THE RELATIONSHIP OF SPECIFIC NUTRIENT DEFICIENCIES TO ANTIBODY RESPONSE IN SWINE

I. VITAMIN A

II. PANTOTHENIC ACID, PYRIDOXINE OR RIBOFLAVIN

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY

Bud G. Harmon

1962

This is to certify that the

thesis entitled

THE RELATIONSHIP OF SPECIFIC NUTRIENT
DEFICIENCIES TO ANTIBODY RESPONSE IN SWINE
I. VITAMIN A
II. PANTOTHENIC ACID, PYRIDOXINE OR RIBOFLAVIN

presented by

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has been accepted towards fulfillment
of the requirements for

Ph D degree in Animal Husbandry

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Date May 18, 1962

O-169

ABSTRACT

THE RELATIONSHIP OF SPECIFIC NUTRIENT DEFICIENCIES
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Four trials were conducted to study and evaluate the relationship of specific deficiencies of vitamin A, pantothenic acid, pyridoxine or riboflavin to antibody production in swine. Each of the trials involved pigs which were weaned to semi-synthetic diets at two weeks of age or less. The first two trials in which 30 pigs were employed were designed to study the antibody response of positive control pigs and pigs deficient in vitamin A. Additional data obtained were weight gain, feed efficiency, serum vitamin A level, serum protein concentration and the electrophoretic components of serum protein.

The pigs receiving the vitamin A free diet exhibited significantly lower (P 0.01) serum vitamin A levels at six weeks of age after having been weaned to the experimental diet at ages of six days and 12 hours respectively in Trials I and II. The pigs in a marginal vitamin A condition (less than 20 micrograms per 100 milliliters of serum) produced significantly lower (P 0.01) antibody titers to experimental intraperitoneal introduction of killed cultures of Salmonella pullorum than did the control pigs. In Trial I the average daily gain and feed efficiency values of the vitamin A deficient and control pigs were not statistically significantly different. However, in Trial II the daily gain was significantly greater and more efficient (P 0.01) in the control pigs. Analysis of the electrophoretic components of serum protein at the time the antibody production

was measured, disclosed that the deficient pigs had significantly higher ($P<0.01$) percentages of alpha and gamma globulin than did the control pigs. This was accompanied by a significant decrease ($P<0.01$) in the percent albumin in the deficient pigs.

Following a repletion phase during which all pigs received a complete natural diet the immunological response was measured with human erythrocytes. All pigs from both previous treatments responded with similar hemagglutination titers. In the second trial the control pigs continued to gain significantly faster ($P<0.01$) during the repletion phase than did the pigs which were previously vitamin A deficient. Serum vitamin A and protein values were similar in all pigs following the repletion phase of each trial.

Trials III and IV, which involved 48 pigs, were designed to study the antibody response of positive control pigs and pigs deficient in pantothenic acid, pyridoxine or riboflavin. Additional data collected were measures of weight gain, feed efficiency, blood cellular components, serum protein concentration and electrophoretic components of serum protein. Also, urinary xanthurenic acid values were obtained from the pyridoxine deficient and control pigs. The pigs in both trials were placed on one of the four experimental diets at four weeks of age after having been weaned to a semi-synthetic diet, deficient in the three B vitamins under consideration, at two weeks of age. In Trial III Salmonella pullorum antigen was injected after the pigs on experimental treatment had been deprived of the particular vitamin for four weeks. In Trial IV the experimental feeding period was extended one week and human erythrocytes were employed as the antigen. The agglutination titers in Trial III and hemagglutination titers in Trial IV were significantly greater ($P<0.01$) in control pigs than for

any of the deficient groups. A series of equated feeding groups (a group included a pig from each of the four treatments) established that inanition was not responsible for decreased antibody production in the deficient pigs since the control pigs on limited feed produced significantly greater ($P < 0.01$) antibody titers.

At the conclusion of the depletion phase of the trials the weight gain of the control pigs was significantly greater and more efficient ($P < 0.01$) than that of any deficient group. Pyridoxine deficient pigs had lower hematocrit, hemoglobin, total erythrocytes and total leukocytes than did the other pigs. The pyridoxine deficient pigs also had significantly higher concentrations ($P < 0.05$) of urinary xanthurenic acid than did the controls, both before as well as after additions of tryptophan to the regular diet.

Analyses of the serum proteins at the conclusion of the depletion feeding phase of the trials established that the alpha globulin was significantly greater ($P < 0.05$) in the pantothenic acid deficient pigs than in the controls.

Following repletion periods of six to seven weeks all pigs responded with similar antibody titers to human erythrocytes in Trial III and Salmonella pullorum in Trial IV. Also at this time, similar serum protein values were measured in all treatments. In Trial III the weight gain of the control pigs was significantly greater ($P < 0.05$) after six weeks on a complete natural diet than all the pigs formerly fed the deficient diets. However, in Trial IV after extending the repletion feeding period one week, the control pigs remained only significantly heavier ($P < 0.05$) than the pigs formerly receiving the pantothenic acid deficient diet.

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By

Bud G. Harmon

A THESIS

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

DOCTOR OF PHILOSOPHY

Department of Animal Husbandry

1962

ACKNOWLEDGMENT

The author wishes to express his very sincere appreciation to Dr. J. A. Hoefer and Dr. E. R. Miller for their invaluable guidance and assistance throughout this study, and for their critical reading of this manuscript. The writer also wishes to extend a sincere note of thanks to the members of his guidance committee, Dr. J. A. Hoefer, Dr. E. R. Miller, Dr. R. W. Luecke, Dr. E. P. Reineke and Dr. C. K. Whitehair for their generous attitude during the completion of his studies.

Sincere gratitude is expressed to Dr. R. H. Nelson and Dr. D. E. Ullrey for the materials and facilities which made this research possible.

The author wishes to thank the herdsman, Mr. G. B. Stafford, and his staff for their assistance in the care and management of the pigs.

Gratitude is expressed to the Rackham Foundation for support of this study. The author also wishes to express his thanks to the National Institute of Health for providing him a Public Health Service Fellowship.

The writer wishes especially to acknowledge his gratitude, appreciation and indebtedness to his wife, Mary Lynne, whose sacrifices and encouragement made this study possible.

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candidate for the degree of

Doctor of Philosophy

DISSERTATION: The Relationship of Specific Nutrient Deficiencies to Antibody Response in Swine

I. Vitamin A

II. Pantothenic Acid, Pyridoxine or Riboflavin

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I. INTRODUCTION

The search for nutritional components which may modify the resistance or susceptibility of a host to infectious diseases has been pursued extensively since the turn of the century.

Evaluation of altered resistance or susceptibility has progressed from simply a percent mortality in the early studies to a series of more sophisticated and specific though less dramatic criteria of host modifications in later investigations.

Active antibody response to a specific antigen is but one of several means of defense by the host in resisting infection. However, antibody formation and immunity are readily measured and the importance of the defense mechanism has been recognized since the time of Jenner(Baron, 1927).

The study of the relationship of dietary factors to antibody production has progressed toward two main purposes: (1) To determine and identify essential components of a nutritional environment for enhancing actively acquired immunity, and (2) To supply possible approaches of investigation for elucidating mechanisms of antibody formation.

Early workers such as Warren (1923a) could find little influence of diet upon antibody production. The early literature, however, does contain many reports in which particular diets were predisposing to increased susceptibility to infection. As various essential nutrients were identified and diets became available in purer forms various groups began to report some relationship between diet and antibody production. Axelrod and his co-workers (1955a) have conducted and reported many studies in which the relationship of vitamin deficiencies and antibody production was examined in rats and guinea pigs.

However, relatively few investigations have been conducted in swine relative to specific antibody production in various nutrient deficiency states. This study was initiated to determine: (1) The effect that deficiencies of vitamin A, pantothenic acid, pyridoxine or riboflavin would have on specific antibody production, and (2) The ability of pigs to produce specific antibodies on a complete diet following a sustained period of consuming diets deficient in the above mentioned vitamins.

II. REVIEW OF LITERATURE

A. Vitamin A

The voluminous amount of research that has been reported concerning the relationship between nutrition and resistance or susceptibility to infection has in many areas been quite contradictory.

In one of the earliest published reports McCollum (1917) reported that rats on a diet low in vitamin A developed severe spontaneous infection. Drummond (1919) also reported an increase in spontaneous infection in vitamin A deficient rats. Mellanby (1919) observed increased susceptibility to pneumonia in pups fed a diet deficient in vitamin A.

In a study with kittens McKay (1921) observed that when the fat of milk was replaced by olive oil there was a high incidence of infections of Dipylidium caninum. Daniels et al. (1923) also reported an increased susceptibility of xerophthalmic rats to spontaneous infections of the respiratory tract. On the other hand Cramer and Kingsbury (1924) were able to demonstrate only small differences between the resistance of vitamin A deficient rats and rats fed a complete diet when exposed to Mycobacterium tuberculosis. These same workers observed no spontaneous outbreaks of pneumonia in the vitamin A deficient rats.

Green and Mellanby (1928) found evidence of infection in a majority of the rats suffering from a vitamin A deficiency. The control animals exhibited no such infections. In 72 percent of the deficient rats, abscesses were observed at the base of the tongue. Also, infections in the urinary tract were quite common. Other areas of infection included the ocular apparatus, respiratory and alimentary tracts, and mastoid and nasal sinuses. Green and Mellanby (1930) fed graded levels of carotene to

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rats on a vitamin A free diet. At levels of .04 milligrams carotene per day and higher, no observable spontaneous infections were present at autopsy. Infections were found in all rats receiving .01 milligrams carotene per day or less. The lesions most often seen were tongue abscesses and upper respiratory infections.

Greene (1933) found spontaneous cultures of Streptococcus pyogenes in nasal passages in rats on vitamin A deficient diets. Cultures of this organism were not isolated from the rats fed the control diets. At autopsy Streptococcus pyogenes was isolated from the lungs of 90 percent of the animals on the deficient diet and from blood taken from the heart in 60 percent of the deficient animals. Greene also found increased spontaneous infections of pneumococcus and Salmonella leptosepticum.

Gerriets (1960) investigated the coccidiostatic effect of vitamin A using graded dosages of vitamin A in a study involving 800 pullets. Appendix coccidiosis was observed to occur spontaneously in many five week old pullets. An experiment was designed with six treatment groups two of which received the vitamin A deficient diet. The mortality to coccidiosis was 100 percent in these two groups. The other lots received the vitamin A deficient basal diet plus 15, 30, 45, and 60 IU (international units) respectively, of vitamin A per day. The mortality in these groups was 67 percent, 38 percent, 24 percent and 6 percent respectively. Gerriet credits much of the prophylactic effect of vitamin A to the maintenance of the epithelium.

Erasmus et al. (1959) found that coccidiosis was more severe in chickens which were on a diet deficient in vitamin A than birds receiving a diet meeting the National Research Council requirements.

Turner et al. (1930) investigated the relationship of vitamin A to the frequency of spontaneous infection of the middle ear and the upper respiratory tract involving Staphlococcus aureus, Escherichia coli, Micrococcus catarrhalis A and Chromagen 6. The latter two, the pyogenic gram negative cocci, were present in greater numbers in the rats on the vitamin A deficient diet.

Stoerk et al. (1952) have stated that the metaplasia of the corneal epithelium invariably led to keratitis and iritis. Further, that tracheitis, pyelitis, cystitis and endometritis were found in association with squamous cell metaplasia of the respective tissue. To further study the cause of spontaneous infection, Stoerk gave daily injections of antibiotics to vitamin A deficient animals. The deficient animals responded to the antibiotic treatment with increased growth and survival which led these workers to conclude that secondary infection is one of the predisposing factors of death in vitamin A deficient animals.

Other workers chose to study the effect of a vitamin A deficiency upon resistance or susceptibility by experimentally infecting the animals. Werkman (1923b) was able to bring about the death of vitamin A deficient rats by intraperitoneal injections of Bacillus anthracis. Positive control rats were not susceptible to anthrax. However, following experimental infection in rats Verder (1928) could isolate Salmonella enteritidis cultures with equal frequency from various tissues of rats fed diets either adequate or deficient in vitamin A.

In an extremely interesting study Boynton and Bradford (1931) were able to show that vitamin A deficient rats experienced a higher mortality rate than did the control animals when injected intraperitoneally with a

bacillus of the Mucosus capsulatus group. In this study 46 rats were divided into two groups and fed either a diet deficient in vitamin A or the same diet supplemented with cod liver oil as a vitamin A carrier. Starting after four weeks on test and at biweekly intervals from five to eight rats per treatment were selected and injected with the bacillus. Survival time following the injection was measured and this time interval served as a criterion for evaluating the effect of vitamin A deficiency upon resistance of the animal. The induced infection was fatal to all but one deficient rat and to 75 per cent of the control animals. Of the rats that died the average survival time was 31 hours for the control animals and 10 hours for the deficient. The growth rate of the vitamin A deficient rats was equal to that of the positive control rats until the experiment had progressed eight weeks. At that time weight gain became suppressed in the deficient animals. In contrast, the deficient animals had depressed resistance to the bacillus injections at least by four weeks after the experiment had started.

McClung and Winters (1932) developed vitamin A deficient rats by feeding a semi-synthetic diet for seven weeks. At that time intraperitoneal injections of Salmonella enteritidis were begun with 1 cc. of a 24 hour broth of which 1.2 cc. had been found to be an MLD at two to four days. At the end of 216 hours one control rat of 20 had died and 13 of 20 of the vitamin A deficient rats had died. The most characteristic lesion was a hemorrhage within the intestinal tract.

