been varied, and few workers have conducted nutritional studies of the type reported here involving the suckling pig.

[1] From a thesis presented by the senior author to the Graduate Faculty, The State College of Washington, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Animal Science, June 3, 1951.

[2] Animal Husbandman and Agricultural Chemist, Idaho Agricultural Experiment Station, respectively.

EXPERIMENTAL PROCEDURE

The methods used in these studies were based upon the procedures of Lehrer, Wiese, Moore, and Pahnish (14). The pigs were confined in individual metal pens (Figures 1 and 2) that were 2.5 by 2.5 feet in size. These pens were equipped with wire bottoms (3/16 by 3/16 inch mesh), and underlying screens facilitated the collection of the solid feces, but allowed the urine to pass into containers provided for that purpose. The animals were housed in an experimental barn that had been thoroughly disinfected. A room temperature of approximately 75° F. was maintained by the use of an oil heater, and the "Jamesway" windows provided a ventilating mechanism.

The pens were scrubbed daily with warm water and then rinsed with a hypochlorite solution. If conditions warranted, the pens were washed more frequently.

The basal synthetic ration (Table 1) used in these studies was a modification of that described by Wiese, Johnson, Mitchell, and Nevens (26) for calf studies. These modifications are presented by Lehrer, Moore, Wiese, and Pahnish (14). The salt mixture (Table 1) was a modification of that used by Phillips and Hart (20).

The synthetic ration was prepared in the following manner: Sodium bicarbonate, in an amount equal to 4.75 percent of the weight of the vitamin-free casein*, was dissolved in distilled water, and then heated to a temperature of 60° C. The casein was then stirred into the solution at a high speed, with a "Lightnin" mixer. This method of dissolving the casein was a modification of the procedure of Bird, Sadler, and Iverson (2). When the casein was completely dissolved, the corn sugar** was added and this was followed by the salt mixture which had previously been dissolved in boiling water. The 95 percent alcoholic solutions of vitamins A, D, E, and K were poured into the melted lard. These components were then mixed into a volume of the casein-corn sugar-salt solution, with a "Waring Blendor" or the "Lightnin" mixer, and were then stirred into the total preparation. The synthetic milk thus prepared was homogenized at a pressure of 1500 to 2000 pounds, pasteurized, and stored in a refrigerator at 5° C. The water-soluble vitamins were dissolved in a 25 percent solution of ethyl alcohol, and an appropriate amount of this solution was fed in a small quantity of synthetic milk twice daily. The synthetic diet contained 13 percent solids and had a pH of 6.5 to 6.8.

The pigs used in these trials were allowed to remain with their dams for 48 hours following birth to assure the receipt of colostrum. They were then placed under experimental conditions. All pigs in each trial were selected from the same litter.

* Labco, manufactured by Borden's, Inc., New York city.
** Cerelose, manufactured by Corn Products Refining Co., New York city.

Table 1

COMPOSITION OF SYNTHETIC RATIONS[1]

Component	Percent
Vitamin-free Casein (Labco)	30.0
Corn Sugar (Cerelose)	37.4
Lard	26.6
Salt Mixture	6.0

Salt Mixture[2]	Grams[11]	Vitamins Fed[3, 4]	Mg. per kg. liquid milk
NaCl	356.0		
K_2HPO_4	773.0	Thiamine	0.65
$CaHPO_4$	1014.0	Riboflavin[5]	0.65
$MgSO_4$	106.0	Nicotinic Acid	2.60
$CaCO_3$	686.0	Inositol	26.00
$FeC_6H_5O_7$	58.0	Choline	260.00
$CuSO_4$	0.6	**para**-Aminobenzoic Acid	2.60
$MnSO_4$	3.0	Pteroylglutamic Acid	0.052
KI	1.7	Biotin[6]	0.01
$ZnCl_2$	0.5	Pyridoxine[7]	0.65
$CoCl_2$	0.2	Calcium Pantothenate[8]	1.30
CaF_2	1.0	Alpha-tocopherol	1.00
		2-methyl-1, 4-naphthoquinone	0.28
	3000.0	Vitamin A[9]	2,000 I.U./kg.
		Vitamin D_3 [10]	200 I.U./kg.

[1] The lard was homogenized with the solution of casein, corn sugar, and salts to produce an emulsion containing 13 percent solids and a pH of 6.5 to 6.8.
[2] Modified salt mixture of Phillips and Hart (20) with added manganese and cobalt.
[3] The water-soluble vitamins were made up in 25 percent ethyl alcohol.
[4] The alpha-tocopheral, 2-methyl-1, 4-naphthoquinone, vitamin A and vitamin D_3 were dissolved in 95 percent ethyl alcohol and mixed with the lard prior to homogenization.
[5] Riboflavin deficient animals received no riboflavin in ration.
[6] Biotin deficient animals received no biotin in ration.
[7] Pyridoxine deficient animals received no pyridoxine in ration.
[8] Pantothenic acid deficient animals received no calcium pantothenate in ration.
[9] Natural ester distilled from fish liver.
[10] Crystalline D_3.
[11] This amount will prepare 3 kilograms of mixture.

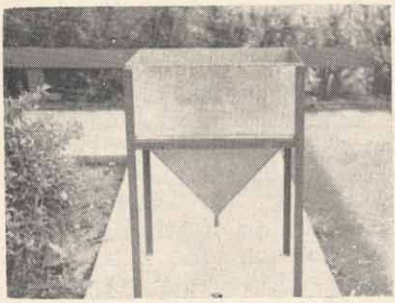

Figure 1. Type of metabolism pen used in these studies.

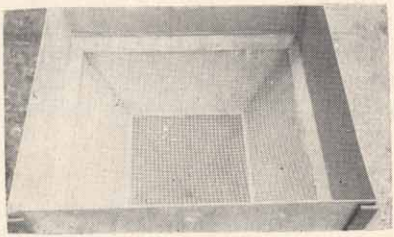

Figure 2. Inside view of metabolism pen showing screens used to separate urine and feces.

The suckling pig readily learns to drink from a trough, but some waste is encountered. Because of this, infants' nursing bottles were used throughout the experimental period (56 days) in order to keep accurate records of the volume of ration fed. The pigs were fed at 3-hour intervals for the first 11 days and then every 4 hours thereafter until the termination of the experiment. The synthetic milk was kept under refrigeration at 5° C. and was heated to 37° C. before feeding.

As a definite feeding schedule was followed, the animals were offered the synthetic milk at each feeding in a quantity that approached maximum consumption. Feed consumption was limited only when justified by evidence of digestive disturbances or lack of appetite. Although no feed consumption records were kept during the first 24 hours of the experimental period, such records were initiated at the end of that time when all pigs were accustomed to hand feeding.

General observations made during the feeding periods; veterinary consultations were arranged when necessary, and weight records kept.

Growth rates compared to the normal curve of Ittner and Hughes (12), plus indications of good health and well-being of the animals were the criteria used to determine the adequacy of the complete basal diet. Any digression from these standards was used to indicate the vitamin deficiency associated with the particular vitamin under study.

Urine samples were obtained from all pigs during the first 24 hours of the experiments. This was followed by periodic collections. Collections from some deficient animals were frequently contaminated due to scouring, and were discarded. This was especially true throughout the entire riboflavin studies. The urine samples were filtered; the volumes were recorded, and adequate portions (preserved with toluene) were refrigerated at a temperature of approximately 5° C. pending analysis for the particular vitamin under study.

The procedures used in the pantothenic acid, biotin, pyridoxine, and riboflavin analyses were based upon the methods of Skeggs and Wright (23), Schull, Hutchings, and Peterson (22), Atkins, Schultz, Williams, and Frey (1), and Snell and Strong (24), respectively.

The animals on the four vitamin deficiency studies reported in this paper received the same synthetic ration as the control animals with the exception that the vitamin under study was not included in the diet (Table 1). The vitamin being studied was later fed as a supplement.

Due to the great amount of work involved in these experiments, control animals in each vitamin study were kept to a minimum. It should be stated, however, that in every case control animals on the complete synthetic ration grew as well or better than the normal

growth of weanling pigs as reported by Ittner and Hughes (12), and were comparable to those animals raised on this diet and reported in the initial paper by Lehrer, Wiese, Moore, and Pahnish (14).

RESULTS AND DISCUSSION

The results and discussion of the four vitamin studies are presented separately.

Pantothenic Acid Deficiency

Three Poland China (Trial I) and four Duroc (Trial II) pigs, 48 hours old, were used as experimental animals.

General Observations

While living on the deficient ration, the pigs in both trials developed certain well defined symptoms attributable to a dietary deficiency of pantothenic acid (Tables 2 and 4). A disturbance in growth rate, anorexia, ataxia, diarrhea, dermatitis, a brownish eye exudate, occasional coughing, alopecia, and reduced urinary pantothenic acid excretion are the symptoms which fall within this group. These symptoms, with the exception of the eye exudate and dermatitis, were observed by Follis and Wintrobe (6) during their study of pantothenic acid deficiencies in pigs that ranged from 16 to 38 days of age when placed under observation. Maynard (17) listed eye exudate and dermatitis as characteristic symptoms of a pantothenic acid deficiency. Figure 3 shows the appearance of deficient pig 6, and Figure 4 compares deficient pig 5 with its control litter mate, pig 4.

The slow rate of growth common to pantothenic acid-deficient pigs is shown in Figure 5. The rate of gain for these animals was much lower than that for the control animals. The loss of weight exhibited by pig 5 was terminated when a pantothenic acid supplement was fed.

A lack of appetite was apparent in all deficient animals (Tables 3 and 5). Feed consumption was increased after the administration of pantothenic acid.

An ataxic condition, which Follis and Wintrobe (6) described as the initial indications of a neural involvement, was observed in these pigs when they had been on the deficient diet for a period of approximately 2 weeks. While the above workers described the peculiar lifting of the hind legs, "goose-stepping," as the first apparent change, pigs in these trials first exhibited a lack of coordination in their movements and several pigs tended to knuckle forward in the pasterns. All pigs eventually showed a tendency to "goose-stepping."

Diarrhea and rectal hemorrhage appeared when the pigs had been on the deficient diet for approximately 28 days and subsided following the oral administration of pantothenic acid.

The dermatitis observed in the deficient animals was of a general nature. It first appeared on the top of the neck and shoulders, and on the ears. Later the entire body was affected. The condition was most severe on the hind legs.

The brownish eye exudate observed during the course of this experiment was described by Maynard (17) as a characteristic symptom of a pantothenic acid deficiency, but the preceding lacrimation noted in these animals (Tables 2 and 4) was not mentioned. However, this lacrimation appears as a characteristic symptom since it occurred in all animals in both trials and was not observed in the controls.

Occasional seizures of coughing were noted in deficient animals. The controls did not exhibit such tendencies.