Tisdall (1950) injected Salmonella typhimurium into rats fed a vitamin A deficient or control diet for four weeks. Forty percent of the rats on the deficient diet survived while 80 percent of the controls sur-

vived. In similar studies with mice using Salmonella typhosa the mortality was 90 percent for the vitamin A deficient mice and 15 percent for the control mice.

Orskov and Moltke (1928) have studied the manner in which an oral infection of Salmonella paratyphi transpired in mice. The bacilli rapidly disappear from the alimentary tract reappearing in the lymph nodes of the mesentery. From the mesentery the bacilli pass via the thoracic duct and blood stream to the lower spleen and peripheral lymph nodes. In normal animals these three areas carry out the destruction of the bacilli. In vitamin A deficient animals the bacilli are not destroyed and a severe secondary infection of the blood stream may follow.

Diehl (1960) investigated the severity of experimental hepatic coccidiosis infection in rabbits receiving a complete diet or one deficient in either vitamin A or vitamin E. The infection was more severe in the vitamin A deficient group than in either of the other treatments.

Niilo and Beyeau (1961) fed chickens a diet deficient in vitamin A in which carotene was added at either 1340 or 5340 IU per kilogram of diet. After four weeks on trial the drinking water was contaminated on three successive days with cultures of Pseudomonas aeruginosa. On the low carotene diet 56 percent of the chickens died while the mortality of the chickens on the high carotene diet was only 14 percent. The vitamin A levels from livers of birds exhibiting positive cultures were 40.4 and 0.9 micrograms per 100 grams respectively in the control and deficient birds.

Sayedain and Kinsy (1960) observed that vitamin A deficient chickens were more susceptible to experimental Candida albicans infections than were the control birds. Sixty percent of the vitamin A deficient birds showed

lesions while only 7 percent of the control birds had moniliasis lesions.

In a more quantitative study Guggenheim and Buechler (1946) made periodic bacteria counts at various intervals following intra-abdominal injections of Salmonella typhimurium. They found significantly larger numbers of extracellular bacteria in the cell-free peritoneal fluid of the vitamin A deficient rats than in the controls. These researchers concluded that the bactericidal action by the vitamin A deficient rats was significantly diminished. Phagocytosis was measured and determined to be significantly greater in the rats receiving vitamin A than in the deficient rats.

Orskov et al. (1928) have concluded that the principal defenses of mice against Salmonella typhimurium and other salmonellas are phagocytosis by leukocytes and by macrophages of the reticuloendothelial system.

Mellanby and Green (1929) reported that the administration of vitamin A in quantities greater than normally included in a diet provided increased resistance to puerperal sepsis and septicemia. These workers concluded from this and other studies that vitamin A is an "anti-infective" vitamin.

Hess et al. (1933) and Clausen (1934) studied the "anti-infective" influence of high levels of vitamin A superimposed upon a diet assumed to provide adequate vitamin A. Neither group could show any increased resistance from the high levels of vitamin A. Schneider (1946) was quite critical of the feeding of additional vitamin A above that normally added to a complete diet as an "anti-infective" measure. He suggests that vitamin A is no more "anti-infective" than many of the B vitamins, and analogizes that to call pyridoxine the "growth vitamin" would be just as proper.

Wohlbach (1942) casts further doubt on the term "anti-infective" that Mellanby and Green (1929) applied to vitamin A. The sublingual abscesses

which the latter workers described in vitamin A deficient animals were shown by Wohlbach (1942) to be simple cysts containing desquamated epithelial cells and detritus.

Other investigators have studied the effects of vitamin A deficiency upon resistance or susceptibility to viruses. Rous (1911) observed that well nourished chicks were more susceptible to fowl sarcoma virus than were chicks which were undernourished.

Squibb and Veros (1961) have studied the effect of varying levels of vitamin A upon the resistance of young chickens to Newcastle disease virus (NDV). All birds were fed a diet free of vitamin A for three to four weeks. The birds were then lotted but continued to receive the same diet. The resultant groups included four which were dosed with from 6250 to 50,000 IU of vitamin A into the crop and two which received no supplemental vitamin A. The mortality following experimental infection was quite high in all treatment groups receiving NDV. In another trial all birds received a complete starter diet for four weeks. At this time seven treatment groups received vitamin A into the crop at levels of from 780 to 50,000 IU of vitamin A. Again two treatment groups received no additional vitamin A other than that contained in the complete starter diet. NDV was again given orally into all birds except the noninfected control group. The mortality again was high in all infected birds. Squibb and Veros (1961) concluded that supplemental vitamin A did not alter the mortality of birds either on a vitamin deficient diet or on a diet that meets National Research Council standards.

Underdahl and Young (1956) studied the influence of dietary intake of fat-soluble vitamins on the intensity of experimental swine influenza virus infection in mice. Mice received diets which were either adequate or

low in vitamin A. After four weeks all rats were intranasally inoculated with swine influenza virus. The mice receiving supplemental vitamin A in the diet showed increased resistance to the swine influenza virus. This was indicated by a lower mortality rate and less severe lesions in the mice showing infection. These workers stated that the increased morbidity of rats on inadequate vitamin A was not due to a lowering of the ability of the mice to produce antibodies.

Several authors have reported the effect of vitamin A deficiency upon resistance of the host animal to parasitic infection. Zinsser et al. (1931) found that rickettsia infections were difficult to initiate in rats receiving vitamin A, whereas, rats exhibiting xerophthalmia developed an exudate rich in the organisms following intraperitoneal injections of the rickettsia.

Ackert et al. (1931) have shown that vitamin A included in a diet increased the resistance of chickens to parasitism by Ascaridia lineatia. In a study involving 200 chickens, Ackert et al. (1931) separated the birds into two treatment groups one of which received a semi-synthetic diet free of vitamin A, and the other the same diet supplemented with vitamin A. After only two weeks on test each bird received 500 embryonated eggs of Ascaridia lineatia. Three weeks later all birds were killed and the intestines removed and flushed clean of the worms. The following table indicates the increased incidence and size of the ascarids in the birds on the vitamin A free diet.

TABLE 1. INFLUENCE OF DIET ON NUMBER OF ASCARIDS

	No. of worms	Length of worms (cm)
Vitamin A deficient diet	58.4	49
Positive control	23.6	12
Commercial diet	11.3	6

Hirashi (1928) of Japan investigated the resistance to human ascaris of swine in an avitaminosis A condition and when fed adequate dietary vitamin A. The pigs on the vitamin A deficient diet became parasitized with the human ascaris while the positive control pigs failed to become infested. Payne et al. (1925) had previously reported that human ascarids would not establish a normal reproductive cycle in swine.

Wright (1935) subjected dogs infected with ascarids to a diet either adequate or deficient in vitamin A for periods up to 106 days. The dogs on the deficient diet harbored about five times as many worms as did the control dogs on an adequate diet.

Pande and Krishnamurty (1959) became interested in the inter-relationship between hypovitaminosis and Ascaridia galli infestation in poultry. The birds deficient in vitamin A developed the characteristic clinico-pathological changes of the epithelium. The altered epithelium favored infestation by the Ascaridis galli which in turn further altered the mucosa of the epithelial tissue.

Mori (1922), Wolbach and Howe (1925), Goldblatt and Benischek (1927), Siefried (1930), Castellanos and Beato (1941) and Follis (1953) have reported the histological changes observed in various tissues of the vitamin A deficient animal. In these pathological studies the vitamin A deficient animals exhibit a metaplasia of the normal columnar type of epithelium to the squamous keratinized type in areas of the respiratory, alimentary, and genito-urinary tracts, the para-ocular glands and the eyes.

Richards (1935) stated that changes in the intestinal epithelium are visible to the naked eye following three weeks feeding of a vitamin A free diet. Cramer and Kingsbury (1924) reported that the intestinal mu-

cosal glands undergo atrophy in severe vitamin A deficiency. Bacterial infections were reported to follow the alteration of the epithelium.

Results of intraperitoneal and intravenous injections of bacterial cultures as reported by Boynton and Bradford (1931), Sayedain and Kinsy (1960), Tisdall (1950) and McClung and Winters (1932) indicated that the deleterious effect of a vitamin A deficiency can not be explained entirely on the basis of altered epithelium or a defense at the outer surface of the body.

Guggenheim and Buechler (1946) reported that in vitamin A deficient rats phagocytosis was not altered when measured two hours after bacterial infection, but after four hours the rats receiving vitamin A exhibited a significantly greater level of phagocytosis.

Werkman (1932a) concluded that phagocytosis was not altered by a lack of vitamin A in the diet. Cottingham and Mills (1943) considerably later found that a lack of the combination of vitamin A and vitamin D resulted in a decreased rate of phagocytosis.

Osborn (1932) found a lowering of complement activity in vitamin A deficient rats. However Axelrod and Pruzansky (1955a) have shown complement level to be inhibited by inanition which could possibly account for the results by Osborn and others.

Greene (1933) carried out complement fixation upon blood from vitamin A deficient rabbits and from rabbits on adequate levels of vitamin A. No differences were recorded between the control rabbits and those on a vitamin A deficient diet. Feller et al. (1942) found no reduction of complement in humans with hypovitaminosis A.

Experiments to measure the effect of vitamin A deficiency upon anti-

body production have been carried out by many investigators. Werkman (1923a) was one of the first investigators to turn to measurements of antibody production as a criterion of nutritional adequacy. In a study with 11 rabbits, six of which were fed a diet low in vitamin A, Werkman measured antibody titer to Salmonella typhosa. The diet in this study, consisted of white corn, linseed oil meal, ground oats, casein (alcohol extracted), tankage, calcium carbonate and sodium chloride. Cod liver oil was used as a source of vitamin A. The rabbits remained on this diet for a period of seven weeks before initiating antigen injections. Five injections of antigen (.2, .3, .4, .6 and 1.0 ml.) were administered to the rabbits at seven day intervals. No differences were found between the antibody titers of the control group and the group on a vitamin A deficient diet. Werkman (1923a) then repeated the experiment with rats using a diet consisting of casein (alcohol extracted), dextrin, salt mixture and yeast. Again the antibody titer was just as high in the rats receiving no supplemental vitamin A.

With the same diets as above, Werkman (1923a) next measured hemolysins using rat erythrocytes in rabbits and rabbit erythrocytes in rats. The vitamin A deficient rabbits immunized with the rat erythrocytes showed the same hemolysis response as the controls. However, the vitamin A deficient rats showed slightly lower hemolysin titers than did the controls.

Cramer and Kingsbury (1924) fed diets to rats deficient in either vitamin A or B complex. They reported no decreased agglutinin formation against Escherichia coli or Salmonella typhosa. However, neither the diet nor the length of time the animals were maintained on the diet was

discussed.

Blackberg (1928) has studied the effect of avitaminosis A and B on the immurity of rats. In the first study, Blackberg injected killed cultures of Salmonella typhosa into rats which were on diets either deficient or adequate in vitamin A. The vitamin A deficient rats consistently developed a lower antibody titer than that measured in the controls. In a second study with treatment groups as before Blackberg injected a broth media culture of Salmonella typhosa. The antibody titer showed a higher value for the rats receiving adequate vitamin A when measured one week after the final injection. With further live Salmonella typhosa culture injections the difference in the antibody titer of the two treatment groups diminished. The deficient animals with sufficient stimulation eventually produced antibody titers equivalent to the control rats. In a third study Blackberg used tetanus toxin as an antigen. The vitamin A deficient rats responded with a very meager antibody production compared to a very high titer in the control animals.

Greene (1933) conducted an extensive study of changes of actively acquired immunity under conditions of a vitamin A deficiency. Greene measured hemolysins to ox and sheep erythrocytes and agglutinins to Salmonella typhosa in rabbits receiving either a vitamin A deficient diet or one supplemented with vitamin A. The rabbits were fed the dietary regime until xerophthalmia had been observed in the vitamin A deficient group for several weeks. Two injections of washed sheep erythrocytes were intravenously injected into the rabbits. Eight days after the second injection the hemolysin titer was measured. In the first trial the deficient animals had from 250 - 2000 hemolytic units and the controls from 5000-200,000

units. In a study with ox erythrocytes the deficient rabbits had from 7.5 - 30 hemolytic units and the controls had from 125 - 1250 units. The same author (1933) injected Salmonella typhosa into xerophthalmic and control rabbits and found a somewhat lower antibody titer in the vitamin A deficient rabbits when compared to the controls.

Lassen (1930,1931) working with Salmonella paratyphoid in vitamin A deficient and control rats reported some reduction in agglutination titers in the vitamin A deficient rats. Natvig (1942) conducted a three year study on the influence of vitamin A and B complex upon resistance of rats to Salmonella danycz and Leptospira icterohemorrhagia. He could find no evidence of reduced antibody production in vitamin A deficient rats. Simola and Brunius (1933) reported that the hemolysin titer to sheep erythrocytes was just as high in vitamin A deficient guinea pigs as in controls.

Feller et al. (1942) examined the relationship between low vitamin A levels in humans and antibody production. This study involved only three human patients studied over a period of one year. The antibody titer for these individuals was not different from control patients; however, the plasma vitamin A level of the three patients never fell below 100 IU per 100 milliliters of blood plasma.

Ludovici and Axelrod (1951a) have compared the level of circulating antibodies in rats receiving a complete semi-synthetic diet or diets lacking in pteroylglutamic acid, niacin-tryptophan, vitamins B_{12}, A or D. After the rats had been maintained on the various diets for four weeks immunization was initiated. A 10 percent suspension of washed type O, Rh positive, human erythrocytes was injected intraperitoneally as the antigen.

Five days after the second of two injections the rats were exsanguinated and the antibody titers were determined. The average hemagglutination titer for the control rats was 1984 as compared to 533 for the vitamin A deficient animals.

Axelrod (1953) and Axelrod and Pruzansky (1955a) again measured hemagglutination response by rats on vitamin deficient diets. Axelrod included ad libitum, pair fed and pair weighed controls. The antigen as in the previous study was type O, Rh positive, human erythrocytes injected intraperitoneally. Once again the vitamin A deficient rats were able to produce antibodies but the hemagglutination response was significantly less than in the control animals. The ad libitum, pair fed and pair weighed controls all produced similarly high levels of hemagglutinins.

A later study was carried out by Pruzansky and Axelrod (1955) in which antibody production was measured to diptheria toxoid in vitamin deficient rats. Inanition controls and ad libitum controls were maintained in this study as well as vitamin deficient rats. The rats received a semi-synthetic diet based on vitamin free casein and sucrose. After the rats had been on feed for 12 days, each was given a single intraperitoneal injection of .15 milligrams of alum precipitated diptheria toxoid. Seventeen days later the rats were bled by cardiac puncture. The antibody titers were determined by hemagglutination of sheep red cells treated with tannic acid and coated with diphtheria toxoid. Just as Axelrod (1953) reported in the previous paper, the vitamin A deficient rats produced a considerable lower antibody titer than did the control rats, 1300 and 3900, respectively.

The literature contains much conflicting data as to the importance

of vitamin A in antibody production. One contributing factor may be that an intended vitamin A deficient diet may not always have resulted in such a deficiency. The early work was subject to error in that the true composition of the natural diet as well as quantitative and qualitative requirements were not well established. Some authors have been prone to use only gross criteria in determining a deficiency status. Levels of serum or liver vitamin A were reported only in rare instances.

B. Pantothenic Acid, Pyridoxine and Riboflavin

In an early study Verder (1928) reported that Salmonella enteritidis did not cross the intestinal wall unless the rats were deprived of vitamin B complex of yeast. Rose (1928) reported that many cases of bacteremia caused by Clostridium perfringes were cured by injections of vitamin B complex. Zinsser et al. (1931) using a diet lacking in all the then known B vitamins found that rats and guinea pigs were much more susceptible to murine typhus infection. Ross and Robertson (1932) placed rats either on diets deficient in the B complex or diets in which supplemental B complex had been added. Salmonella murotitis organisms were introduced orally into the rats after the rats had been on trial one week. The mortality was much greater in the rats receiving a diet deficient in the B vitamin complex.

Rose and Rose (1936) observed that dogs receiving from 13 - 33 percent of the required amounts of the B vitamins known at that time became much less resistant to infections of Staphlococcus aureus than were dogs receiving the recommended amounts of B vitamins.

Pinkerton and Bessey (1939) reported that rats maintained on a riboflavin free diet for seven weeks were more susceptible than controls to

murine typhus and were more severely affected by the infection.

Seeler and Ott (1944) have reported a decreased susceptibility to Plasmodium lophurae infection in chicks suffering from riboflavin deficiency. In this study two levels of riboflavin were fed, 20 and 2000 micrograms per 100 grams of diet respectively. After 11 days on the respective diets one-half of the chicks in each treatment was infected with Plasmodium lophurae. The progress of the infection was measured by determining the frequency of parasitized erythrocytes in circulation and mortality of the birds. Three percent of the erythrocytes from the riboflavin deficient birds were parasitized while 17 percent of the cells from the chicks fed high riboflavin levels were parasitized. In a second trial the feed of the chicks on the high riboflavin diet was limited to the quantity of that consumed by the chicks on the low riboflavin diet. The results with the pair fed controls were the same as that reported for the ad libitum feeding trial. Although the parasitism of the riboflavin deficient birds was low, the mortality of these birds was higher (61 percent) than it was among the birds on the high riboflavin diet (29 percent). Uninfected controls on either dietary treatment had a low mortality. The increased mortality of the infected birds was manifested in a manner other than increased parasitism of erythrocytes.

Wooley and Sebrell (1942) found that mice maintained on a diet deficient either in riboflavin or thiamin were more susceptible than ad libitum controls to intranasal infections of Diplococcus pneumoniae. In a similar experiment using intraperitoneal introduction of Diplococcus pneumoniae, Day and McClung (1945) fed a diet deficient in pantothenic acid for from 19 to 38 days. The pantothenic acid deficient rats were no more suscep-

tible than were ad libitum control rats. Seventy-four percent of the pantothenic acid deficient and 69 percent of the controls died. Mortality was the only reported criteria.

Robinson and Siegel (1944) placed rats on a complete control diet and diets deficient either in pantothenic acid or riboflavin. All rats were subjected to infection of Diplococcus pneumoniae just as Woolley and Sebrell (1942) had done. No differences were observed in the resistance by the rats on any of the diets. West et al. (1944) found that pantothenic acid deficient rats exhibited an increased resistance to experimental infection of Diplococcus pneumoniae when introduced by nasal insufflation. They concluded that the pneumococcus required pantothenic acid for growth, and that in the deficient animal the pathogen was unable to obtain sufficient pantothenic acid for maximum growth.

Kligler et al. (1944) demonstrated that when mice were fed a diet deficient in riboflavin the susceptibility to Salmonella typhimurium was greatly increased. Guggenheim and Buechler (1946) supported these findings in a study in which rats were fed a diet low in riboflavin, thiamin or vitamin A. All rats were intra-abdominally injected with Salmonella typhimurium following four to five weeks feeding of the respective diet. A significantly larger number of extracellular bacteria was present in the peritoneal fluid of the riboflavin deficient rats. Guggenheim and Buechler (1946) concluded, without using pair fed controls, that the reduced bactericidal activity of the riboflavin, thiamin and vitamin A deficient rats was due to the reduced feed intake.

Fitzpatrick (1948) found an increased susceptibility of rats to murine typhus infection when fed diets deficient in pantothenic acid, pyri-

doxine or riboflavin. A deficiency of thiamin did not appear to increase the severity of the infection from that of the controls.

Smith and Reynolds (1961) studied the influence of dietary levels of riboflavin from 0 - 150 milligrams per kilogram of diet upon resistance to Leptospira pomona in hamsters. The diets were maintained six to eight weeks prior to experimental intraperitoneal infection. The pathogenicity of the virus culture varied in the three different trials from causing no mortality in the first trial to complete mortality at six days post injection in the third trial. No differences in resistance were recorded between any of the levels of dietary riboflavin.

Watt (1944) introduced infections of Nippostrongylus muris into rats fed ad libitum either a control diet or a diet deficient in riboflavin. The control rats exhibited more resistance to an infection of the parasites than did the riboflavin deficient rats. A second experimental infection of Nippostrongylus muris was introduced and the control rats presented almost a complete immunity to the organism while the riboflavin deficient rats were severely affected by the second infection. Watt (1944) suggested that the decreased resistance to the second infection may be due to a low antibody titer against Nippostrongylus muris.

Several workers have shown that deficiencies of the B vitamins do not lower the resistance to experimental viral infections. Rasmussen et al. (1944) studied the influence of riboflavin deficiency in mice upon experimental poliomyelitis infection. After the mice had received the experimental diet for 14 days they were injected with either Lansing poliomyelitis strain or encephalomyelitis GD VII. No difference in resistance was observed between the control and the riboflavin deficient animals follow-

ing encephalomyelitis GD VII injections; however, the riboflavin deficient mice were less severely affected by the Lansing strain poliomyelitis injections than were the controls.

Lichstein et al. (1944) working on the same research team reported the influence of pantothenic acid deficiency on resistance of mice to experimental virus infection. Cultures of either Lansing strain poliomyelitis, or Theilero encephalomyelitis were injected intracerebrally following two weeks on the pantothenic acid deficient diet. In this study the pantothenic acid deficient mice were less severely affected by the Theilero encephalomyelitis than were the controls. No difference in resistance was observed when Lansing strain poliomyelitis was injected.

Mirick et al. (1949) injected Lansing strain poliomyelitis into rats maintained on a pyridoxine deficient diet. A slight increase in resistance was reported for the pyridoxine deficient rats. Lichstein et al. (1945) could demonstrate no difference between the resistance of pyridoxine deficient mice and the controls following an intracerebral injection of Lansing strain poliomyelitis.

Seronde et al. (1956) studied the pathogenicity of Corynebacterium C-197 in rats maintained on diets deficient in pantothenic acid, pyridoxine or thiamin. The incidence of spontaneous as well as experimental infection was measured involving this normally non-pathogenic organism. Inanition controls, ad libitum controls and infected and non-infected deficient treatments were included in this study. Spontaneous infection of Corynebacterium C-197 was observed only in the rats on the pantothenic acid deficient diets. After the rats had received the experimental diet for 30 days the Corynebacterium C-197 was intraperitoneally injected into the

rats. The experimental infection was fatal to many pantothenic acid deficient rats. The pyridoxine deficient and thiamin deficient rats were less severely affected and no apparent infection was established in the control rats.

In an attempt to determine the humoral factors which may become altered in B vitamin deficiencies many authors have turned to studies of antibody production primarily in rodents. Werkman (1923a) as was pointed out in the vitamin A section, measured antibody production to Salmonella typhosa in rats and rabbits deficient in vitamins A and B. The deficiency of either vitamin did not result in a decreased antibody titer, when the diets were made up of natural foodstuffs.

Morey and Spies (1942) measured antibody response in humans showing clinical manifestation of pellagra, beri beri and riboflavin deficiency. Avirulent forms of Pasteurella tularensis were given in three daily injections to patients testing negative to this organism prior to the initiation of the study. The titers were measured periodically following the antigen injections. The more severe the symptoms of the three B vitamin deficiencies the lower were the measured antibody titers. Also the titers were maintained a shorter period of time in these patients showing the more severe symptoms.

Ruchman (1946) determined the effect of particular vitamin deficiencies on the development of neutralizing antibodies against the virus of Western equine encephalomyelitis in mice. Mice received the control diet or experimental diets deficient in riboflavin, thiamin or total B vitamins for 17 days prior to vaccination with a 4 percent formalin solution of Western equine encephalomyelitis virus. Two weeks later all mice were

bled out and the serum antibody titers measured. The antibody titers were similar for all treatments. The results obtained by Ruchman are consistent with the failure of a deficiency of B vitamins to lower resistance of mice to viral infections.

Stoerk and Eisen (1946) evaluated the influence of a pyridoxine deficiency upon antibody production in rats. In addition to the deficient diet treatment, pair weighed and ad libitum controls were included. All rats received a semi-synthetic diet. During the fifth week of the study all animals were injected with the first of three intraperitoneal injections of sheep erythrocytes. Five days later all rats were exsanguinated and hemagglutination and hemolysin determinations were made. The average hemagglutination titers were 0.4 and 64.0 for the pyridoxine deficient and the pair weighed controls respectively. The hemolysin titers were 13.0 and 412.0 in the same order as above . Six of the nine pyridoxine deficient rats had no measurable antibody titer.

Stoerk et al. (1947) in a similar study measured serum antibody titer in rats or diets deficient in pyridoxine, thiamin, riboflavir, partothenic acid or protein. A semi-synthetic diet was fed to all rats with appropriate omissions for the deficient diets. The first of three intraperitoneal injections of sheep erythocytes was begun during the fifth week of the trial followed by two additional injections on alternate days.

The pyridoxine deficient rats exhibited a significantly lower hemagglutination titer than did the pair fed or ad libitum controls. The response to the sheep erythrocytes was low in all treatments.

Axelrod et al. (1947) measured the titer of circulating antibodies in rats deficient in pantothenic acid, pyridoxine or riboflavin. Pair

fed and ad libitum controls were included along with the deficient diets. All diets were semi-synthetic comprised of vitamin free casein and sucrose fortified. After seven weeks on the experimental diets the rats were immunized with intraperitoneal injections of type O human erythrocytes. Five days later all rats were exsanguinated and hemagglutination titers were determined. The pantothenic acid and the pyridoxine deficient groups failed to produce a measurable hemagglutination titer. Forty-two percent of the riboflavin deficient rats failed to produce a measurable titer and the remainder produced low titers. In contrast, to the work by Stoerk et al. (1947) with sheep erythrocytes the titers of the pantothenic acid deficient group was also inhibited.

Agnew and Cook (1949) measured antibody production in pyridoxine deficient rats. The study in addition included ad libitum, pair fed and pair weighed controls. The rats remained on the respective experimental treatments for six weeks prior to the antigen injection with sheep erythrocytes in one study and formalinized Salmonella typhosa in the second study. The hemagglutination response to the sheep erythrocytes and the agglutin response to the Salmonella typhosa were significantly lower in the rats fed the diet free of pyridoxine than for any other treatment.

The circulating antibodies were compared by Stoerk (1950) in rats receiving a pyridoxine deficient diet and the same diet with deoxypyridoxine added. All rats remained on the experimental treatment three weeks prior to immunization with sheep erythrocytes. Five days following immunization the hemagglutination titers were determined. The rats on the deoxypyridoxine treatment produced a significantly lower titer than did the uncomplicated pyridoxine deficient rats. In a second experiment Stoerk (1950)

immunized both rats and mice 20 weeks before the start of the feeding trial. All rats received deoxypyridoxine and one-half the rats received pyridoxine in the diet. The experimental feeding period was continued three weeks before a second injection of the original antigen was administered. The anamnestic response one week following the injection of the antigen was high in the rats and mice on the adequate diet and not detectable in the deficient animals.