Alopecia was common to all deficient animals. It was most apparent along the median line of the back and over the rump. This condition, however, was general in nature and was accompanied by a dull, lifeless condition of the hair coat.

Other abnormalities were noted in the deficient animals (Tables 2 and 4) ; but due to a lack of sufficient evidence of their association with this vitamin deficiency, it seems inadvisable to include them in the same category with the symptoms previously discussed.

A possible impairment of the suckling reflex was observed in three of the pigs at various times. After short periods of bottle nursing, the tongues of both animals seemingly ceased to function properly. However, this condition appeared during periods of decreased appetite; and the removal of synthetic milk, when the impairment became evident, resulted in no indication that the appetite was not satisfied. Therefore, this apparent condition may

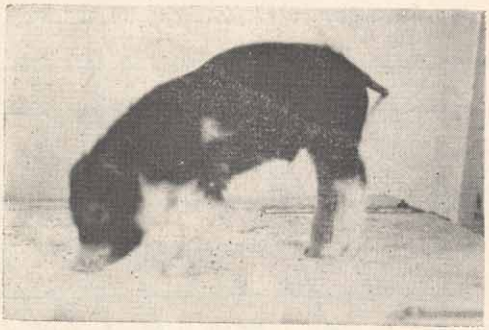

Figure 3. Pig 6 after being on pantothenic acid-deficient diet for 30 days.

have been only the result of an indifferent attitude toward the feed offered. On the other hand, these observations may be in accord with those of Follis and Wintrobe (6) who reported but did not describe changes in the tongue with pantothenic acid deficiencies.

At the time of autopsy, an increased heart rate was noted in pigs on Trial I. Since this condition has not been described previ-

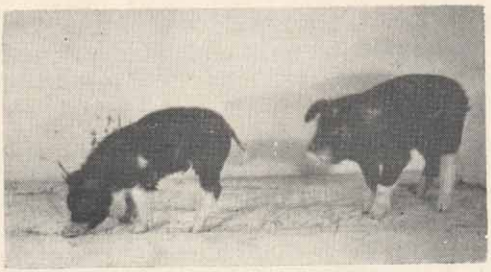

Figure 4. Comparison of pantothenic acid-deficient Pig 5 on left, and litter mate control Pig 4 on right.

ously as a characteristic symptom of a pantothenic acid deficiency in pigs, it would be inadvisable to consider it as such without further observations.

A slight enlargement of the heart and kidneys was noted in deficient pigs in both trials. As these organs apparently were normal in other respects, size may not be indicative of a pathological condition.

Ulcerative colitis was noted in varying degrees. These findings coincide with those of Follis and Wintrobe (6) who consistently found ulcerative colitis associated with pantothenic acid deficiencies.

The sciatic nerves in the deficient animals were of a smaller diameter than those taken from the controls. While these observations seem to be somewhat in accord with Follis and Wintrobe (6), who reported nerve degeneration in pigs fed a pantothenic acid-deficient diet, the smaller nerves normally would be expected by virture of the difference in the size of the animals at the time of autopsy. The lesions of the sheath of the sciatic nerves are more in line with the findings of Follis and Wintrobe (6).

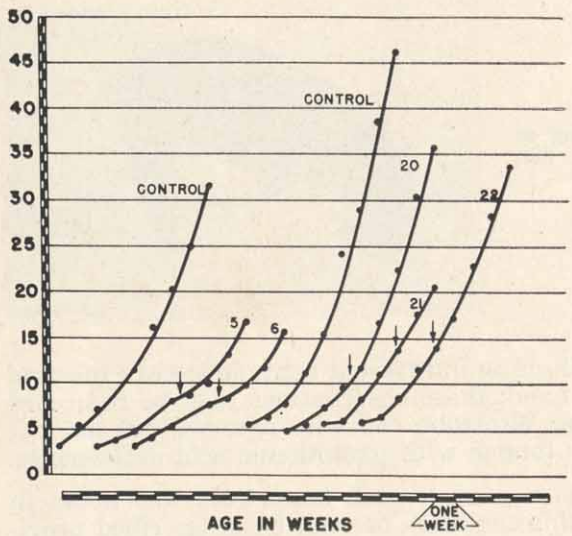

Figure 5. Growth of pigs on pantothenic acid-deficient diet. The arrows indicate when feeding of calcium pantothenate began.

The absence of subcutaneous and internal fat was characteristic of the deficient pigs, and the bones were easily fractured. Since vitamin D_3 and the salt mixture were incorporated in the synthetic milk, perhaps these abnormalities can be attributed to the low level of food intake.

Trial I.—The treatment of the pantothenic acid-deficient pigs 5 and 6 was initiated on the 33rd day when 1 mg. of calcium pantothenate was fed the deficient animals (Table 2). Since some of the deficiency symptoms did not respond to supplementation, it appeared evident that the quantity of pantothenic acid administered from the 33rd day through the 50th did not constitute a curative dose. As the weights of these pigs increased during this 18-day period of treatment, the quantity of pantothenic acid administered per pound of body weight varied.

When it became evident that the previously described dosage of calcium pantothenate was an inadequate one, 10 mg. doses—equivalent to 9.2 mg. of pure pantothenic acid—were given (Table 2) in an attempt to overcome the deficiency symptoms. This increase was begun on the 51st day. The appetite of both animals was improved somewhat following this increase, and the urinary excretion of the vitamin increased markedly in pig 5 within a period of 2 days (Table 6). However, the urinary pantothenic acid excretion of pig 6 increased only to a slight degree, and this animal developed an almost complete posterior paralysis on the 53rd day of the trial. This condition improved very little, if any, by the time that the observations were terminated.

Since the major portion of the daily intake of 10 mg. of calcium pantothenate was retained (Table 6), it appears that the tissues of pigs 5 and 6 were highly deficient in this vitamin at the time that this increased level was offered; but due to the short period of observation, the ultimate effect of this increase was not determined.

One gram of liver concentrate (Wilson's 1 to 20 Liver Powder)* was fed, as a crude vitamin carrier, to pig 6 from the 51st through the 54th day. The carrier was offered because of the severity of the condition exhibited by this animal. The general course followed by pig 6 during this period is described above; and here again, due to the short period of observation, the ultimate value of the liver concentrate was undetermined.

The fact that it was impossible to cure the deficiencies in Trial I completely may be due either to the low amount of calcium pantothenate fed as a supplement, or to the fact that the pigs had been so severely depleted that they were no longer able to respond to calcium pantothenate administration.

Trial II.—This experiment was undertaken to check more closely the deficiency symptoms observed in Trial I and to attempt to cure these symptoms with larger supplementations of calcium pantothenate.

* Manufactured by Wilson and Company, Chicago.

The supplementation of deficient pigs on this trial began on the 23rd day when 5 mg. of calcium pantothenate were fed to pig 20, followed by feedings of 500 μg and 200 μg. Pigs 21 and 22 were each fed 1 mg. of calcium pantothenate for one day. Since this supplementation gave only partial relief to the deficiency symptoms exhibited (Table 4), amounts of calcium pantothenate were increased on the 30th day. At this time pig 20 received 10 mg. per day for 12 days, and pigs 21 and 22 each 5 mg. per day for 3 days, respectively. The supplementation of pig 22 was later increased to 10 mg. and continued at this level for 8 days. Both pigs 20 and 22 were fed 20 mg. of calcium pantothenate on the 45th day of the experiment; this higher level was continued until the termination of the experiment on the 56th day at which time these animals appeared normal in every respect. Autopsy of pig 20 revealed no macroscopic or microscopic abnormalities except for stunted growth.

Pig 21 received lower levels of calcium pantothenate and for shorter periods of time. Following the 10 mg. feedings, as indicated above, this animal was fed 10 mg. of calcium pantothenate on the 35th day, 1 mg. on the 41st through the 45th day, and 25 mg. on the 46th day. When it became obvious that this animal was in severe pain and following the same deficiency course as those animals in Trial I on low, infrequent levels of calcium pantothenate, it was thought advisable to sacrifice the animal to determine whether internal macroscopic lesions existed. *Post mortem* studies revealed enlarged heart and kidneys, lack of calcification in the ribs and long bones of the legs, absence of subcutaneous fat and internal fat, and ulcerative colitis. Histologic examination revealed lesions of the sheath of the sciatic nerves. (Table 4).

Urinary excretion studies of pigs 20 and 22 indicate conclusively that calcium pantothenate retention had reached a high level and at the close of the experiment overflow was being excreted (Table 6).

Biotin Deficiency

Three Chester White (Trial I) and four Duroc (Trial II) suckling pigs which were permitted to receive colostrum for 48 hours were used as experimental animals.

General Observations

Contrary to the reports of McRoberts and Hogan (18), and Lindley and Cunha (15) pigs on a biotin-deficient synthetic diet developed certain deficiency symptoms which were attributed to a lack of this vitamin (Tables 7 and 9). The symptoms associated with this deficiency were: dermatitis, alopecia, skin ulceration, brown eye exudate, diarrhea, transverse cracking and bleeding of feet, and inflammation of mucous membrane of the mouth. There was not, however, a reduction in urine biotin excretion (Table 11), nor a retardation of growth (Figure 6). Appetites of deficient

animals were normal and comparable to litter mate controls (Tables 8 and 10).

Cunha, Lindley, and Ensminger (5) in their studies with dessicated egg white, reported somewhat similar deficiency symptoms with pigs 12 to 22 weeks of age. These workers, however, did not report skin ulceration and inflamed mucous membranes as characteristic symptoms. Figure 7 clearly illustrates the syndrome associated with a biotin deficiency of the suckling pig fed a purified diet, and Figure 8 its normal litter mate receiving 0.01 mg. of biotin per kg. of liquid diet.

The deficiency symptoms, in the studies reported here, followed a uniform course; dermatitis first appeared on the ear, neck, top of shoulder, and tail, eventually spreading over the animal's entire body. The dermatitis was followed by general alopecia and finally ulceration of the hams and belly.

Transverse cracking and subsequent bleeding of the soles and top of the hoof head gradually became apparent. This abnormal condition increased in severity as the experiments progressed until the animals could walk only with difficulty and pain.

Inflammation of the mucous membranes of the mouth, while very pronounced, was not associated with loss of appetite or difficulty in sucking and thus apparently was not of a painful nature.

Dark brown eye exudate and diarrhea, generally associated with most water-soluable vitamin deficiencies, occurred at an early date. The diarrhea was easily alleviated by the feeding of sulfathalidine. (Table 7).

Deficient animals in both trials weighed approximately the same as controls (Figure 6) and *post mortem* studies revealed no macroscopic abnormalities (Tables 7 and 9).

The control animals did not develop any of the above symptoms and were normal in every respect.