Axelrod et al. (1961) determined the circulating antibodies present in guinea pigs fed diets deficient in pyridoxine, containing deoxypyridoxine or control diets. Injections of .15 milliliters of alum-precipitated diphtheria toxoid preparation were given parenterally after four weeks feeding of the respective diets. Three weeks later the guinea pigs were bled and a second dosage of diphtheria toxoid was administered for measuring secondary response. The antibody titer in both the deficient group and the group receiving the antagonist were severly inhibited when the primary response was measured and somewhat less inhibited when the secondary response was measured.

Ludovici et al. (1949) measured the circulating antibodies in rats deficient in pantothenic acid and pair weighed controls. The study was divided into three groups with each containing both the deficient rats and control rats. The three groups were maintained on the respective diets for three, five or seven weeks before immunization. Type O, Rh positive, human erythrocytes were intraperitoneally injected into the rats and the hemagglutination titers were measured five days post injection. The hemagglutination titer was severely inhibited in all pantothenic acid deficient rats irrespective of the time of immunization from three to seven

weeks. Later, this same group (1951b) investigated possible methods by which a pantothenic acid or a pyridoxine deficiency could inhibit the level of circulating antibodies. They (1951b) first determined that a deficiency of either of the vitamins was not responsible for an increased destruction of antibodies. Fourteen days after being started on a diet containing both pantothenic acid and pyridoxine, rats were immunized with type O, Rh positive, human erythrocytes. After determining the hemagglutination titer, the rats were allotted to a complete diet or to a diet free of either pantothenic acid or pyridoxine. Determination of the titers continued at intervals of two to four weeks until the deficient rats became moribund, which amounted to 12 weeks for the pyridoxine deficient and 18 weeks for the pantothenic acid deficient rats. There was no significant difference between the antibody titers of the pantothenic acid deficient rats, the pyridoxine deficient rats or the control rats at any time during the experiment. Ludovici concluded that deficiencies of pantothenic acid or pyridoxine did not accelerate the destruction of antibodies.

In the second trial Ludovici and his colleagues (1951b) illustrated that deficiencies of pantothenic acid or pyridoxine are not involved in the release of preformed antibodies. Rats fed a diet deficient in either pantothenic acid or pyridoxine for five to six weeks, were immunized with type O, Rh positive, human erythrocytes. After the hemagglutination titers were determined each of the pantothenic acid deficient rats were injected with 10 milligrams of calcium pantothenate and fed 300 micrograms per day for the remainder of the experiment. At the same time the pyridoxine deficient rats received an injection of five milligrams of pyridoxine and an oral dose of 50 micrograms per day. In both deficient groups the growth

stimulating effects of the injected vitamins were immediate. The effects of the injected pantothenate upon serum antibody levels were very gradual as no increase in the antibody titers was observed until 23 days post injection. The titer continued to increase until it reached that of normal controls at about 51 days post injection. The pyridoxine injection into the deficient animals brought about a more rapid initial increase in antibody titer than had been observed for the pantothenic acid deficient group. However, the titer did not increase to that of the normal controls until about 45 days had passed. Ludovici et al. (1951b) concluded from this study that neither of these vitamins appeared to be involved in the release of preformed antibodies.

The enhancing effect of added DL-methionine upon the antibody response of pantothenic acid deficient rats was established by Ludovici et al.(1951a). Two separate trials were conducted in this experiment varying only in the amount of methionine added to the particular diet. Each trial consisted of a positive control diet, positive control plus DL-methionine, pantothenic acid deficient diet and the deficient plus methionine. The levels of DL-methionine added were 2.7 percent in the first trial and 1.4 percent in the second trial. The rats were immunized during the seventh week of the trial with human erythrocytes. None of the unsupplemented pantothenic acid deficient animals possessed serum antibody titers higher than 320. In contrast 60 percent of the pantothenic acid deficient rats supplemented with DL-methionine at either level exhibited titers of 640 or higher. The controls had titers that measured at least 2560. Supplemental DL-methionine did not alter the antibody titers of the normal controls. The addition of methionine to the pantothenic acid deficient

diet did not alleviate the growth depression. In a similar study B-alanine was added to a pantothenic acid deficient diet. The addition had no observable effect upon the antibody titer or increase in weight.

Ludovici and Axelrod (1951b) have also substituted pantothenol for pantothenic acid and found the antibody titer to human erythrocytes was just as high as the normal pantothenic acid controls while the deficient group failed to produce a measurable titer.

Wertman and Sarandria (1951a) using the same semi-purified diets as used by Stoerk (1946) and Ludovici (1949) reported no inhibition of antibody production in rats receiving 10 percent of normally recommended amounts of all the B vitamins. The diet was fed for six weeks prior to the initiation of a series of weekly injections of formalinized Rickettsiae typhi. The rats on the B vitamin limited diet did not grow normally but the serum antibody titer was not inhibited.

In a second study the treatments included diets totally lacking in B vitamins, deficient in pantothenic acid, deficient in thiamin and a normal control. The procedure was just as before except that the titer was measured one week after the first injection and one week after the third injection. Only the pantothenic acid deficient group exhibited a lower titer after the first injection; after the third injection the rats on the deficient diets had similar titers which were only slightly lower than the normal controls.

Wertman and Sarandria (1951b) and Wertman et al. (1952) compared the effects of deficiencies of pyridoxine, nicotinic acid, riboflavin and folic acid upon levels of complement-fixing murine typhus antibodies. After three weeks on the respective diets a series of five weekly injections of forma-

linized suspension of Rickettsiae typhi was commenced. The serum was examined for antibodies one week after the first and fifth injection. The pyridoxine and folic acid deficient rats produced an extremely low complement-fixing titer. The riboflavin deficient rats produced a titer mid-way between that of the pyridoxine deficient rats and the control rats. The niacin deficient, pair fed control and ad libitum controls all produced a uniformly high complement-fixation titer.

Zucker et al. (1956) have measured serum antibody levels in rats fed diets deficient in pantothenic acid, pyridoxine and thiamin. Following 30 days on the experimental diets all rats received the first of three alternate daily intraperitoneal injections of a formalinized suspension of Corynebacterium kutscheri. Five days after the last injection blood was collected from the tail for antibody determination. The pyridoxine deficient rats failed to produce measurable antibody titers, the pantothenic acid deficient rats produced an average titer of less than five and the pair weighed controls had an agglutination titer of 155.

Many investigators have studied the effects of deficiencies of pantothenic acid, pyridoxine or riboflavin on the blood cellular components. Wintrobe et al. (1943) developed pyridoxine deficiency in swine and found a severe anemia which was characterized by microcytosis, an increase in polychromatophilia, nucleated red cells in the blood, a rise in serum iron and bone marrow hyperplasia. Cartwright et al. (1944) found that the anemia was not caused by increased hemolysis but due to an inhibition of hemoglobin formation. The blood studies reported by Hughes and Squibb (1942) showed the characteristic microcytic hypochromic anemia. This work is supported by Fouts et al. (1938) who found that puppies fed a diet de-

ficient in pyridoxine developed a microcytic hypochromic anemia.

Moustgaard (1953) reported a reduction of hemoglobin in pyridoxine deficient animals. Also in this same study Moustgaard observed a decrease in serum gamma globulin in the deficient animals. Stoerk et al. (1952) could find no change in the gamma globulin in pyridoxine deficient animals.

In a cellular study Wertman et al. (1955)observed no differences between the erythrocyte counts of pyridoxine deficient rats and either pair fed or ad libitum controls. However, total leukocytes were increased in the deficient animals and the percent neutrophils was increased. Street et al. (1941) reported that adding pyridoxine to a deficient rat diet brought about an increase in erythrocytes and hemoglobin of the rats.

Beck et al. (1950) also reported decreasing erythrocyte counts and hemoglobin values in pyridoxine deficiencies. They also found a decrease in total leukocytes. This is in agreement with Axelrod and Pruzansky (1954) who also found a decrease in total leukocytes. Miller et al. (1957a) found a significantly decreased hemoglobin and a slightly decreased red blood cell count in pyridoxine deficient pigs.

The presence of xanthurenic acid was first identified in the urine of pyridoxine deficient pigs by Cartwright et al. (1944). Wintrobe et al. (1943) a year earlier had reported a green pigment producing substance in the urine of pyridoxine deficient animals.

Wintrobe et al. (1944) reported a moderate anemia in swine fed a riboflavin free diet. In a study of riboflavin deficient pigs Mitchell et al. (1950) concluded that the most sensitive index of riboflavin deficiency is the increase in concentration of neutrophilic granulocytes. Miller et al. (1956) confirmed these findings and also found an increased

total leukocytes in riboflavin deficient pigs.

Wertman et al. (1957) reported only a slight increase in polymorphonuclear neutrophils and a slight leukopenia in riboflavin deficient rats. No change in the total erythrocyte count was observed. Complement activity was decreased in the riboflavin deficient as well as inanition control rats. Shukers and Day (1943) supported the observation that a riboflavin deficiency was followed by a leukopenia. Axelrod and Pruzansky (1954) found a decreased total leukocyte count.

Wintrobe (1943) reported that a deficiency of pantothenic acid was associated with the development of only a moderate normocytic anemia. Axelrod and Pruzansky (1954) could find no change in total leukocyte count in pantothenic acid deficient rats. Stothers et al. (1955) observed an increase in leukocytes in pantothenic acid deficiency. Recently Furness and Axelrod (1959) have reported a leukopenia and lymphopenia in pantothenic acid deficient animals.

The effect of vitamin deficiencies upon disease resistance has been the subject of a multitude of papers. Just as was the case with vitamin A, research groups have not all agreed as to the importance of particular B vitamins in maintaining resistance to infection.

Agglutination provides just one of the many lines of defense within the body. However, due to the importance and also to the ease of determination, antibody production in relationship to nutrient deficiencies has been one of the defenses more thoroughly studied. Axelrod and Pruzansky (1955a, 1955b) have reviewed much of the research carried on since 1944, mainly with rats, which relates vitamin deficiencies and immunological response as a criterion of disease resistance. He concluded that deficien-

cies of pantothenic acid, pyridoxine or folic acid severely inhibited antibody response while riboflavin and vitamin A deficiencies affected this response somewhat less severely.

III. EXPERIMENTAL PROCEDURE

A. General

The objectives of this experiment were to study the effects of specific nutrient deficiencies upon the serum level of circulating antibodies in swine. Four trials were carried out in this investigation. In trials I and II the effect of a vitamin A deficiency upon serum antibody titers was measured. Trials III and IV consisted of studies in which the effect of deficiencies of pantothenic acid, pyridoxine and riboflavin upon specific serum antibody titers was measured.

The pigs in all trials were weaned at two weeks of age or younger and placed in individual metal cages with wire mesh bottoms and solid sides. These were located in a permanent structure in which the environmental temperature could be maintained at approximately 72° F. In addition, heat lamps were placed over the cages to provide supplemental heat.

All pigs were started on an homogenized liquid synthetic milk diet. Later the same diet with minor modifications was fed in the dry form. Solka-floc was added to the dry ration at 4 percent to increase the bulk of the ration and sodium bicarbonate was removed from the dry rations. The ingredient composition of the ration is listed in Tables 2, 3 and 4. The diet is essentially that used by Miller et al. (1954).

The synthetic milk was prepared by mixing seven kilograms of the dry formula in 28 liters of warm water (70° - 75° C.) which provided a milk with 20 percent solids. The sodium bicarbonate was provided to simplify suspending the casein in solution. Next the cerelose was mixed into solution followed by the lard which had been heated. The fat soluble vitamins were added to the heated lard. The solution was mixed with an

TABLE 2

INGREDIENT COMPOSITION OF RATION

Ingredient	Liquid preparation[a] %	gm.	Dry preparation %
Casein (vitamin free)	30	2100	30
Lard	10	700	10
Minerals[b]	5	350	5
Sodium bicarbonate	1.5	105	-
Cerelose	53.5	3745	51
Solka-floc	-	-	4
Vitamins[c,d]	+	+	+
	100.0	7000	100.0

[a]Formulated to make up a 20 percent solids milk

[b]Mineral mixture composition contained in Table 3

[c]B vitamin mixture composition contained in Table 4

[d]Vitamin A study-Vitamin A palmitate (3960 IU per kilogram of dry diet)
Vitamin D as Viosterol (2480 IU per kilogram of dry diet), menadione and alpha tocopherol added
B vitamin study-Cod liver oil (6070 IU vitamin A and 607 IU vitamin D per kilogram dry diet), menadione and alpha tocopherol added

electric stirrer for 30 minutes and then passed thru a Manton Gaulin homogenizer at a pressure of 2500 - 3000 pounds per square inch. The milk was rapidly cooled in a milk can cooler. To avoid the destruction of any heat labile vitamins the B vitamin solution was not added until the milk had been completely cooled.

Each study consisted of a vitamin depletion phase followed by a vitamin repletion period. The pigs remained in the individual metal cages

TABLE 3

MINERAL MIXTURE OF SYNTHETIC DIET[a]

Ingredient	1000 grams
$CaCO_3$	400
NaCl (.01 / KI)	100
KH_2PO_4	210
$MgSO_4 \cdot H_2O$	50
$MnSO_4 \cdot H_2O$	2
$ZnSO_4 \cdot H_2O$	5
$CuSO_4 \cdot 5H_2O$	1
$FeSO_4 \cdot 7H_2O$	10
$CoCl_2 \cdot 6H_2O$	1
Cerelose	221
	1000

[a]Formulated to constitute 5 percent of the diet

throughout the depletion phase of the trial. After concluding the depletion phase the pigs were grouped according to size and housed in a 20' x 24' Doane-type portable hog house. All the pigs received a corn-soybean oil meal diet fortified to meet the National Research Council (1959) recommendations during repletion. The composition of that diet is included in Table 5. This ration and water were ad libitum fed.

Antigen Administration and Antibody Production

Each depletion and repletion period was concluded when the pigs had been challenged with one of two antigens and the resultant specific serum antibody titers measured. The antigens were injected intraperitoneally on six consecutive days, and one week following the last injection the appro-

TABLE 4

VITAMIN MIXTURE OF SYNTHETIC DIET[a,b]

Ingredient	Amount	Amount per kg. solids
Thiamin	325 mg.	4.6 mg.
Riboflavin	1000 mg.	14.3 mg.
Pyridoxine	325 mg.	4.6 mg.
Ca-pantothenate	1500 mg.	21.4 mg.
Nicotinic acid	1500 mg.	21.4 mg.
Para-amino benzoic acid	1300 mg.	18.6 mg.
Biotin	5 mg.	71.5 mcg.
Folic acid	26 mg.	372.0 mcg.
l-inositol	13 gm.	186.0 mg.
Ascorbic acid	8 gm.	114.0 mg.
Choline chloride	130 gm.	1.86mg.
B_{12} with mannitol (0.1/ B_{12})	10 gm.	143.0 mcg.B_{12}

[a]To be dissolved in 250 milliliters ethyl alcohol and 2250 milliliters water.

[b]Formulated to add 35.7 milliliters per kilogram dry diet

priate agglutination determinations were made. At the conclusion of the repletion phase the same procedure was carried out with a different antigen.

The first antigen was a Salmonella pullorum test antigen which was prepared by Vineland Poultry Laboratories, Vineland, New Jersey for poultry testing. The stock solution had a density equivalent to 50 x McFarland nephelometer, tube one. Prior to injection the stock antigen was di-

TABLE 5

INGREDIENT COMPOSITION OF REPLETION RATION

Ingredient	Percent
Corn	54.3
Soybean oil meal	28.0
Rolled oats	10.0
Dried skim milk	5.0
Trace mineral salt (0.5% zinc)	.5
Limestone	1.0
Dicalcium phosphate	.8
Nopcosol M-1[a]	.25
Aurofac 10	.15
	100.00

[a]Composition of Nopcosol M-1 (5 pounds of pre-mix)

Vitamin A, IU	3,000,000	Niacin, gm	20
Vitamin D_2, IU	1,500,000	Choline Chloride, gm	100
Vitamin E, IU	2,000	Vitamin B_{12}, mg	20
Riboflavin, gm	4	Arsanilic Acid, gm	90
d-Pantothenic acid, gm	8	Zinc bactracin, gm	20

Butylated hydroxy toluene, Manganese, Zinc, Iron, Copper, Iodine, and Cobalt

luted 1:40 in physiological saline. The amount of antigen administered to each pig was proportional to the estimated blood volume of the pigs as determined by Hansard et al. (1951, 1956).

To minimize the opportunity for cross agglutination, an altogether different type antigen was used in the second immunological measurement. The second antigen was type O, Rh positive, erythrocytes obtained from the author. The blood was collected into a citrated flask, spun down, and the plasma drawn off. The remaining cellular material was rinsed twice with

physiological saline and centrifuged. Alsevers solution was finally added to the cells in a sufficient quantity to give a 20 percent erythrocyte solution. Serum antibody titers were determined according to the tube serial dilution technique described by Stafseth et al. (1959). A 0.2 milliliter quantity of serum was serially diluted from 1:5 - 1:5120 and tested with an equal volume of diluted antiger suspension. All agglutination titers reported represent net values determined by deducting the pre-injection titer from the final titer. The prepared tubes for the Salmonella pullorum antibody tests were shaken and then allowed to incubate at 37° C. for 24 hours. They were then placed in a cold room (4° C.) for one hour and then the agglutination titer was determined. The tests for the type O erythrocytes were prepared as the above and allowed to incubate for one hour. The tubes were then cooled and the hemagglutination titer determined.

Serum Protein and Electrophoresis

The determination of serum protein and the electrophoretic components was carried out at the time of initiation of antigen injection, at the close of the depletion, and at the initiation of antigen injection in the repletion phase of the trial.

The serum protein was determined according to the method first described by Waddell (1956). Five lambda of serum was diluted to five milliliters (1:1000) with 0.9 percent NaCl. The reading was made at wavelengths of 215 mμ and at 225 mμ on a Beckman Model DU Spectrophotometer. The absorbance at 225 mμ was subtracted from that at 215 mμ. This difference multiplied by 144 gave the protein concentration in the serum expressed in micrograms per milliliter. This value was then converted to grams percent.

The serum protein fractions were separated on a Spinco, Model R, paper electrophoresis system (Spinco Technical Bulletin 6027A) at room temperature. A constant current of three milliamperes per cell was maintained for 16 hours on Spinco number 300-846 paper strips using a veronal buffer of pH 8.6 and an ionic strength of 0.075. This buffer was made up of 2.26 grams of di-ethyl barbituric acid and 15.4 grams of sodium diethyl barbiturate per liter of distilled water.

Approximately 0.006 milliliters of serum was applied to each paper strip. Following the electrophoretic separation, the strips were dried for 30 minutes at 110° C., dyed with brom phenol blue, rinsed with acetic acid and dried once again. The basic color was developed with ammonium hydroxide and the relative intensities of the separated proteins were determined by scanning with a Spinco Model RB Analytrol with a number five cam.

Serum Vitamin A Determination

Serum samples collected in Trials I and II were analyzed for vitamin A according to the method by Sobel and Snow (1947). Slight modifications were made to adjust the reagents since two milliliters of serum were used. The samples were kept in amber glassware throughout the extraction and the evaporation periods. The serum samples were all analyzed colorimetrically using a Beckman Model DU Spectrophotometer. The color producing reagent in all analyses was 1,3-dichloro-2 propanol(glycerol dichlorhydrin). The samples were analyzed as rapidly as possible, usually within two weeks. None of the samples remained frozen (-23° C.) longer than six weeks before analysis. Heaney (1960) reported only slight loss in vitamin A content after six months of freezing.

Hemoglobin Determination

Hemoglobin was determined by the cyanmethemoglobin method described by Crosby et al. (1954). A 0.02 milliliter sample of blood was collected with a Sahli pipette and diluted in five milliliters of Drabkins solution. Drabkins contains

$NaHCO_3$	1.0 gm.
KCN	50 gm.
$K_3Fe(CN)_6$	200 gm.

diluted to one liter with double distilled water. The light absorbance of the sample was read at 540 mμ on a Bausch and Lomb Spectronic 20.

Hematocrit Determinations

The hematocrit values were determined by the procedure outlined by McGovern et al. (1955). The blood was collected in capillary tubes, sealed with flame and centrifuged at a speed of 12,000 RPM's for five minutes in an International hematocrit centrifuge. The hematocrit readings were taken on an International micro-capillary reader.

Erythrocyte and Leukocyte Counts

The blood for these counts was drawn into the respective "Zero error Hellige tru count" pipettes and diluted accordingly with physiological saline for the erythrocytes and three percent acetic acid for the leucocytes. The counts for erythrocytes and leucocytes were made on a Neubauer counting chamber by the method of Ham (1956). Slides for differential counts were prepared according to the procedure of Wintrobe (1956) using Wright's stain. Values are presented for the percent lymphocytes and polymorphonuclear neutrophils. Two counts of 100 cells each were made on the blood smears by the method described by Ham (1956).

B. Trials I and II. The Effect of Vitamin A Deficiency In Swine Upon Specific Serum Antibody Titer Response

This experiment consisted of two studies involving pigs from three litters in each trial. In Trial I, 21 Hampshire and Hampshire-Duroc pigs were weaned to semi-synthetic milk diet free of vitamin A at five days of age and placed in individual metal cages. The diet was bottle fed five times daily until the pigs were two weeks old. At that time the 16 thriftiest pigs were allotted according to weight, sex and litter to either a complete semi-synthetic milk diet containing 3960 IU vitamin A per kilogram of dry diet or to a vitamin A free diet. The number of feedings was reduced to four per day at this time. Weights were taken weekly throughout the depletion study. During the fifth week the diet was gradually switched to a dry form.

Serum vitamin A levels were determined at weekly intervals starting when the pigs were six weeks old. The original plan had been to initiate antigen injections when the average serum vitamin A for the deficient pigs of a litter dropped to 10 micrograms percent. However, it became obvious that the mortality rate of such pigs would be quite high. In subsequent litters the initiation of antigen injection began when the serum vitamin A level was in the range of 15 - 20 micrograms per milliliter. Prior to the first antigen injection, blood was drawn for initial or background antibody response, total serum protein and electrophoretic protein fractionation. Serum protein and antibody titers for Salmonella pullorum were determined as described, and the pigs were then moved to the Doane-type house and put on the starter ration based primarily on corn and soybean oil meal. The repletion period continued from 45 - 60 days. Once again the serum vitamin A level was determined and the human erythrocyte injections were initiated.

Measurement of the serum hemagglutination titer concluded the repletion phase of the trial.

In Trial II the pigs were again selected from three different litters. However, in an attempt to produce a low serum vitamin A at an earlier age the pigs were removed from the sows at 12 hours of age. It was hoped that by limiting the intake of colostrum which Braude (1951) and Davis et al. (1950) have shown to be extremely high in vitamin A, the pig would acquire less vitamin A for liver storage. As in Trial I all pigs were bottle fed the vitamin A free synthetic milk until two weeks of age at which time they were allotted to either the vitamin A free diet or to the complete diet. Serum vitamin A level determinations were commenced at six weeks of age. In contrast to the findings in Trial I the serum vitamin A values of all the deficient pigs dropped below 15 micrograms per 100 milliliters at six weeks of age. The intraperitoneal injections of Salmonella pullorum were commenced immediately. As in the previous trial pre-injection serum antibody titers and protein values were obtained. Following the serum agglutination determinations the pigs began the repletion phase which continued for 30 - 50 days. Once again antibody response to human erythrocytes was determined.

C. Trials III and IV. The Effect of Deficiencies of Pantothenic Acid, Pyridoxine or Riboflavin in Swine Upon Specific Serum Antibody Titer Response

The studies with the particular B vitamin deficiencies were carried out in two trials. The pigs were weaned to a synthetic milk diet at two weeks of age. The only change in the synthetic diet in trials III and IV other than omission of the particular B vitamin was to provide vitamins A and D in cod liver oil rather than specific sources of each.

Miller et al. (1954,1957a) and Stothers et al. (1955) have shown that deficiencies of the three B vitamins under question can be brought about much more rapidly than can a vitamin A deficiency. Also Harmon et al. (1959), Brown et al. (1961) and Miller et al. (1962) have shown that the young pig is inefficient in producing measurable quantities of antibodies before six weeks of age. In an earlier study Miller et al. (1957b) were unable to show an effect of pantothenic acid, pyridoxine or riboflavin deficiency upon hemagglutinin levels of three to four week old pigs. However, extremely low antibody titer values were measured in all the pigs. Therefore, to avoid developing a deficiency before the pigs were capable of producing measurable antibody titers, weaning to a deficient synthetic diet was delayed until the pigs were two weeks old.

Twenty-four Yorkshire-Hampshire pigs from three litters were started on a synthetic milk diet deficient in the three B vitamins under consideration. The milk was pan fed four times a day until the pigs were four weeks old. At this time 23 of the pigs were allotted to four different dietary treatments: positive control, pantothenic acid deficient, pyridoxine deficient, and riboflavin deficient. The pigs were then gradually switched to the dry form of the diet. After two weeks on the respective diets or a total period for each vitamin deficiency of four weeks, a series of six daily injections of Salmonella pullorum was begun.

Immediately prior to the antigen injections the pigs were bled for determinations of initial or background antibody response, erythrocytes, leukocytes, differentials, total protein and electrophoretic protein fractions. Also at this time urinary xanthurenic acid levels were determined in the pyridoxine deficient group and the controls, according to the meth-

od of Wachstein and Gudaitis (1952), to give an indication of the severity of the deficiency. Twenty-four hour urine collections were made before and after the feeding of 100 milligrams of DL-tryptophan per kilogram of body weight. The pigs were housed in individual metabolism units during the period of urine collection.

Seven days after the last antigen injection, the pigs were bled and the serum agglutination titer, total serum protein and electrophoretic protein fractions were determined once again. The pigs were then moved to the Doane-type house and put on the starter ration. After six weeks the pigs were bled to determine background antibody response and protein values and challenged with six daily injections of a 20 percent solution of type O, Rh positive, human erythrocytes. The hemagglutination titers were measured one week after the last injection to conclude the repletion phase of trial three.