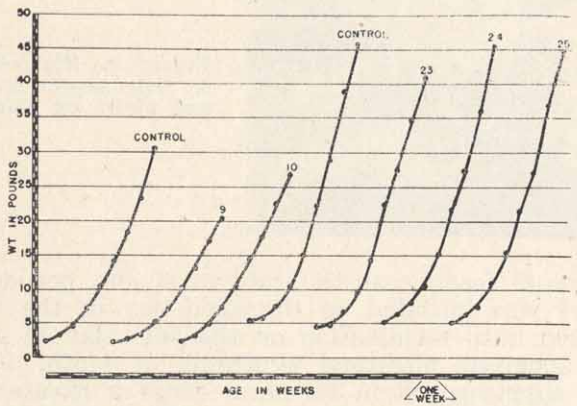

Figure 6. Growth of pigs on biotin-deficient diet.

Trial I.—Table 7 presents in order of appearance the symptoms observed in this trial associated with a lack of a dietary intake of biotin. Deficiency symptoms were more severe in pigs 9 and 10 in Trial I than in pigs 23, 24, and 25 in Trial II.

The urinary biotin excretion of the control pig showed the expected increase with increased intake (Table 11). The deficient pigs, on the other hand, failed to show the expected decrease of biotin excretion and continued to excrete the vitamin although at a reduced rate. Since the symptoms of biotin deficiency were apparent at this time (29 days), sulfathalidine was added to the diet at the rate of 0.5 gm. per feeding (3 gm. per day) in an attempt to decrease possible intestinal synthesis; supplementation was continued for 20 days. It is apparent from Table 11 that the animals continued to excrete biotin in spite of this intake of sulfathalidine.

From the 46th day and through the final day of the experiment (56th day), 10 μg of biotin was fed daily to each deficient animal. Neither pig showed any great response to this supplementation nor was there any increase in biotin excretion in the urine during this period. Further investigation showed that the urine contained an unknown ether soluble material which stimulated the growth of the microorganism used in the microbiological assay for biotin. Consequently, the urine samples obtained from the pigs in Trial II were extracted with ether before being assayed for biotin. The results obtained in Trial II for the urinary excretion of biotin give a truer indication of biotin excretion under the conditions of this experiment.

Trial II.—Pigs on this trial followed the same general patterns as pigs on Trial I in developing biotin-deficient symptoms, although the symptoms were not as severe. (Table 9).

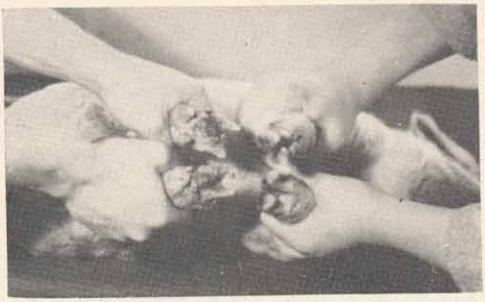

Figure 7. Biotin-deficient Pig 9. Note severe cracks on feet and ulcers on hams.

Sulfathalidine feeding at the rate of 6 gm. per day (1 gm. per feeding) was initiated on the 42nd day of the experiment and continued until termination on the 56th day in an attempt to inhibit apparent intestinal synthesis of biotin (Table 11). Again this supplementation failed to cause a greater reduction in biotin excretion or any change in deficiency symptoms. This

Figure 8. Litter mate control Pig 8 received adequate amounts of biotin.

may have been due to the fact that sulfathalidine did not inhibit the organisms responsible for intestinal synthesis of biotin.

The fact that the biotin-deficient animals in both trials grew at a rate comparable to their litter mate controls, would indicate that the pigs were obtaining some biotin from either intestinal or tissue synthesis. The amount of biotin the pigs obtained from this source while sufficient for growth was not, however, enough to prevent the physical symptoms observed.

The supplementation of biotin in Trial I did not alleviate the deficiency symptoms.

PYRIDOXINE DEFICIENCY

The studies of this vitamin consisted of two trials. Three Chester White pigs were used in Trial I and four Duroc-Poland China crossbred pigs in Trial II. In both trials the litter mate baby pigs received colostrum and were then placed on the experiments at two days of age.

General Observations

After the animals had been on a pyridoxine-deficient diet for approximately two weeks, various symptoms associated with a lack of this vitamin became apparent (Tables 12 and 14). By the fourth week of the experiments, deficiency symptoms had become most severe. Those observed in these experiments and

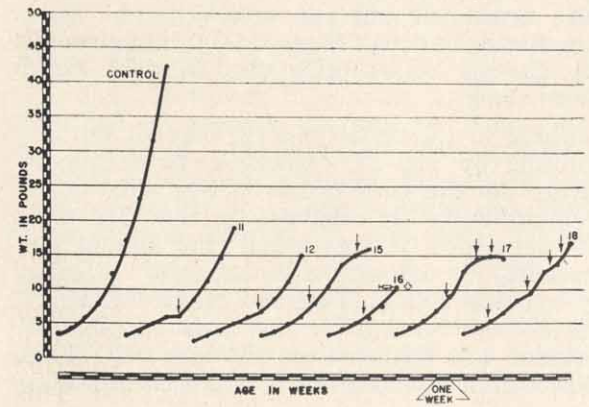

Figure 9. Growth of pigs on pyrdoxine-deficient diet. The arrows and syringe indicate supplementation of pyridoxine. The cross shows when Pig 16 died.

attributed to a pyridoxine deficiency were: poor growth, incoordination of muscles, spastic gait, epileptiform fits, comas, rough hair coats, brown exudate around the eyes, and impairment of eyesight.

Trial I.—During the first 17 days on experiment, pig 11 showed only subnormal appetite (Table 13) and resultant slow growth (Figure 9). On the 17th day the pig vomited, exhibited trembling, showed incoordination of the legs and a tendency to run backwards (Table 12). These symptoms gradually became more pronounced until the 21st day when the animal had a mild epileptiform seizure. The seizure was quite typical. The pig trembled, was unable to stand, and rolled over on its side or back with legs thrashing wildly. Although weak, the animal was able to stand normally within three to five minutes after a fit. As the experiment progressed, these seizures occurred more frequently and became more intense. Excitement seemed often to have been a precipitating factor in bringing on a convulsion. During this period the animal showed poor growth and lack of appetite, developed a rough hair coat, was unthrifty in appearance, very inactive, and had a dark brown exudate around the eyes. On the 23rd day, the epileptiform seizures became more numerous and severe and the animal became comatose. These findings were contrary to those of Wintrobe and co-workers (30), who reported their animals failed to pass into a coma. Ten mg. of pyridoxine were fed on the 28th day, followed by a daily supplementation of 5 mg. for the next 11 days. On the 28th day blood samples indicated this animal had microcytic hypochromic anemia.

The pig showed an immediate response to pyridoxine therapy; the appetite improved (Table 13) and growth increased (Figure 9); epileptiform seizures gradually became less frequent and severe. Six days after pyridoxine supplementation was begun, the seizures stopped and the deficiency symptoms disappeared. The pyridoxine supplement was removed from the ration on the 39th day. The animal did not develop deficiency symptoms again up to the time the experiment was terminated on the 56th day. However, at the end of the trial and removal of the pig from its experimental pen, it was noted the pig ran into objects. Upon close examination, it was discovered that there was an impairment of eyesight. Apparently the pig was totally blind since it could not distinguish light from dark.

The other deficient animal in this trial, pig 12, had all the deficiency symptoms exhibited by pig 11; however, these abnormalities were not as severe nor did they occur on the same days, (Table 12). The first epileptic-like fit occurred on the 23rd day. As deficiency symptoms developed, it was noted this animal ate with difficulty. The inability to eat normally appeared to have been due to a loss of the sucking reflex. This and other deficiency symptoms were immediately corrected after the daily supplementation of 5 mg. of pyridoxine was initiated on the 34th day. This supplementation was discontinued on the 45th day. The animal,

similar to pig 11, did not develop any new deficiency abnormalities up to the time the experiment was terminated. However, like pig 11, it exhibited impaired eyesight, but was not totally blind since it could distinguish light from dark.

Autopsy of pigs 11 and 12 revealed no macroscopic abnormalities. Histologic studies revealed lesions of the sheath of the optic nerves.

Trial II.—After the completion of Trial I, further work was thought advisable and Trial II was undertaken. Crossbred Duroc-Poland China pigs, used in Trial II, showed pyridoxine deficiency symptoms similar to those exhibited by the two Chester White baby pigs in Trial I.

Pig 15 exhibited the characteristic slow growth (Figure 9) and poor appetite (Table 15) during the first stage of the trial. On the 16th day this animal started to vomit, tremble, and seemed to be very weak. It had an unthrifty appearance and a rough hair coat, showed excessive lacrimation and a dark brown exudate around the eyes. These deficiency symptoms were similar to those exhibited by pigs in the first trial (Tables 12 and 14). Starting on the 22nd day and continuing through the 28th day (until the pyridoxine supplement was fed) this pig had an average of two observed epileptiform seizures and comas per day. The day pyridoxine supplementation was begun (28th day), the animal had six severe epileptiform seizures and became comatose. Upon close examination it was established that the pig was completely blind. Immediate improvement of most deficiency symptoms (Table 14) were noted after feeding 5 mg. of pyridoxine. By the 30th day the epileptiform seizures and comas ceased completely. As the experiment progressed, all deficiency symptoms disappeared with the exception of impaired eyesight. From the 50th day until the termination of the experiment on the 56th, daily feedings of 40 μg of pyridoxine were given (Table 16). Throughout this period, careful eye examinations were made, but no improvement in eyesight was observed.

Pig 16 showed loss of appetite on the 12th day and was given 5 mg. of pyridoxine on the 17th day. Although this pig exhibited impaired eyesight on the 26th day and started to vomit on the 35th day, it exhibited no epileptiform seizures until the 37th day. However, it had the other characteristic symptoms as described for pigs 11, 12, and 15. Following this seizure, the pig was moribund. Although an intramuscular injection of 1 mg. of pyridoxine was administered, the pig died a few hours later. Necropsy revealed an enlarged heart and kidneys, lack of subcutaneous fat and internal fat, and slight ulceration of the intestines.

Similar pyridoxine deficiency symptoms were observed in pig 17. This animal exhibited epileptiform seizures from the 22nd through the 25th day. Close examination revealed impaired eyesight; like pig 16, this animal was partially blind. Pyridoxine supplementation was started on the 26th day and continued

periodically until the termination of the experiment (Table 14). Complete absence of seizures, rough hair coat, poor appetite, brown eye exudate and weakness was noted after supplementation. However, the eyesight impairment was not corrected.

Loss of appetite was observed by pig 18 on the 12th day of the experiment. Five mg. of pyridoxine were administered on the 15th day and then discontinued. As the experiment progressed, typical deficiency symptoms were observed. Vomiting started on the 35th day and on the following day 200 μg of pyridoxine were fed, epileptiform seizures were observed frequently thereafter until the end of the trial (Table 14) even though 10 μg of pyridoxine were fed daily beginning on the 50th day. Impaired eyesight was not improved by supplementation.

Wintrobe and associates (31) reported that prolonged feeding of a diet deficient in pyridoxine is associated with the development of fatty infiltration of the liver. The studies reported here did not substantiate these findings. These workers also noted temporary blindness in pyridoxine-deficient pigs rather than the apparent permanent partial and complete blindness found in these studies.

Urinary excretion of pyridoxine and pyridoxine dietary supplementation are presented in Table 16. In both trials the urinary excretion of pyridoxine fell to a low level during the period of severest deficiency symptoms. Upon adequate supplementation, symptoms were alleviated and urinary excretion of pyridoxine increased.

Riboflavin Deficiency

These studies were divided into two trials. Trial I consisted of four Duroc, and Trial II of three Duroc, 48 hour old litter mate baby pigs.

General Observation

Signs of nutritional deficiency became apparent within the first one to two weeks of these experiments. The animals failed to gain in weight (Figure 10), lost their appetite (Tables 18 and 20), and normal appearance (Tables 17 and 19). Their hair coats and skin which were at first clean, smooth, and sleek, became thin, coarse, and unthrifty in appearance. The skin became dry and scaly and hair fell out readily. As the trials progressed, diarrhea and scours, prevalent in all deficient animals became very severe and in some cases ultimately resulted in rectal hemorrhaging and inflammation of the anus. Animals also exhibited an apparent eye sensitivity to light. All the above symptoms, with the exception of rectal hemorrhaging and inflammation of anal mucosa, were reported by Wintrobe, Buschke, Follis, and Humphreys (32). These workers reported lens opacities after pigs had been on a riboflavin-deficient diet for 96 days. This eye abnormality may have been similar

to the condition noted in animals reported in these trials.

An abnormal gait was noted after animals had been on the experimental diet for four weeks. The gaits of the pigs appeared stiff, mincing and hesitant; at times they walked on the tips of the hoofs. These findings coincide with the reports of Wintrobe, Buschke, Follis, and Humphreys (32), who started pigs on a riboflavin-deficient diet at 21 days of age.

The slow rate of growth (Figure 10), and low dietary intake (Tables 18 and 20) are consistent with the findings of Mitchell, Johnson, Hamilton, and Haines (19).

Urinary excretion studies with riboflavin-deficient animals was impossible because of continual scouring and subsequent contamination of samples.

Figure 11 shows poor growth, unthrifty appearance and rough thin hair of pig 37 after being on the riboflavin-deficient diet for 35 days. A comparison of pig 38 and its normal control litter mate receiving adequate amounts of riboflavin (0.65 mg. per kg. liquid diet) is presented in Figure 12.

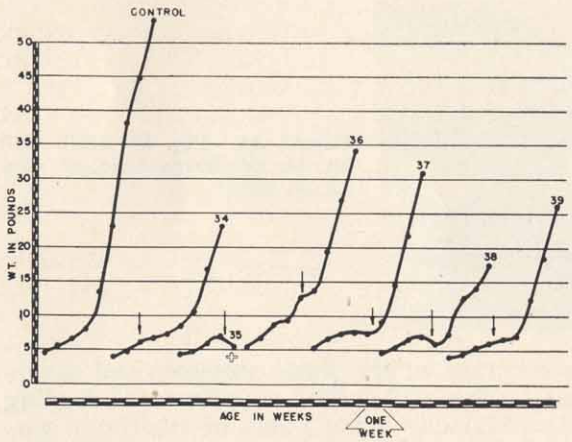

Figure 10. Growth of pigs on riboflavin-deficient diet. The arrows indicate when supplementation of riboflavin was started. The cross shows when Pig 35 died.

Trial I.—The treatment of riboflavin-deficient pig 35 began on the 27th day. At the time of supplemental feeding, this pig was extremely weak and refused to eat, thus force feeding had to be practiced. This pig was fed 500 μg of riboflavin daily up to the time it died on the 30th day.

The remaining two deficient pigs, 34 and 36, were fed 500 μg of riboflavin beginning on the 31st and 33rd days, respectively. This supplementation was continued for 6 days. Following supplementation, appetites of both animals improved; scouring ceased and hair coats became more lustrous. A few days after discontinuing supplementations (46th day), however, both pigs exhibited recurrence of deficiency symptoms (Table 17). On the 50th day, 500 μg of riboflavin were fed;

this amount was added daily to their dietary intake of liquid diet until termination of the trial on the 56th day.

Macroscopic examination of these animals at the end of the trial revealed small size, scaly skins, and rough sparse hair coats. Necropsy showed necrosis and sloughing of the corium with hemorrhage in the proliferative germinal layers of the skin. The liver and kidneys showed blotchy hemorrhagic areas on the surface which microscopically showed subcapsular inflammation. The liver showed leucocytic infiltration and many polymorphonuclear leucocytes in the liver blood. The kidneys exhibited a subacute glomerulonephritis and cloudy swelling with destruction of the tubular epithelium. The lungs showed pneumonia and microscopically there was leucocytic and erythrocytic infiltration, with many polymorphonuclear leucocytes present.

Trial II.—When it became evident that the dosage of riboflavin described above was inadequate, this trial was undertaken to recheck the riboflavin-deficiency symptoms observed in Trial I, and to attempt to more completely alleviate the symptoms with larger supplementations of riboflavin.

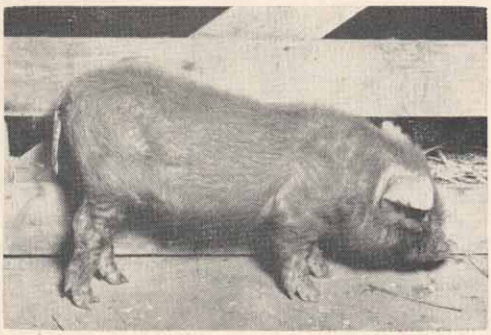

Figure 11. Pig 37 after being on riboflavin-deficient diet for 35 days.

Deficiency symptoms occurred in the same sequence and severity as did those in Trial I (Table 19). The supplemental feeding of riboflavin began on the 31st day when 1 mg. of riboflavin was fed to all deficient animals; supplementation at this amount was continued for the following 8 days. This amount of riboflavin gave immediate relief to all deficiency symptoms. However, immediately upon removal of riboflavin from the diet all animals again

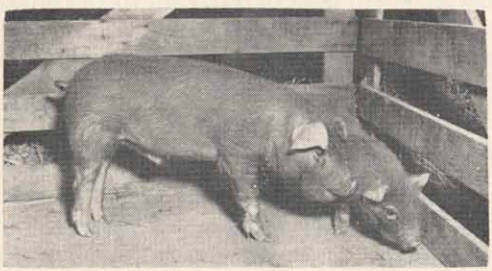

Figure 12. Comparison of control pig 33 on left, and litter mate riboflavin-deficient pig 38 on right.

began scouring and vomiting. On the 48th day to alleviate these symptoms, and throughout the duration of this trial, pigs 37 and 39 were fed 1.5 mg. and pig 38 was fed 1 mg. per day of riboflavin, respectively.

At the termination of this trial, all pigs appeared to be normal in every respect except for their small size. Table 20 shows animals had improved appetites and Table 19 indicates all observed deficiency symptoms were no longer visible. **Post mortem** examination, however, revealed many abnormalities. The liver and kidneys showed blotchy and subcapsular macroscopic hemorrhages. Microscopically, the liver showed a granulocytic infiltration of the interlobular spaces with intercolumnar congestion in the subcapsular region. There were many polymorphonuclear leucocytes in the blood. The kidneys showed an interstitial congestion with many polymorphs in the blood. The lungs had a finger-sized solidified area of pneumonia in the lower portion. Microscopically, they showed granulocytic and erythrocytic infiltration with many polymorphs present.

Hughes (9), using 80 pound pigs, indicated the minimum riboflavin requirement for the growing pig was 1.0 to 3.0 mg. per 100 pounds body weight. From the studies reported above, the suckling pig apparently has a requirement nearer the maximum amount reported by Hughes (9) than the minimum.

SUMMARY

Pigs which were allowed to nurse their mothers for 48 hours have been successfully raised on a synthetic milk diet through the suckling period. The growth of these pigs, used as controls in vitamin-deficiency studies, over an 8-week experimental period was normal and no deficiency symptoms were observed. In view of the above findings it is assumed that the basal diet presented is adequate for growing baby pigs.

Vitamin-deficient diets for the studies reported herein were obtained by leaving the vitamin being studied out of the synthetic milk ration. The vitamin under study was later fed as a supplement.

Pantothenic acid deficiency in the young pig is characterized by poor growth, anorexia, diarrhea, lacrimation, dermatitis, coughing, loss of the sucking reflex, a dark brown exudate around the eyes, spastic gait, "goose-stepping", alopecia, low urinary excretion of pantothenic acid, ulcerative colitis, and damage to the sheath of the sciatic nerves. All these symptoms of pantothenic acid deficiency have been previously reported by other workers with the exception of excessive lacrimation, loss of sucking reflex, coughing, and low urinary pantothenic acid excretion.

The daily feeding of 1 mg. of calcium pantothenate resulted in an improvement of appetite and growth and cessation of diarrhea. There was no great improvement of the other symptoms.

However, daily supplementation of 10 to 20 mg. of calcium pantothenate resulted in complete recovery from symptoms and great improvement of appetite and growth.

In the suckling pig biotin deficiency is apparently associated with ulceration of the skin, transverse cracking of the soles and top of the hoof, inflammation of the mucous membranes of the mouth, dematitis, and alopecia. Other workers have reported these deficiency symptoms with the exception of skin ulceration and inflammation of mouth mucosa. The urinary excretion of biotin failed to show the expected decrease and was not materially affected by the feeding of sulfathalidine. This may indicate that sulfathalidine does not inhibit the organisms responsible for intestinal synthesis of biotin in the suckling pig.

This study indicates the suckling pig apparently obtains biotin from intestinal or tissue synthesis, but not enough to meet its over-all requirements.

In these studies pyridoxine deficiency in baby pigs was characterized by poor appetites, incoordination of the muscles, spastic gait, poor growth, epileptiform fits, comas, anemia, rough hair coats, brown exudate around the eyes, impairment of eyesight, low urinary excretion of pyridoxine, and internal abnormalities. With the exception of impairment of eyesight, comas, and low urinary pyridoxine excretion, these symptoms have been previously reported.

The adequate supplementation of pyridoxine cured all symptoms with the exception of the impairment of eyesight and damage to the optic nerves.