In the final trial 25 Yorkshire-Hampshire pigs from three litters were weaned at two weeks to a synthetic milk diet deficient in pantothenic acid, pyridoxine or riboflavin. At four weeks of age the pigs were placed on dry diets and allotted to the four treatments as in Trial III. In addition, three groups of four pigs (one from each of the three deficient rations and a control constituting a group) were established as pair fed groups with the intake by all pigs within one group being limited to that amount eaten by the pig consuming the least. All pigs were bled at seven weeks of age in this trial for antibody background titer, protein values and cellular components. At that time a series of six daily injections of type O, human erythrocytes was commenced. The order of the use of the antigens was reversed in this study to determine if the choice of antigens had any influ-

ence on the antibody response. The hemagglutination titers were determined seven days post-injection and then a repletion period of between seven and eight weeks was begun. At the close of this period serum antibody levels to Salmonella pullorum were determined one week after the last of six daily injections of the antigen.

IV. RESULTS AND DISCUSSION

A. Vitamin A Studies

Trial I

A summary of the results obtained in this trial is contained in Table 6. The differences between means throughout this trial as well as all the vitamin A and specific B vitamin trials were tested for significance by the studentized range method of Duncan(1955). In particular instances, litter effect differences were removed by using the method of Snedecor (1956) for analyzing multiple classification variance. As evidenced in Figure 1

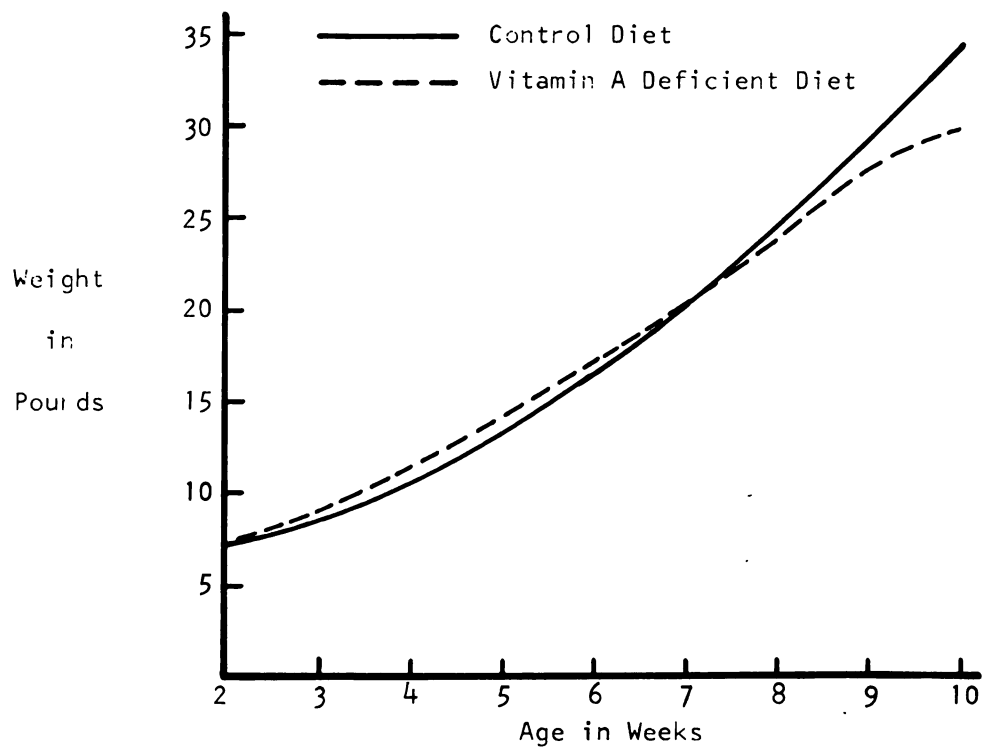

FIGURE 1 GROWTH CURVES OF PIGS ON VITAMIN A DEFICIENT AND CONTROL DIET

TABLE 6

TRIAL I. SUMMARY OF DATA IN VITAMIN A STUDY

	Exptl. Depletion Phase[a]				Exptl. Repletion Phase[a,b]	
	At time of antigen injection		At time of agglutination determination		Final value	
	vitamin A deficient	vitamin A fortified	vitamin A deficient	vitamin A fortified	Previously vitamin A deficient	Previously vitamin A fortified
No. pigs	9	7	9	7	6	7
Av. weight, lbs.	27.3 (±4.2)[c]	27.1 (±4.3)	34.6 (±6.1)	37.5 (±4.4)	92.7 (±14.3)	93.6 (±10.2)
Av. daily gain, lbs.			.46 (±.04)	.54 (±.04)	1.10 (±.19)	1.11 (±.17)
Feed efficiency, lbs.			1.43 (±.15)	1.44 (±.09)		
Av. serum vitamin A(mcg. /)	12.6 (±2.2)	24.1[d] (±3.9)	13.4 (±1.9)	26.5[d] (±3.2)	39.8 (±1.5)	35.3 (±1.5)
Av. serum protein (gm. /)	5.03 (±.21)	4.65 (±.23)	4.72 (±.45)	5.10 (±.28)	5.27 (±.23)	5.20 (±.20)
Av. / albumin	43.8 (±1.93)	43.4 (±2.50)	31.6 (±2.37)	45.1[d] (±1.65)	37.6 (±1.62)	37.0 (±2.45)
Av. / alpha globulin	27.6 (±1.51)	26.6 (±1.59)	34.6[d] (±1.51)	26.8 (±1.21)	23.1 (±.74)	25.4 (±1.37)
Av. / beta globulin	17.3 (±.22)	17.6 (±.78)	19.0 (±1.66)	18.1 (±1.20)	14.4 (±.87)	14.2 (±.68)
Av. / gamma globulin	12.7 (±1.46)	10.3 (±.52)	16.9[d] (±1.41)	9.8 (±1.20)	25.0 (±2.02)	23.1 (±1.61)
Av. net antibody titer			31.1 (±9.2)	377.0[d] (±96.7)	53.3 (±27.8)	77.1 (±19.4)

[a]Diets: Depletion - Semi-synthetic listed in Table 2
Repletion - Natural diet listed in Table 5

[b]All pigs received complete natural diet during repletion phase

[c]Standard error of the mean in parenthesis under mean value

[d]Significantly greater than corresponding value for other treatment ($P<0.01$)

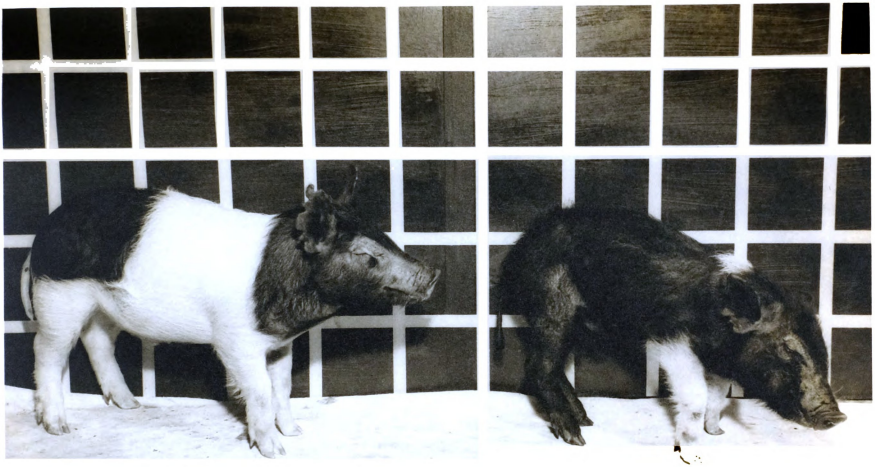

FIGURE 2. TRIAL I. EXAMPLES OF A CONTROL (LEFT) AND A VITAMIN A DEFICIENT (RIGHT) PIG AFTER BEING ALLOTTED TO THE RESPECTIVE TREATMENT FOR NINE WEEKS

and Appendix Tables 1 and 2, there is no statistically significant difference between the weights of the vitamin A deficient group and the control treatment at anytime during the study. However, more weight variation occurred in the pigs on the deficient diet. Daily gain and feed efficiency values were also quite similar in the two treatments. Figure 2 shows a control pig and a deficient pig, which displayed the more overt symptoms of vitamin A deficiency. These symptoms include hyperkeratinization of the epidermis, apparent xerophthalmia and a heavy accumulation of an exudate around the eye. The symptoms were similar to those described by Follis (1958) for vitamin A deficient animals.

Three of the deficient pigs from litter 78 succumbed immediately following the conclusion of the depletion feeding phase of the trial. These pigs had serum vitamin A values of 8.6 micrograms per 100 milliliters or less. Severe diarrhea was observed in each of these pigs shortly before death. Following this experience the series of antigen injections were commenced when the serum vitamin A level of deficient pigs dropped below 20 micrograms per 100 milliliters. This occurred at 10 weeks of age in litter 79 and eight weeks in litter 81. No other pigs died during this trial.

The serum vitamin A levels in all pigs are shown for various ages in Figure 3 and Appendix Table 3. Some fluctuation in the serum vitamin A concentration is evident, but for the most part the deficient pigs show a downward progression of values from week to week throughout the depletion phase of the trial. The serum vitamin A level of the control pigs was significantly higher ($P \leq 0.01$) than that of the deficient pigs at all weekly intervals from 6 to 12 weeks of age. After the pigs had received a com-

plete natural diet for six to seven weeks, the serum vitamin A level of the pigs formerly on the deficient diet increased to the level of the control pigs.

Appendix Tables 4 and 5 contain the individual values of total serum protein and electrophoretic protein components. The total protein values for the vitamin A deficient and control treatments were not statistically different at anytime during the trial. However, at the time the response to Salmonella pullorum antigen was measured (at 11, 12 and 10

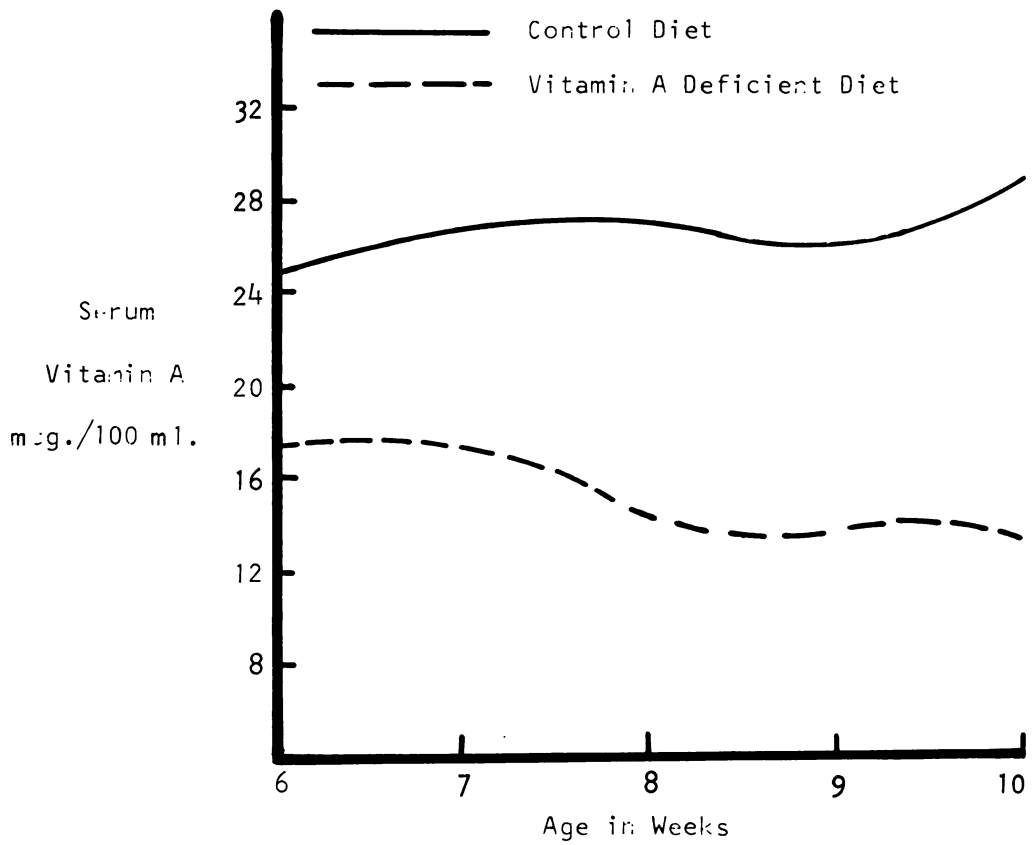

FIGURE 3 SERUM VITAMIN A VALUES OF PIGS ON VITAMIN A DEFICIENT AND CONTROL DIETS

weeks respectively for litters 7[illegible], 79 and 81) the alpha and gamma globulin fractions of the deficient pigs were significantly higher ($P<0.01$) than the same fractions in the control animals. The albumin on the other hand was significantly lower ($P<0.01$) in the deficient pigs. After receiving the complete diet for seven weeks all pigs on either treatment had similar values of serum protein electrophoretic components.

The individual antibody titers produced in response to Salmonella pullorum injections during the depletion phase are shown in Appendix Table 6. Some variation in response to the antigen was experienced in both treatments, however, the antibody titer was significantly higher ($P<0.01$) in the control pigs than in the deficient pigs. The correlation between antibody titers and serum vitamin A levels was found to be .60 and highly significant. In contrast, the correlation between serum vitamin A and rate of gain was .44 and not statistically significant. Similarly, the correlation between agglutination titer and rate of gain was .2[illegible] and not statistically significant.

The response to the injection of human erythrocytes following the repletion feeding period was found to be similar in all pigs. However, the level of antibody response to human erythrocytes by any of the pigs was not as great as the response to Salmonella pullorum by the control pigs.