Riboflavin deficiency of baby pigs is associated with alopecia, anorexia, poor growth, rough hair coat, dermatitis, scours, ulcerative colitis, inflammation of anal mucosa, vomiting, light sensitivity, unsteady gait, and many abnormal internal complications. These studies coincide with other workers with the exception that rectal hemorrhaging and inflammed rectal mucosa have not been reported before.

The continual supplementation with riboflavin at a 1 to 1.5 mg. daily intake cured all macroscopic external abnormalities associated with a deficiency of this vitamin, but at these levels failed to alleviate internal disorders.

These studies further indicate the need of calcium pantothenate, biotin, pyridoxine, and ribloflavin in the daily ration of the growing suckling pig.

TABLE 2

RESULTS OF PANTOTHENIC ACID DEFICIENCY, SYMPTOMS, TREATMENTS, AND RESPONSE

TRIAL I

Days on Diet	Pigs 5 and 6		
	Symptoms Observed	**Treatments**	**Treatment Response**
9	Both pigs exhibited labored breathing, and excessive lacrimation.		
10	Pigs showed anorexia, and occasional coughing.		
11	Pig 5 had dermatitis on neck and shoulders; rear pasterns knuckled forward, and slight incoordination of the hind legs.		
12	Dermatitis began spreading over body on Pig 5.		
14	Pig 6 exhibited dermatitis, alopecia and incoordination.		
20	Pigs 5 and 6 had pronounced dermatitis on hind legs, head, neck, shoulders, and back. Hair dull in appearance. Exhibited marked incoordination of hind legs.		
22	Pig 6 had alopecia; Pig 5 "goose-stepped".		
25	Brown eye exudate replaced previous lacrimation of both pigs.		
28	Pigs had diarrhea.		
29	Pig 6 exhibited impaired sucking reflexes.		
30	Pig 5 exhibited impaired sucking reflexes.		
31	Pig 6 "goose-stepped".		

(Continued on next page)

TABLE 2 (continued)
RESULTS OF PANTOTHENIC ACID DEFICIENCY, SYMPTOMS, TREATMENTS, AND RESPONSE
TRIAL I

Days on Diet	Pigs 5 and 6		
	Symptoms Observed	Treatments	Treatment Response
32	Final day on deficient diet. All pigs had anorexia, impairment of sucking reflexes, occasional coughing, a brownish eye exudate, general alopecia, a dull lifeless hair coat, general dermatitis, diarrhea, incoordination of hind legs, rear pasterns knuckled forward, loss of weight, low urinary excretion of pantothenic acid.		
33		Daily supplements of 1 mg. calcium pantothenate fed. Continued for 18 days.	
35			The pigs showed increase in activity and appetite, cessation of diarrhea, restoration of sucking reflexes, increase in rate of gain, slight increase in pantothenic acid excretion.
51		Daily fed supplement of calcium pantothenate increased to 10 mg. Continued until end of experiment. Pig 6 fed 1 gram Wilson's 1 to 20 liver powder for a period of 4 days.	Moderate increase in appetite observed in both animals.
53	Pig 6 had posterior paralysis.		
56	Both animals had an increase in heart rate, excessive salivary secretion. Autopsy performed on both animals. Revealed absence of subcutaneous and internal fat, lack of calcification in ribs and long bones of the legs, lack of color in the bone marrow, and small diameter of the sciatic nerves. Evidence of mild ulcerative colitis.		

TABLE 3

RESULTS OF PANTOTHENIC ACID DEFICIENCY, RELATIONSHIP OF DIETARY INTAKE TO GROWTH

TRIAL I

Days on Diet	Control			Deficient					
	Pig 4			Pig 5			Pig 6		
	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)
7	3.908	1.75	2.23	2.801	0.75	3.73	2.969	1.00	2.97
14	5.683	1.75	3.25	3.351	1.00	3.35	4.387	1.50	2.92
21	7.010	2.50	2.80	5.510	2.25	2.45	5.877	2.00	2.94
28	8.390	1.75	4.79	6.310	0.75	8.41	4.988	0.25	19.95
35	12.727	4.75	2.68	4.941	0.75	6.58	4.205	0.50	8.41
42	16.236	4.25	3.82	8.583	1.25	6.87	8.067	1.50	5.38
49	18.914	4.50	4.20	9.852	3.25	3.03	8.264	2.00	4.13
56	23.560	6.75	3.49	11.762	3.50	3.36	10.542	3.50	3.01

TABLE 4

RESULTS OF PANTOTHENIC ACID DEFICIENCY, SYMPTOMS, TREATMENTS, AND RESPONSE

TRIAL II

Days on Diet	Pigs 20, 21, and 22		
	Symptoms Observed	Treatments	Treatment Response
10	All pigs had anorexia and alopecia.		
13	All pigs exhibited dermatitis on ears, neck, and tail, and excessive lacrimation.		
15	Pig 20 exhibited bowed rear legs.		
17	All pigs had severe dermatitis over entire body, and incoordination of hind legs. Pig 20 had most severe symptoms.		
21	All pigs were coughing and scouring.		
23	Pig 21 exhibited weakness in rear legs. Pig 20 "goose-stepped".	Pig 20 fed 5 mg. calcium pantothenate.	
24	Pigs had brown eye exudate. Pig 21 walked with difficulty and "goose-stepped".		Pig 20 exhibited more activity and increased appetite.
25	Pig 20 began hemorrhaging rectally. Pig 21 and Pig 22 had slight paralysis of hind quarters.		
26	All pigs exhibited impairment of sucking reflexes.		
27	Pig 22 "goose-stepped".	Pig 20 fed 500 μg calcium pantothenate.	
28		Pig 20 was fed 200 μg calcium pantothenate.	
29		Pigs 21 and 22 each fed 1 mg. calcium pantothenate.	
30		Supplementation of calcium pantothenate began as follows: Pig 20, 10 mg. for 12 days Pig 21, 5 mg. for 3 days Pig 22, 5 mg. for 3 days.	Pig 20 ceased hemorrhaging. Pigs 21 and 22 no longer showed paralysis or incoordination. Dermatitis on all animals clearing up.

(Continued on next page)

TABLE 4 (continued)

RESULTS OF PANTOTHENIC ACID DEFICIENCY, SYMPTOMS, TREATMENTS, AND RESPONSE

TRIAL II

Days on Diet	Pigs 20, 21, and 22		
	Symptoms Observed	Treatments	Treatment Response
31	Pig 21 began severe rectal hemorrhaging. This condition continued until the animal was sacrificed on the 47th day of experiment.		
35		Pigs 21 and 22 fed 10 mg. calcium pantothenate.	Pig 22 stopped hemorrhaging.
36		Pig 22 fed 10 mg. calcium pantothenate for 7 days.	
41	Pig 21 exhibited nervousness.		
42		Pig 21 fed 1 mg. calcium pantothenate for 4 days.	
45		Pigs 20 and 22 each fed 20 mg. calcium pantothenate for the remainder of the experiment.	
46	Pig 21 exhibited severe trembling, incoordination of muscles, and could not stand. Lost ability to control its reflexes.	Pig 21 fed 25 mg. calcium pantothenate.	
47	Sacrificed Pig 21. Autopsy revealed enlarged heart and kidneys. Lack of calcification in the ribs and long bones of the legs. Absence of subcutaneous fat and internal fat. Light color in bone marrow. Histologic studies revealed lesions of the sheath of the sciatic nerves. Severe ulcerative colitis.		
48			Pigs 20 and 22 appeared to be normal and were very active.
56	Autopsy of Pig 20 revealed no macroscopic abnormalities with the exception of small size for age. Histologic studies of the sciatic nerves revealed no abnormalities.		Final day of experiment. Pigs 20 and 22 normal and active. Showed no apparent deficiency symptoms.

TABLE 5

RESULTS OF PANTOTHENIC ACID DEFICIENCY,
RELATIONSHIP OF DIETARY INTAKE TO GROWTH

TRIAL II

Days on Diet	Control						Deficient					
	Pig 19			Pig 20			Pig 21			Pig 22		
	Period Intake (liters)	Period Gain (lb.)	Intake per lb. Gain (liters)	Period Intake (liters)	Period Gain (lb.)	Intake per lb. Gain (liters)	Intake (liters)	Period Gain (lb.)	Intake per lb. Gain (liters)	Period Intake (liters)	Period Gain (lb.)	Intake per lb. Gain (liters)
7	3.640	0.50	7.28	3.630	0.50	7.26	3.590	0.50	7.18	3.640	0.50	7.28
14	7.380	1.75	4.22	7.460	2.00	3.73	7.410	2.25	3.29	7.360	2.25	3.27
21	10.815	3.00	3.61	8.285	2.25	3.68	10.960	3.00	3.65	10.960	3.00	3.65
28	16.400	4.50	3.64	9.090	2.00	4.55	10.945	2.50	4.38	11.230	2.50	4.49
35	26.225	9.00	2.91	18.340	4.75	3.86	13.215	3.25	4.07	14.995	3.00	4.99
42	34.650	4.50	7.70	25.930	5.50	4.71	19.410	3.50	5.55	22.230	6.00	3.71
49	39.900	10.00	3.99	30.160	8.50	3.55				24.030	5.50	4.37
56	42.000	7.00	6.00	34.780	5.25	6.62				35.210	5.50	6.40

TABLE 6

URINARY EXCRETION OF PANTOTHENIC ACID*

Days on Diet	TRIAL I						TRIAL II							
	Control		Deficient				Control		Deficient					
	Pig 4		Pig 5		Pig 6		Pig 19		Pig 20		Pig 21		Pig 22	
	Intake μg/day	Excre. μg/day	Intake μg/day	Excre. μg/day	Intake μg/day	Excre. μg/day	Intake μg/day	Excre. μg/day	Intake μg/day	Excre. μg/day	Intake μg/day	Excre. μg/day	Intake μg/day	Excre. μg/day
2	410	104	0	49	0	92	390	585	0	423	0	296	0	432
3	630	59	0		0	42								
7	770	46	0	80	0	44	700	410	0	90	0	138	0	159
12	1100	58	0	11	0	14								
15							1560	293	0	23	0	50	0	27
21							2030	106	0	40	0	16	0	22
27							3120	90	500	53	0	75	0	151
28							3120	169	200	56	1000	62	1000	43
30							3900	127	10000	110	5000	91	5000	136
33			1000	3	1000	4								
34			1000	33	1000	10								
35			1000	12	1000	19	5460	432	10000	987	10000	148	10000	4
36	2510	420	1000	8	1000	41								
42							6630	277	10000	1356			10000	689
48	2990	524	1000	65	1000	12			20000	2493			20000	3216
54	3900	666	10000	188	10000	17			20000	10512				

* Microbiological assay using **Lactobacillus arabinosus** based on the procedures of Skeggs and Wright (23)

TABLE 7

RESULTS OF BIOTIN DEFICIENCY, SYMPTOMS, TREATMENTS, AND RESPONSE

Trial I

Days on Diet	Symptoms Observed	Pigs 9 and 10 Treatments	Treatment Response
11	Both pigs vomited and coughed frequently.		
14	Both pigs had slight dermatitis, especially on neck, ears, and shoulders.		
18	Pig 10 started scouring.		
22	Pig 9 started scouring.	2 gms. sulfathalidine fed to both pigs for scours.	
23	Biotin excretion in both Pigs 9 and 10 was the same as control pig; indicated possible intestinal or tissue synthesis.		Both pigs ceased scouring and vomiting.
29	Dermatitis became quite severe, covered entire bodies of both animals. Both had inflammation of mucous membrane of the mouth.	Began feeding sulfathalidine at the rate of 0.5 gm. per feeding (3 gms. per day). This supplementation continued until the 48th day. Sulfathalidine fed in an attempt to reduce possible intestinal synthesis of biotin.	
32	Both pigs had rough hair coat. Hair stood erect over entire body. Both had brown exudate around eyes. Animals seemed to have tender feet.		Feeding of sulfathalidine did not seem to reduce urine biotin in either pig, and did not lead to any marked change in deficiency symptoms.

(Continued on next page)

TABLE 7 (continued)

RESULTS OF BIOTIN DEFICIENCY, SYMPTOMS, TREATMENTS, AND RESPONSE

Trial I

Days on Diet	Pigs 9 and 10		
	Symptoms Observed	Treatments	Treatment Response
34	Slight transverse cracking of feet prevalent in both animals.		
38	Both pigs exhibited very severe cracking of feet, ulceration of skin on bellies and hams, and alopecia.		
46		Began to feed 10 μg biotin daily to both animals. This supplementation continued until end of experiment.	
48		Ceased feeding sulfathalidine.	
56	At end of trial both animals exhibited dark brown exudate around eyes; thin rough hair coats, dermatitis, soles and top of feet cracked and apparently very sore. Ulceration of hams and bellies. Autopsy revealed no apparent abnormalities. Growth and weight of animals was normal.		Neither pig exhibited any marked response to feeding of biotin at a level of 10 μg for 10 days.

TABLE 8

RESULTS OF BIOTIN DEFICIENCY,
RELATIONSHIP OF DIETARY INTAKE TO GROWTH

Trial I

Days on Diet	Control			Deficient					
	Pig 8			Pig 9			Pig 10		
	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)
7	2.934	0.75	3.91	2.421	0.50	4.84	3.011	1.00	3.01
14	4.246	1.25	3.40	3.746	0.75	4.99	4.265	1.50	2.84
21	6.240	2.25	2.77	4.724	1.75	2.70	6.230	2.25	2.77
28	9.360	3.50	2.67	6.720	1.75	3.84	9.360	3.00	3.12
35	13.690	3.50	3.91	9.330	3.00	3.11	13.654	4.25	3.21
42	15.720	4.25	3.70	11.600	3.00	3.87	15.463	3.50	4.42
49	19.350	5.00	3.87	15.037	3.75	4.01	18.915	4.25	4.45
56	26.974	7.25	3.72	15.433	3.25	4.75	20.119	4.25	4.73

TABLE 9

RESULTS OF BIOTIN DEFICIENCY, SYMPTOMS, TREATMENTS, AND RESPONSE

Trial II

Days on Diet	Pigs 23, 24, and 25		
	Symptoms Observed	Treatments	Treatment Response
6	Pigs 24 and 25 had slight diarrhea.		
12	Dermatitis was on ears, necks and tails of all pigs. Pig 24 had slight cracking of soles of feet.		
13	Pig 25 had mild cracks on soles of feet.		
16	Pig 23 had small cracks on soles of feet.		
23	All pigs exhibited moderate cracks on soles and tops of feet, brown eye exudate, and mild ulceration of skin on bellies and hams. All three pigs excreted similar amounts of biotin as control animal. This indicated possible intestinal or tissue synthesis.		
48		Started feeding sulfalthalidine at the rate of 1 gm. per feeding (6 gms. per day) in an attempt to reduce possible intestinal synthesis of biotin. Sulfathaliadine fed until end of trial.	
56	All pigs exhibited mild cracking of feet, ulceration of skin on hams and bellies, dermatitis, rough thin hair coats, alopecia, brown eye exudate and inflammation of mucuous membrane of the mouth. Autopsy revealed no apparent abnormalities. Growth was not affected.		Sulfathalidine feeding merely reduced urine biotin slightly, but did not accentuate deficiency symptoms.

TABLE 10

RESULTS OF BIOTIN DEFICIENCY,
RELATIONSHIP OF DIETARY INTAKE TO GROWTH

Trial II

Days on Diet	Control			Deficient								
	Pig 26			Pig 23			Pig 24			Pig 25		
	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)
7	3.640	0.50	7.28	3.640	0.50	7.28	3.070	0.50	6.14	3.445	0.50	6.89
14	7.380	1.75	4.22	7.410	2.25	3.29	6.960	1.75	3.98	7.070	2.00	3.54
21	10.815	3.00	3.61	10.910	2.75	3.97	10.940	2.50	4.38	10.960	3.00	3.65
28	16.400	4.50	3.64	16.710	4.75	3.52	13.910	5.00	2.78	16.140	4.25	3.80
35	26.225	9.00	2.91	24.380	8.50	2.87	19.585	5.25	3.73	24.450	6.25	3.91
42	34.650	4.50	7.70	28.835	4.25	6.78	28.465	6.25	4.55	30.400	6.00	5.07
49	39.900	10.00	3.99	33.990	7.00	4.86	34.530	8.75	3.95	36.620	8.75	4.19
56	42.000	7.00	6.00	33.680	6.50	5.18	35.210	9.25	3.81	37.410	8.00	4.68

TABLE 11

URINARY EXCRETION OF BIOTIN[1]

Days on Diet	Trial I					Trial II					
	Control		Deficient			Control		Deficient			
	Pig 8		Pig 9		Pig 10	Pig 26		Pig 23		Pig 24	Pig 25
	Intake μg/day	Excre. μg/day	Intake μg/day	Excre. μg/day	Excre. μg/day	Intake μg/day	Excre. μg/day	Intake μg/day	Excre. μg/day	Excre. μg/day	Excre. μg/day
2	4.0	0.29	0	0.43	0.15	3.0	1.23	0	1.02		1.27
9	6.0	0.32	0	0.95	0.19	6.0	1.41	0	1.60	0.63	0.35
15						12.0	1.52	0	2.17	1.57	1.78
21	11.0		0	0.60	1.60	16.0	2.33	0	2.26	1.66	1.63
27						24.0	3.74	0	3.22	2.86	2.70
28						24.0	4.23	0	1.63	1.43	1.36
29[2]	18.0	1.90	0	2.00	2.70						
35						42.0	8.40	0	5.26	2.50	4.85
39	24.0	3.90	0	2.40	3.10						
42[3]						51.0	9.97	0	2.42	1.13	1.48
48[4]	30.0	6.80	10	3.40	3.30	60.0	12.16	0	1.08	0.53	0.61
56	42.0	15.00	10	2.90	3.50	78.0	8.98	0	0.50	0.32	0.34

[1] Microbiological assay using **Lactobacillus arabinosus** modification of the procedure of Shull, Hutchings, and Peterson (22).
[2] Began feeding pigs 9 and 10 Sulfathalidine at the rate of 0.5 gm. per feeding (3 gms. per day).
[3] Began feeding pigs 23, 24, and 25 Sulfathalidine at the rate of 1 gm. per feeding (6 gms. per day).
[4] Ceased feeding Sulfathalidine to pigs 9 and 10.

TABLE 12

RESULTS OF PYRIDOXINE DEFICIENCY, SYMPTOMS, TREATMENTS, AND RESPONSE

Trial I

Days on Diet	Pigs 11 and 12		
	Symptoms Observed	Treatments	Treatment Response
11	Both animals exhibited mild dermatitis, alopecia, and anorexia.		
16	Pig 11 had an attack of trembling, ran backwards, showed lack of muscle coordination. Both pigs had subnormal appetites and exhibited poor growth.		
17	Pig 11 trembled, exhibited incoordination of legs, vomited, and appeared very nervous. Pigs 11 and 12 had slight dermatitis and rough hair coats.		
21	Pig 11 had a mild epileptiform seizure. Pigs 11 and 12 had poor appetites.		
22	Pig 11 had two epileptiform seizures. Animal laid on side and thrashed wildly for about two minutes. Upon recovery showed extreme weakness. Had dark brown exudate around eyes.		
23	Pig 11 had three severe epileptiform seizures and subsequently became comatose. These seizures continued for the next 5 days. Pig 12 had a mild seizure. Both pigs exhibited microcytic hypochromic anemia.		
28	Pig 11 refused to eat, appeared weak, listless and nervous, had rough hair coat.	Pig 11 fed 10 mg. pyridoxine.	
29	Pig 12 lost sucking reflex.	Pig 11 fed 5 mg. pyridoxine for next 10 days.	

(Continued on next page)

TABLE 12 (continued)

RESULTS OF PYRIDOXINE DEFICIENCY, SYMPTOMS, TREATMENTS, AND RESPONSE

Trial I

Days on Diet	Pigs 11 and 12		
	Symptoms Observed	Treatment	Treatment Response
30	Pig 11 had one mild epileptiform seizure.		Pig 11 resumed eating.
31	Both Pigs 11 and 12 had mild epileptiform seizures. Pig 12 had brownish exudate around eyes, appeared nervous and had rough hair coat.		
32	Both pigs appeared very nervous.		
33	Pig 11 had one mild epileptiform seizure and was inactive.		
34		Pig 12 fed 5 mg. pyridoxine. This supplementation continued for 11 days.	
35			Pig 11 appeared normal in every respect except for small size.
39		Pigs 11 and 12 each fed 5 mg. pyridoxine.	
50			Pig 12 appeared normal.
55			Both pigs exhibited impaired eyesight. Pig 11 was totally blind and Pig 12 partially blind.
56	Autopsy revealed no macroscopic abnormalities. Histologic studies revealed lesions of sheath of optic nerves. Blood normal.		