Trial II

Results of the criteria determined in this trial are summarized in Table 7. The pigs were weaned to a vitamin A deficient synthetic diet at 12 hours of age. As seen in Figure 4 and Appendix Table 7 early weaning appeared to be effective in bringing about a rapid lowering of serum vitamin A level in the deficient pigs. At six weeks of age the serum vitamin

TABLE 7

TRIAL II. SUMMARY OF DATA IN VITAMIN A STUDY

	Exptl. Depletion Phase[a]				Exptl. Repletion Phase[a,b]	
	At time of antigen injection		At time of agglutination determination		Final value	
	vitamin A deficient	vitamin A fortified	vitamin A deficient	vitamin A fortified	Previously vitamin A deficient	Previously vitamin A fortified
No. pigs	7	7	7	7	4	7
Av. weight, lbs.	13.9 (±.40)[c]	14.2 (±.68)	16.4 (±1.01)	21.2[d] (±.74)	55.6 (±6.02)	76.28[e] (±4.80)
Av. daily gain, lbs.			.21 (±.029)	.42[d] (±.009)	.24 (±.08)	1.32[e] (±.045)
Feed efficiency, lbs.			2.31[f] (±.360)	1.24 (±.037)		
Av. serum vitamin A (mcg. %)	12.6 (±1.00)	26.3[d] (±.84)	10.9 (±1.11)	26.6[d] (±1.12)	30.9 (±1.28)	32.4 (±1.33)
Av. serum protein (gm. %)	4.54 (±.09)	4.96 (±.04)	5.24 (±.11)	4.73 (±.19)	5.78 (±.10)	5.70 (±.11)
Av. % albumin	43.9 (±1.01)	49.2[d] (±1.54)	34.9 (±2.21)	45.6[d] (±1.04)	47.5 (±1.32)	51.4 (±1.65)
Av. % alpha globulin	27.4[d] (±.55)	24.1 (±.54)	33.6[d] (±2.01)	27.6 (±.72)	20.4 (±1.09)	20.4 (±.80)
Av. % beta globulin	17.9 (±.89)	1 .2 (±1.60)	17.8 (±1.00)	16.5 (±1.40)	17.9 (±1.15)	18.2 (±1.57)
Av. % gamma globulin	10.8 (±.59)	8.77 (±.93)	13.5[e] (±.70)	10.4 (±1.03)	14.3 (±.58)	9.9 (±.92)
Av. ret antibody titer			16.4 (±4.3)	2[illegible].6[d] (±71.4)	87.5 (±12.0)	74.3 (±9.5)

[a]Diets: Depletion - Semi-synthetic diets listed in Table 2
Repletion - Natural diet listed in Table 5

[b]All pigs received complete natural diet during repletion phase

[c]Standard error of the mean in parenthesis under mean value

[d]Significantly greater (P 0.01) than corresponding value for other treatment

[e]Significantly greater (P 0.05) than corresponding value for other treatment

[f]Significantly more (P 0.05) pounds of feed per pound of gain

A level was significantly lower ($P<0.01$) in the deficient pigs and within the predetermined range (10 - 15 mcg. %) for initiating antigen injections.

In contrast to Trial I the weights of the deficient pigs were significantly lower ($P<0.05$) by seven weeks of age and the differences were highly significant ($P<0.01$) at eight weeks of age. The feed efficiency during this same phase of Trial II was significantly improved ($P<0.05$) in the control over the deficient pigs. After all the pigs had received the complete natural diet for five to six weeks the control pigs still remained significantly heavier ($P<0.05$) than the pigs formerly fed the deficient

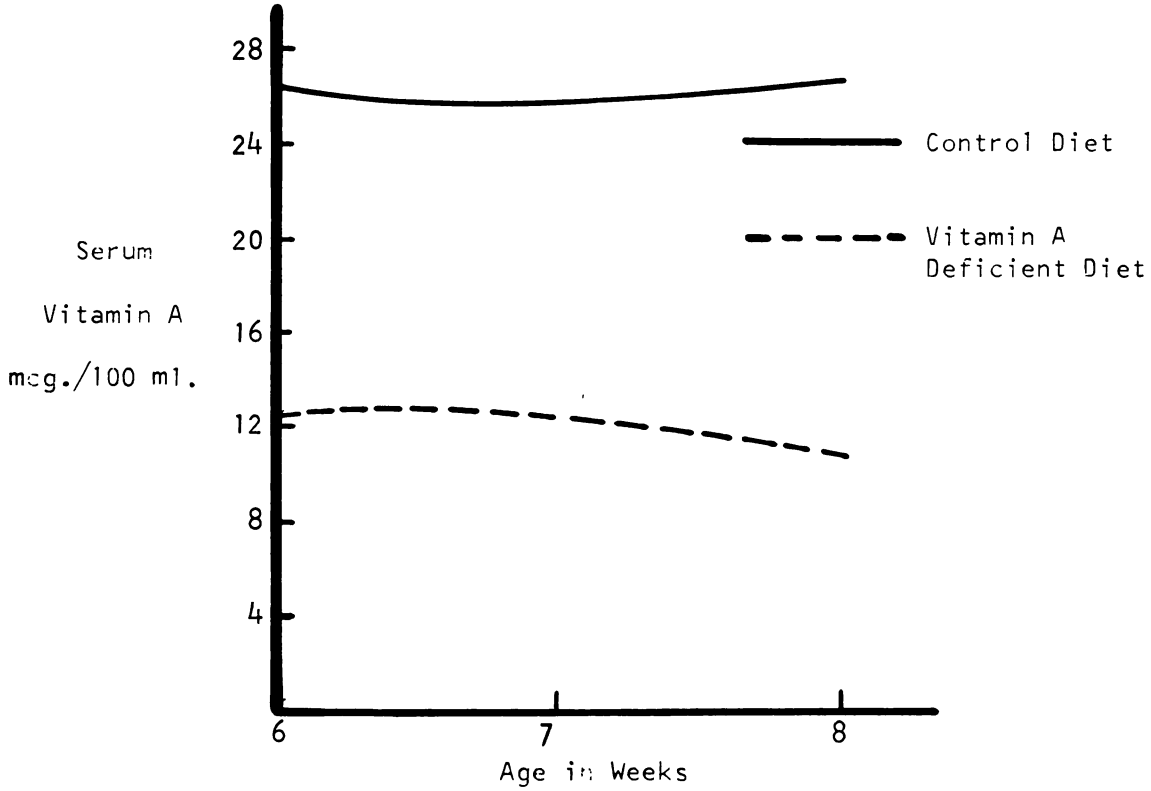

FIGURE 4 SERUM VITAMIN A VALUES OF PIGS ON VITAMIN A DEFICIENT AND CONTROL DIETS

diet; however, the serum vitamin A levels were similar in all pigs. The complete growth data are contained in Appendix Tables 8 and 9 and a graphic presentation is shown in Figure 5.

One deficient pig died at seven weeks of age which was seven days after the first antigen injection and two others died shortly following the antibody response determination. These three pigs evinced much hyperkeratinization of the epidermis, apparent xerophthalmia, faulty locomotion and severe diarrhea. Figure 6 shows a deficient pig that succumbed later in the trial and a control pig.

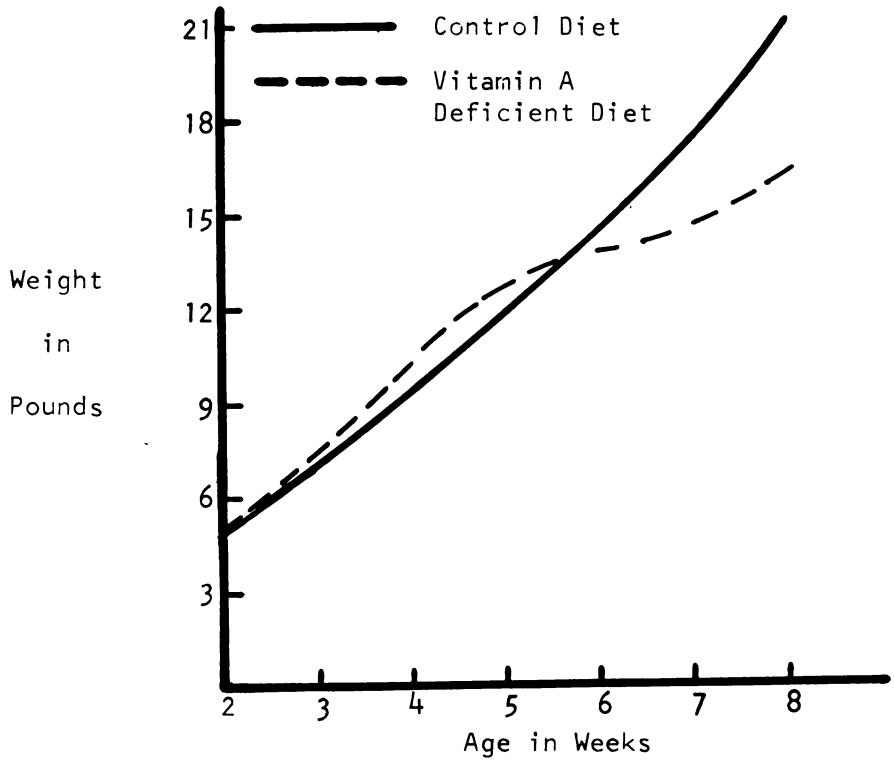

FIGURE 5 GROWTH CURVES OF PIGS ON VITAMIN A DEFICIENT AND CONTROL DIETS

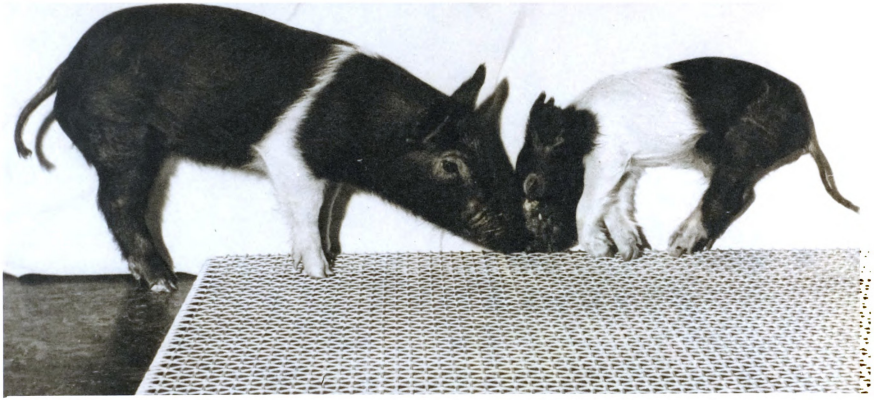

FIGURE 6. TRIAL II. EXAMPLES OF A CONTROL (LEFT) AND A VITAMIN A DEFICIENT (RIGHT) PIG AFTER BEING ALLOTTED TO THE RESPECTIVE TREATMENT FOR SIX WEEKS

The serum analyses determined at different intervals during the trial indicated that the total protein concentration was not affected by the removal of vitamin A from the diet. On the other hand, the alpha and gamma globulin fractions as presented in Appendix Table 10 were found to be significantly greater ($P<0.01$) and ($P<0.05$) respectively in the vitamin A deficient pigs and the albumin in these same pigs was significantly lower ($P<0.01$). The highly significant differences were found after both six and eight weeks of feeding the vitamin A deficient diet. However, after five to six weeks on the complete natural diet as presented in Appendix Table 11 the percentages of the protein electrophoretic components were similar in all pigs.

The mean values for the net agglutination titers to Salmonella pullorum following depletion were significantly higher ($P<0.01$) for the control than for the deficient pigs(240 and 16 respectively).

Statistical analysis of the antibody titers and serum vitamin A values showed a statistically significant correlation of .67 between the two. In this study the rate of gain and serum vitamin A had a correlation of .86 which was much higher than was found in Trial I. Following the repletion feeding period, challenge with human erythrocytes brought about hemagglutinin responses which were not significantly different in the two treatments. All individual antibody titers for both phases of the trial are compiled in Appendix Table 12.

The serum vitamin A values in each of the trials for the control pigs agree closely with the normal values of between 15 and 32 micrograms per 100 milliliters as reported by Hentges (1952). In each of the trials the serum vitamin A level in the deficient pigs had fallen to significantly lower ($P<0.01$) levels by the time vitamin A determinations were begun at

six weeks of age. Apparently the drop in serum vitamin A was most rapid during early deprivation. Foot et al. (1939) found that the liver vitamin A stores of baby pigs became exhausted at eight weeks of age when the dam received a vitamin A deficient diet for two weeks prior to parturition. In the second trial weaning to a synthetic vitamin A free diet at 12 hours of age (thereby limiting the total intake of vitamin A rich colostrum) resulted in a considerably lower serum vitamin A level at six weeks than was meas-

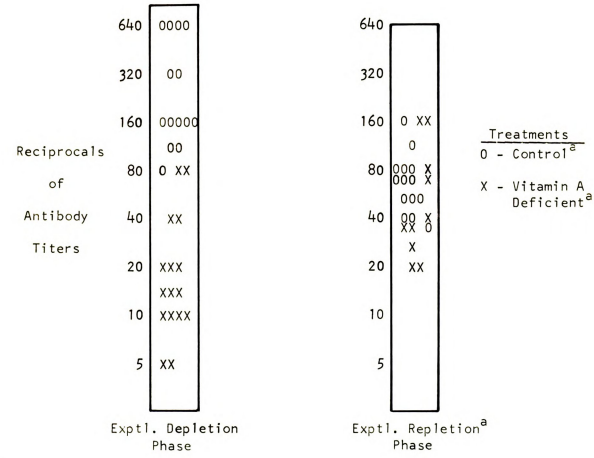

FIGURE 7 TRIAL I AND II. INDIVIDUAL NET ANTIBODY TITERS PRODUCED AGAINST SALMONELLA PULLORUM IN THE DEPLETION PHASE AND HUMAN ERYTHROCYTES IN THE REPLETION PHASE

[a]All pigs received complete natural diet during repletion

ured in Trial I. Moore and Berry (1944) with dairy calves and Heaney (1960) with baby pigs have reported as much as a five fold increase in serum vitamin A the first 24 hours of life.