TABLE 13

RESULTS OF PYRIDOXINE DEFICIENCY, RELATIONSHIP OF DIETARY INTAKE TO GROWTH

Trial I

Days on Diet	Control			Deficient					
	Pig 7			Pig 11			Pig 12		
	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)
7	3.590	0.75	4.79	2.536	0.50	5.07	2.396	0.75	3.19
14	5.945	1.75	3.39	4.013	1.00	4.01	3.490	0.75	4.65
21	7.970	1.75	4.55	4.270	1.75	2.44	3.600	1.00	3.60
28	12.925	4.50	2.87	4.493	0.75	5.99	5.044	1.00	5.04
35	17.630	4.75	3.71	6.545	2.00	3.27	4.583	0.75	6.11
42	21.170	6.25	3.39	10.684	3.00	3.56	6.270	2.00	3.14
49	27.670	8.50	3.26	13.700	3.25	4.22	9.415	2.50	3.77
56	34.840	10.50	3.32	14.758	4.25	3.47	11.720	3.75	3.13

TABLE 14

RESULTS OF PYRIDOXINE DEFICIENCY, SYMPTOMS, TREATMENTS, AND RESPONSE

Trial II

Days on Diet	Pigs 15, 16, 17, and 18		
	Symptoms Observed	Treatment	Treatment Response
10	Pigs 15, 16, and 17 exhibited anorexia,		
12	Pigs 16 and 18 began scouring and regurgitating, and lost appetite.		
13	Pig 18 was very weak.		
14	Pig 17 exhibited anorexia.		
15	All pigs exhibited alopecia and dermatitis.	Pig 18 fed 5 mg. pyridoxine.	
16	Pig 15 started vomiting, trembling, and exhibited excessive lacrimation. All pigs had brown exudate around eyes and rough hair coat.		Pig 18 had ceased scouring and vomiting.
17		Pig 16 fed 5 mg. pyridoxine.	
19	All pigs were nervous and inactive.		Pig 16 had ceased vomiting and scouring.
22	Pigs 15 and 17 had mild epileptiform seizures and comas. These maladies continued in ranging severity; Pig 15 had seizures for 6 days and Pig 17 for 3 days.		
26	Pigs 16 and 17 had impaired eyesight.	Pig 17 fed 2 mg. pyridoxine.	
28	Pig 15 had 6 severe epileptiform seizures and became comatose. Pig 15 totally blind.	5 mg. pyridoxine fed to pig 15.	
30			Pig 15 stopped having epileptiform seizures.
34	Pig 16 was extremely weak, regurgitated food. Pig 17 had two severe epileptiform seizures and subsequent coma.		
35	All pigs began vomiting.		
36		Pig 19 fed 200 μg pyridoxine.	

(Continued on next page)

TABLE 14 (continued)

RESULTS OF PYRIDOXINE DEFICIENCY, SYMPTOMS, TREATMENTS, AND RESPONSE
Trial II

Days on Diet	Pigs 15, 16, 17 and 18		
	Symptoms Observed	**Treatment**	**Treatment Response**
37	Pig 16 died at 1 p.m. immediately after an epileptiform seizure. Autopsy revealed: enlarged heart and kidneys, lack of subcutaneous fat and internal fat, slight ulceration of the intestines.	Pig 16 given 1 mg. pyridoxine subcutaneously at 9:30 a.m.	
38	Pig 17 had three severe epileptiform seizures and comas. Pig 18 began vomiting.	Pig 17 fed 2 mg. pyridoxine for 2 days. Pig 18 fed 200 μg pyridoxine.	
40		Pig 17 fed 400 μg pyridoxine.	
45	Pig 18 had an epileptiform seizure. Animal completely blind.		
46	Pig 18 became very weak.	Pig 18 fed 100 μg pyridoxine.	
48	Pigs 17 and 18 had many severe seizures.		
50	Pigs 17 and 18 continued to have many seizures. All had poor appetites.	Pigs fed the following daily amounts of pyridoxine for the remainder of the experimental period: Pig 15, 40 μg; Pig 17, 20 μg; and Pig 18, 10 μg.	
51	Pig 18 had an epileptiform seizure and coma which lasted for 7 minutes.		
52	Pig 18 had two severe seizures. One lasted 6 minutes, the other 3 minutes.		
54	Pig 18 continued having seizures.		Pigs 15 and 17 appeared normal in every respect with the exception of impaired eyesight.
56	Autopsy of Pigs 15 and 18 revealed lack of internal and subcutaneous fat. Enlarged heart and kidneys. Histologic studies revealed lesions of the sheath of the optic nerves. Blood normal.		Pigs 15 and 17 appeared normal with exception of small size and impaired eyesight. Pig 18 did not respond to pyridoxine therapy.

TABLE 15

RESULTS OF PYRIDOXINE DEFICIENCY, RELATIONSHIP OF DIETARY INTAKE TO GROWTH

Trial II

Days on Diet	Deficient											
	Pig 15			Pig 16			Pig 17			Pig 18		
	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)
7	3.341	0.50	6.68	3.590	0.75	4.79	3.514	0.75	4.69	3.500	0.75	4.67
14	3.615	1.00	3.62	3.685	0.75	4.91	4.655	1.25	3.72	3.925	1.00	3.93
21	4.835	1.00	4.84	4.570	0.75	6.09	4.815	1.25	3.85	5.245	1.00	5.25
28	5.255	1.00	5.20	6.690	2.00	3.35	6.345	2.25	2.82	6.095	2.00	3.05
35	7.965	3.50	2.28	6.165	2.75	2.24	9.695	3.50	2.77	7.565	1.00	7.57
42	9.555	3.25	2.94				8.282	1.75	4.73	9.750	3.25	3.00
49	8.035	1.50	5.36				5.765	0.25	23.06	6.800	1.00	6.80
56	8.450	0.75	11.27				7.085	-0.25		11.610	3.00	3.87

TABLE 16

INTAKE AND URINARY EXCRETION OF PYRIDOXINE*

Days on Diet	Trial I						Trial II							
	Control		Deficient				Deficient							
	Pig 7		Pig 11		Pig 12		Pig 15		Pig 16		Pig 17		Pig 18	
	Intake μg/day	Excre. μg/day	Intake μg/day	Excre. μg/day	Intake μg/day	Excre. μg/day	Intake μg/day	Excre. μg/day	Intake μg/day	Excre. μg/day	Intake μg/day	Excre. μg/day	Intake μg/day	Excre. μg/day
2	0	3	0	7	0	5	0	4	0	4	0	3	0	3
4	273	4					0	2	0	2	0	2	0	4
9			0	34	0	18								
12	546	22					0	5	0	3	0	2	0	8
13	546	35					0	4	0	4	0	4	0	4
15													5000	
16			0	7	0	13								
17									5000					
21			0	4	0	10								
22	780	44					0	3	0	14	0	2	0	5
24	1024	57					0	2	0	9	0	14	0	10
26											2000			
28	1267	149					5000	1036	0	38	0	150	0	11
29			10,000	400	0	7	0	9	0	1	0	10	0	3
36	1755	154											200	
37									1000					
39			5,000	400	5,000	400								
40											400			
44	2223	158					0	10			0	10	0	9
46													100	
47			0	29	0	75								
49							0	8				8	0	16
50							40				20		10	
51	2925	97					40	9			20	6	10	2
52							40	8			20	8	10	1
53	3315	267					40	8			20	5	10	4
54							40				20		10	
55	3900	275					40	9			20	15	10	13
56			0	28	0	16								

* Yeast microbiological assay with **Sacchromyces carlsbergensis.** Modification of the procedure of Atkins, Schultz, Williams and Frey (1).

TABLE 17

RESULTS OF RIBOFLAVIN DEFICIENCY, SYMPTOMS, TREATMENTS, AND RESPONSE

Trial I

Days on Diet	Pigs 34, 35, and 36		
	Symptoms Observed	Treatment	Treatment Response
6	All pigs exhibited alopecia and anorexia.		
11	All pigs exhibited rough hair coats, dermatitis, some diarrhea.		
20	Pigs 34 and 35 began scouring and vomiting.		
22	Pig 36 started scouring and vomiting.		
26	Pigs 34, 35, and 36 had very inflamed anuses. Eyes were sensitive to light; hid their eyes and whimpered.		
27	Pig 35 exhibited unsteady gait, very rough hair coat, severe dermatitis. Refused to eat.	Pig 35 fed 500 μg riboflavin by pipette. Force fed milk and 500 μg riboflavin until it died on 30th day.	
30	Pig 35 died.		
31	Pigs 34 and 36 began scouring severely. Both passed some blood.	500 μg riboflavin fed to Pig 34 for 6 days.	
33		Pig 36 fed 500 μg riboflavin for 6 days.	
34			Pig 34 stopped scouring. Appetite improved. Dermatitis cleared up. Hair coat more lustrous.

(Continued on next page)

TABLE 17 (continued)

RESULTS OF RIBOFLAVIN DEFICIENCY, SYMPTOMS, TREATMENTS, AND RESPONSE

Trial I

Days on Diet	Pigs 34, 35, and 36		
	Symptoms Observed	Treatment	Treatment Response
40			Pig 36 exhibited improved appetite, scours had ceased. Animal appeared more normal.
46	Pig 36 vomited, lost appetite. Pig 34 scoured and lost appetite.		
50		500 μg riboflavin fed to Pigs 34 and 35 for remainder of trial.	
53			Pig 34 showed improved appetite. Scours ceased.
56	Pigs 34 and 36 were small in size. Skins were very scaly. Autopsy: microscopic examination revealed necrosis and sloughing of the corium with hemorrhage in the proliferative germinal layers of the skin. The liver and kidneys showed blotchy hemorrhage areas on the surface which microscopically showed subcapsular inflammation. The liver showed leucocytic infiltration and many polymorphonuclear leucocytes in the blood. The kidneys exhibited a subacute glomerulonephritis and cloudy swelling with destruction of the tubular epithelium. The lungs showed pneumonia and microscopically there was leucocytic and erythrocytic infiltration, with many polymorphonuclear leucocytes present.		

TABLE 18

RESULTS OF RIBOFLAVIN DEFICIENCY, RELATIONSHIP OF DIETARY INTAKE TO GROWTH

Trial I

Days on Diet	Control			Deficient								
	Pig 33			Pig 34			Pig 35			Pig 36		
	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)
7	3.065	1.00	3.07	3.075	0.75	4.10	3.075	0.50	6.15	3.075	1.25	2.46
14	4.800	1.00	4.80	4.690	1.25	3.75	4.690	1.25	3.75	4.690	1.50	3.13
21	6.430	1.50	4.29	5.230	0.50	10.46	5.620	1.00	5.62	6.430	1.00	6.43
28	7.780	3.75	2.07	5.180	0.75	6.91	4.360	-1.25		7.580	3.00	2.53
35	14.370	9.25	1.55	5.975	1.00	5.98				8.130	2.00	4.07
42	24.090	15.00	1.61	9.890	2.25	4.40				13.870	6.00	2.31
49	23.410	6.50	3.60	9.790	6.00	1.63				18.320	7.00	2.62
56	33.760	8.50	3.97	14.360	6.50	2.21				22.980	7.50	3.06

TABLE 19

RESULTS OF RIBOFLAVIN DEFICIENCY, SYMPTOMS, TREATMENTS, AND RESPONSE

Trial II

Days on Diet	Pigs 37, 38, and 39		
	Symptoms Observed	Treatment	Treatment Response
8	Pigs had poor appetites.		
12	All pigs had diarrhea, vomited, had dermatitis, and alopecia.		
19	All hair coats were rough and skins were scaly.		
24	Diarrhea of all pigs had become more severe. Hair coats were very rough and coarse.		
26	Pigs 37, 38, and 39 began scouring and vomiting more frequently. Pig 39 had very inflamed anus.		
28	Pig 38 lacked coordination, right eye pasted shut. Pig 39's eyes were very sensitive to light, hid eyes and whimpered.		
31	Pigs 37 and 38 had inflamed anuses and passed some blood rectally.	All pigs fed 1 mg. riboflavin. Supplementation continued 8 days.	
33	Pig 39 passed blood rectally and had an inflamed anus.		
36			Pigs 37 and 38 showed improved appetites, scouring and blood passing had ceased. Hair coats were more lustrous. Anus appeared normal.
39			Pig 39 was greatly improved, appetite near normal. Scouring and blood passing had stopped.

(Continued on next page)

TABLE 19 (continued)

RESULTS OF RIBOFLAVIN DEFICIENCY, SYMPTOMS, TREATMENTS, AND RESPONSE

Trial II

Days on Diet	Pigs 37, 38, and 39		
	Symptoms Observed	**Treatment**	**Treatment Response**
40	Pig 37 began vomiting and scouring again.		
42	Pig 38 began vomiting and scouring.		
46	Pig 39 began vomiting and scouring.		
48		Began feeding Pigs 37 and 39. 1.5 mg. riboflavin and Pig 38, 1 mg. riboflavin; supplementation continued to end of trial.	
50			All pigs had ceased vomiting and scouring. Hair coats improved. Appetites improved.
56	All pigs appeared normal except for small size. Autopsy revealed normal skins. Liver and kidneys showed blotchy subcapsular hemorrhages macroscopically. Microscopically, the liver showed a granulocytic infiltration of the interlobular spaces with intercolumnar congestion in the subcapsular region. There were many polymorphonuclear leucocytes in the blood. The kidneys showed an interstitial congestion with many polymorphs in the blood. Lungs had a fingersized solidified area of pneumonia in the lower portion. Microscopically, they showed granulocytic and erythrocytic infiltration with many polymorphs present.		

TABLE 20

RESULTS OF RIBOFLAVIN DEFICIENCY, RELATIONSHIP OF DIETARY INTAKE TO GROWTH

Trial II

Days on Diet	Deficient								
	Pig 37			Pig 38			Pig 39		
	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)	Period Intake (liters)	Period Gain (lb.)	Intake Per lb. Gain (liters)
7	3.075	1.00	3.08	3.005	0.75	4.01	3.025	0.50	6.05
14	4.690	1.00	4.69	4.690	1.00	4.69	4.690	1.25	3.75
21	6.430	0.25	25.72	6.430	0.50	12.86	5.780	0.50	11.56
28	5.725	-0.25		3.955	-1.00		5.470	0.50	10.94
35	6.425	1.75	3.67	5.595	1.75	3.20	5.150	0.50	10.30
42	12.080	5.50	2.20	10.810	4.75	2.28	9.075	5.00	1.82
49	13.130	7.25	1.81	7.560	1.50	5.04	10.930	6.00	1.82
56	18.715	8.75	2.14	11.320	3.50	3.23	15.660	7.75	2.02

Literature Cited

1. Atkins, L., Schultz, A. S., Williams, W. L., and Frey, G. N. Yeast Microbiological Method for Determination of Vitamins. Pyridoxine. Ind. and .Eng. Chem., Anl. Ed., 15:141-144, 1943
2. Bird, E. W., Sadler, H. W., and Iverson, C. A. The Preparation of a Non-Desiccated Sodium Caseinate Sol and its Use in Ice Cream. Iowa Agr. Expt. Sta. Bul. 187: 177-208, Iowa State College, Ames, 1935
3. Bustad, L. K., Ham, W. E., and Cunha, T. J. Preliminary Observations on Using a Synthetic Milk for Raising Pigs from Birth. Arch. Biochem. 17: 249-260, 1948
4. Chick, H., Macrae, T. F., Martin, A. J. P., and Martin, C. J. The Water-Soluble B-Vitamins other than Anurin (Vitamin B_1), Riboflavin, and Nicotinic Acid Required by the Pig. Biochem J. 32: 2207-2224, 1938
5. Cunha, T. J., Lindley, D. C., and Ensminger, M. E. Biotin Deficiency Syndrome in Pigs Fed Desiccated Egg White. J. Animal Science 5: 219-225, 1946
6. Follis, R. H. Jr., and Wintrobe, M. M. A Comparison of the Effects of Pyridoxine and Pantothenic Acid Deficiencies on the Nervous Tissues of Swine. J. Expt. Med. 81:539-552, 1945
7. Hughes, E. H., The Vitamin B-Complex as Related to Growth and Metabolism in the Pig. Hilgardia 11:595-610, 1938
8. Hughes, E. H. Minimum Requirements of Thiamine for the Growing Pig. J. Nutrition 20:239-241, 1940
9. Hughes, E. H. The Minimum Requirements of Riboflavin for the Growing Pig. J. Nutrition 20:233-237, 1940
10. Hughes, E. H., and Ittner, N. R. Minimum Requirements of Pantothenic Acid for the Growing Pig. J. Animal Science 1:116-119, 1942
11. Hughes, E. H., and Squibb, R. L. Vitamin B_6 (Pyridoxine) in the Nutrition of the Pig. J. Animal Science 1:320-325, 1942
12. Ittner, N. R., and Hughes, E. H. A Normal Growth Curve for Swine. J. Heredity 29:385-386, 1938
13. Johnson, B. C., James, M. F., and Krider, J. L. Raising Newborn Pigs to Weanling Age on a Synthetic Diet with Attempt to Produce a Pterolyl-glutamic Acid Deficiency. J. Animal Science 7:486-493, 1948
14. Lehrer, W. P. Jr., Moore, P. R., Wiese, A. C., and Pahnish, O. F. A Synthetic Milk Ration for Baby Pigs. J. Animal Science 8:107-111, 1949
15. Lindley, D. C., and Cunha, T. J. Nutritional Significance of Inositol and Biotin for the Pig. J. Nutrition 32:47-59, 1946
16. Livestock Advisory Committee. Research and Marketing Proposals for the Livestock Industry. United States Department of Agriculture Report: 1-50. United States Department of Agriculture, Washington, D. C. 1947
17. Maynard, L. A. Animal Nutrition. pp. 222, McGraw-Hill Book Company, Inc., New York City, 1947
18. McRoberts, V. F., and Hogan, A. C. Adequacy of Simplified Diets for the Pig. J. Nutrition 28:165-174, 1944
19. Mitchell, H. H., Johnson, B. C., Hamilton, T. S., and Haines, W. T. The Riboflavin Requirement of the Growing Pig at Two Environmental Temperatures. J. Nutrition 41:317-338, 1950
20. Phillips, P. H., and Hart, E.B. The Effect of Organic Dietary Constituents Upon Chronic Fluorine Toxicosis in the Rat. J. Biol. Chem. 109: 657-663, 1935

21. Russell, W. C., Terri, A. E., and Unna, K. Growth and Reproduction of Swine on a Purified Diet. J. Nutrition 35:321-332, 1948

22. Schull, G. M., Hutchings, B. L., and Peterson, W. H. A Microbiological Assay for Biotin. J. Biol. Chem. 142:913-920, 1942

23. Skeggs, H. R., and Wright, L. D. The Use of Lactobacillus Arabinosus in the Microbiological Determination or Pantothenic Acid. J. Biol. Chem. 156:21-26, 1944

24. Snell, E. E., and Strong, F. M. A Microbiological Assay for Riboflavin. Ind. and Eng. Chem., Anal. Ed., 11:346-350, 1939

25. Van Etten, C., Ellis, N. R., and Madsen, L. L. Studies on Thiamin Requirements of Young Swine. J. Nutrition 20:607-622, 1940

26. Wiese, A. C., Johnson, B. C., Mitchell, H. H., and Nevens, W. B. Synthetic Rations for the Dairy Calf. J. Dairy Science. 30:87-94, 1947

27. Wintrobe, M. M. Nutritive Requirements of Young Pigs. Amer. J. Physiol. 126:375-387, 1939

28. Wintrobe, M. M., Mitchell, D. L., and Kolb, L. C. Sensory Neuron Degeneration in Vitamin Deficiency. J. Expt. Med. 68:207-219, 1938

29. Wintrobe, M. M., Miller, J. L. Jr., and Lisco, H. The Relation of Diet to the Occurrance of Ataxia and Degeneration in the Nervous System of Pigs. John Hopkins Bul. 67:377-394, Johns Hopkins Hospital, Baltimore, 1940

30. Wintrobe, M. M., Miller, M. H., Follis, R. H. Jr., Stein, H. J., Mushatt, C., and Humphreys, S. Sensory Neuron Degeneration in Pigs. IV Protection Afforded by Calcium Pantothenate and Pyridoxine. J. Nutrition. 24:345-366. 1942

31. Wintrobe, M. M., Follis, R. H. Jr., Miller, H. H., Stein, H. J., Alcayago, R., Humphreys, S., Suksta, A., and Cartwright, G. E. Pyridoxine Deficiency in Swine, With Particular Reference to Anemia, Epileptiform Convulsions, and Fatty Liver. Johns Hopkins Bul. 77:1-26, Johns Hopkins Hospital, Baltimore, 1943

32. Wintrobe, M. M., Buschke, W., Follis, R. H. Jr., and Humphreys, S. Riboflavin Deficiency in Swine. Johns Hopkins Bul. 75:102-114, Johns Hopkins Hospital, Baltimore, 1944.

Acknowledgments

The authors wish to acknowledge the assistance of Dr. L. A. Scrivner, Dr. W. B. Ardrey, Dr. P. R. Moore, Mr. O. F. Pahnish, Mr. W. V. Hartwell, Mrs. June Anderson, Mrs. Sybil Brislain, and Dr. P. G. Eldredge for assistance with various phases of these studies.

The authors are also indebted to Merck and Company, Rahway, New Jersey, through the courtesy of Dr. D. F. Green, for supplies of thiamine, riboflavin, pyridoxine, nicotinic acid, inositol, choline, p-Aminobenzoic acid, biotin, calcium pantothenate and tocopherol; to Lederle Laboratories Inc., Pearl River, New York, through the courtesy of Dr. E. R. L. Stockstad, for pteroylglutamic acid; to E. I. Dupont De Nemours and Company, New Brunswick, New Jersey through the courtesy of Dr. J. Waddell, for crystalline vitamin D_3 and to Wilson and Company, Chicago, Illinois, through the courtesy of Dr. S. W. Hier, for the 1:20 liver powder.