The deficient pigs upon receiving the complete natural diet containing 2300 IU per pound responded rapidly in appearance and in serum vitamin A levels. The correlation between antibody titer and serum vitamin A levels in Trials I and II were similar, .60 and .67 respectively. However, the correlation between weight gain and serum vitamin A was low (.44) in the Trial I and quite high (.86) in Trial II. The results of Trial II strongly suggest that serum vitamin A and antibody titers respond more rapidly to vitamin A additions than does weight gain. A scatter diagram in Figure 7 clearly shows the differences in antibody production of control and deficient pigs in the depletion phase and the increased similarity following repletion.

The inhibiting effect of vitamin A deficiency upon antibody production in pigs was even more pronounced in this study than was reported by Axelrod et al. (1947) with rats. However, Ludovici and Axelrod (1951) fed a deficient diet for only four weeks and no attempt to measure either serum or liver vitamin A values was reported.

B. Pantothenic Acid, Pyridoxine, and Riboflavin Studies

Trial III

A summary of the results obtained in this trial is presented in Tables 8, 9 and 10. The riboflavin and pyridoxine deficient pigs were significantly ($P<0.05$) lighter than the control pigs after having received the deficient diet for only three weeks. One week later, at six weeks of

TABLE 8

TRIAL III. SUMMARY OF GROWTH AND SERUM PROTEIN VALUES IN B VITAMIN STUDY

	Exptl. Depletion Phase[a] Dietary Treatment				Exptl. Depletion Phase[a,b] Dietary Treatment			
		Deficiency				Deficiency		
	Complete	B_6	Ribo	P. A.	Complete	B_6	Ribo	P. A.
No. pigs	5	5	5	5	5	5	4	4
Final weight, lbs.	26.8[d] (±1.97)[c]	20.4 (±2.03)	17.6 (±2.26)	16.8 (±1.65)	87.2[d] (±2.99)	70.5 (±6.40)	65.8 (±4.18)	70.2 (±2.28)
Av. daily gain, lbs.	.45[d] (±.08)	.25 (±.10)	.17 (±.09)	.15 (±.04)	1.46[d] (±.04)	1.20 (±.12)	1.15 (±.05)	1.24 (±.05)
Feed efficiency, lbs.	1.58 (±.11)	2.61 (±.23)	5.25[e] (±1.37)	3.34 (±.70)				
Av. serum protein, gm. %	6.34 (±.06)	6.58 (±.25)	6.02 (±.12)	6.01 (±.31)	7.00 (±.24)	6.62 (±.22)	6.32 (±.21)	6.70 (±.19)
Av. % albumin	41.2 (±1.52)	46.4 (±2.26)	46.3 (±2.58)	41.7 (±4.38)	48.5 (±1.16)	51.9 (±3.05)	51.4 (±2.81)	48.9 (±1.99)
Av. % alpha globulin	23.0 (±.50)	22.3 (±2.17)	24.0 (±.43)	25.1 (±4.97)	19.5 (±.81)	16.9 (±1.11)	18.3 (±.40)	18.6 (±1.12)
Av. % beta globulin	19.1[d] (±.46)	14.1 (±.58)	15.2 (±.57)	16.4 (±1.15)	13.1 (±.65)	11.9 (±.73)	12.1 (±.69)	12.4 (±.73)
Av. % gamma globulin	16.7 (±1.52)	17.3 (±2.57)	13.5 (±1.93)	16.8 (±2.70)	18.9 (±.70)	19.3 (±2.17)	18.2 (±2.06)	20.1 (±1.64)

[a]Diets: Depletion - Semi-synthetic diets listed in Table 2
Repletion - Natural diet listed in Table 5

[b]All pigs received complete natural diet during repletion phase

[c]Standard error of the mean in parenthesis under mean value

[d]Significantly greater ($P<0.05$) than corresponding value for all other treatment groups

[e]Significantly more pounds of feed per pound of gain than control or pyridoxine deficient groups ($P<0.05$)

age, the pigs on each of the experimental treatments weighed significantly less ($P<0.05$) than did the control pigs and the differences were highly significant ($P<0.01$) between the riboflavin deficient pigs and the controls. Appendix Table 13 and Figure 8 present the changes in weight with the progression of the trial. The riboflavin deficient pigs not only gained slowly but also required significantly ($P<0.05$) more feed per pound of gain during depletion than did the control or pyridoxine deficient pigs.

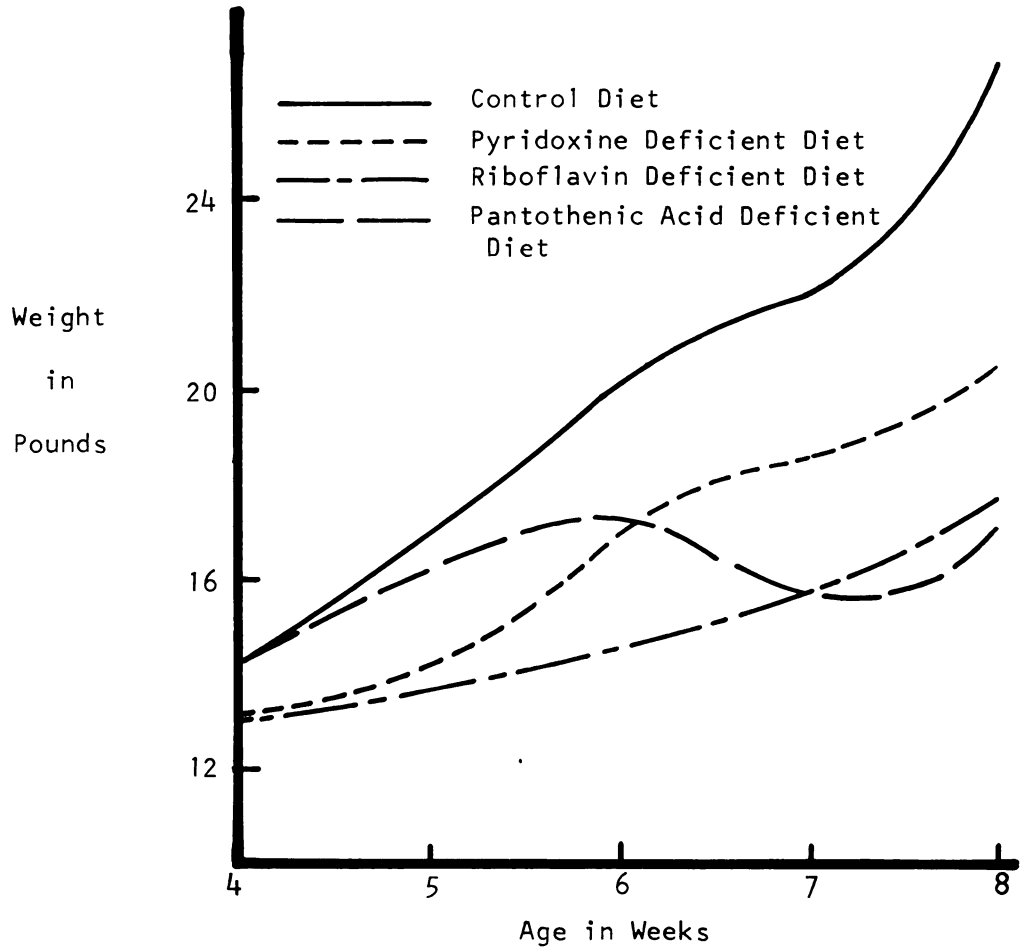

FIGURE 8 GROWTH CURVES OF PIGS ON PANTOTHENIC ACID, PYRIDOXINE, RIBOFLAVIN DEFICIENT OR CONTROL DIETS

TABLE 9

TRIAL III. BLOOD CELLULAR VALUES IN B VITAMIN STUDY

	Experimental Depletion Phase			
	Dietary Treatment[a]			
		Deficiency		
	Complete	B_6	Ribo	P. A.
Hematocrit, %	44.1 (±1.20)[b]	34.0[c] (±3.14)	44.8 (±1.72)	37.7 (±3.67)
Hemoglobin, gm. %	12.93 (±1.00)	10.10 (±1.05)	13.16 (±.51)	13.04 (±1.34)
Erythrocytes, 10^6	7.13 (±.42)	6.39 (±.70)	8.69 (±.54)	8.61 (±1.05)
Leukocytes, 10^3	25.86 (±1.86)	18.57[c] (±1.91)	26.58 (±2.44)	24.09 (±5.50)
Differential				
Lymphocytes, %	49.0 (±2.60)	61.2 (±6.60)	37.8[d] (±5.44)	52.2 (±2.06)
Neutrophils, %	48.2 (±2.05)	37.5 (±5.55)	61.0[e] (±5.60)	44.4 (±2.82)

[a]Diets listed in Table 2

[b]Standard error of the mean in parenthesis under mean value

[c]Significantly lower ($P<0.05$) than corresponding value for riboflavin deficient or control pigs

[d]Significantly lower ($P<0.05$) than corresponding value for pyridoxine deficient pigs

[e]Significantly higher ($P<0.05$) than corresponding value for pyridoxine or pantothenic acid deficient pigs

Depressed gains observed in pigs deficient in pantothenic acid, pyridoxine or riboflavin were not quickly overcome by feeding a complete natural diet. As shown in Appendix Table 13, after six weeks of feeding a complete diet, the pigs formerly designated controls were still significantly heavier ($P<0.05$) than were the pigs that had been on the three

deficient diets. Two pigs which were on the pantothenic acid deficiency treatment died during the course of this study.

The data obtained from blood cellular studies as presented in Appendix Table 14 and summarized in Table 9 show the hematocrit of the pyridoxine deficient pigs to be significantly lower than for the control or riboflavin deficient pigs. The pyridoxine deficient pigs had a lower average value for hemoglobin and total erythrocytes though the differences were not significant. The average leukocyte count in the pyridoxine deficient pigs was significantly lower ($P<0.05$) than in the riboflavin deficient or control pigs. A further analysis of the leukocytes by differential count showed the riboflavin deficient pigs to have significantly lower ($P<0.05$) lymphocytes than pyridoxine deficient pigs and higher ($P<0.05$) polymorphonuclear neutrophils than the pantothenic acid or pyridoxine deficient pigs. The latter observation agrees with the findings of Mitchell et al. (1950) of an increased percent of neutrophils in a riboflavin deficient condition. Miller (1956) also reported an increased percentage of neutrophils in baby pigs fed no riboflavin.

The feeding of diets deficient in pantothenic acid, pyridoxine or riboflavin for either four or six weeks did not result in a change of total serum protein, albumin, alpha globulin or gamma globulin. However, the percentage of serum beta globulin was significantly higher ($P<0.05$) in the control pigs than in any other treatment. The statistical differences between mean values of the serum beta globulin fraction were no longer evident after four weeks feeding of a complete natural diet. The individual protein determinations made periodically in this trial are contained in Appendix Tables 15, 16 and 17.

TABLE 10

TRIAL III. RECIPROCALS OF NET ANTIBODY TITERS IN B VITAMIN STUDY

	Dietary Treatment[a]			
		Deficiency		
	Complete	B_6	Ribo	P. A.
Experimental depletion phase	176[c] (±16.0)[b]	33 (±9.5)	23 (±7.2)	15 (±6.3)
Experimental repletion phase	60 (±12.6)	65 (±24.5)	56 (±14.4)	33 (±4.8)

[a]Diets: Depletion - Semi-synthetic diets listed in Table 2
Repletion - Natural diet listed in Table 5

[b]Standard error of the mean in parenthesis under mean value

[c]Significantly greater ($P < 0.01$) than corresponding value for all other treatment groups

Data obtained from urine studies indicate that urinary xanthurenic acid concentration is an excellent indication of pyridoxine insufficiency. The pigs receiving a diet free of pyridoxine for six weeks had average xanthurenic acid levels of 278 micrograms per milliliter while the control fed pigs had only 20 micrograms per milliliter These values were obtained after feeding of an oral dose of tryptophan However, even without additional tryptophan the pyridoxine deficient pigs excreted significantly greater amounts ($P < 0.05$) of xanthurenic acid than did the controls.

The antibody production to the Salmonella pullorum antigen in pigs on the three vitamin deficiency treatments was extremely low as is shown in Table 10 and Appendix Table 18 and the means were highly significantly lower ($P < 0.01$) than for the control pigs. However, as had been observed in the vitamin A study all pigs were able to form hemagglutinins after receiving complete natural diets for six weeks. Figure 9 presents the

different net agglutinin and hemagglutinin titers in a graphic form.

Trial IV

The data compiled in this study are summarized in Tables 11, 12 and 13. In contrast to the previous study the average weight of the control pigs did not become significantly heavier ($P<0.05$) until the deficient pigs had been on test seven weeks. The pigs were nine weeks of age at this time. Appendix Table 19 contains the individual feed and growth data for this trial and Figure 10 presents the growth data graphically. The control pigs

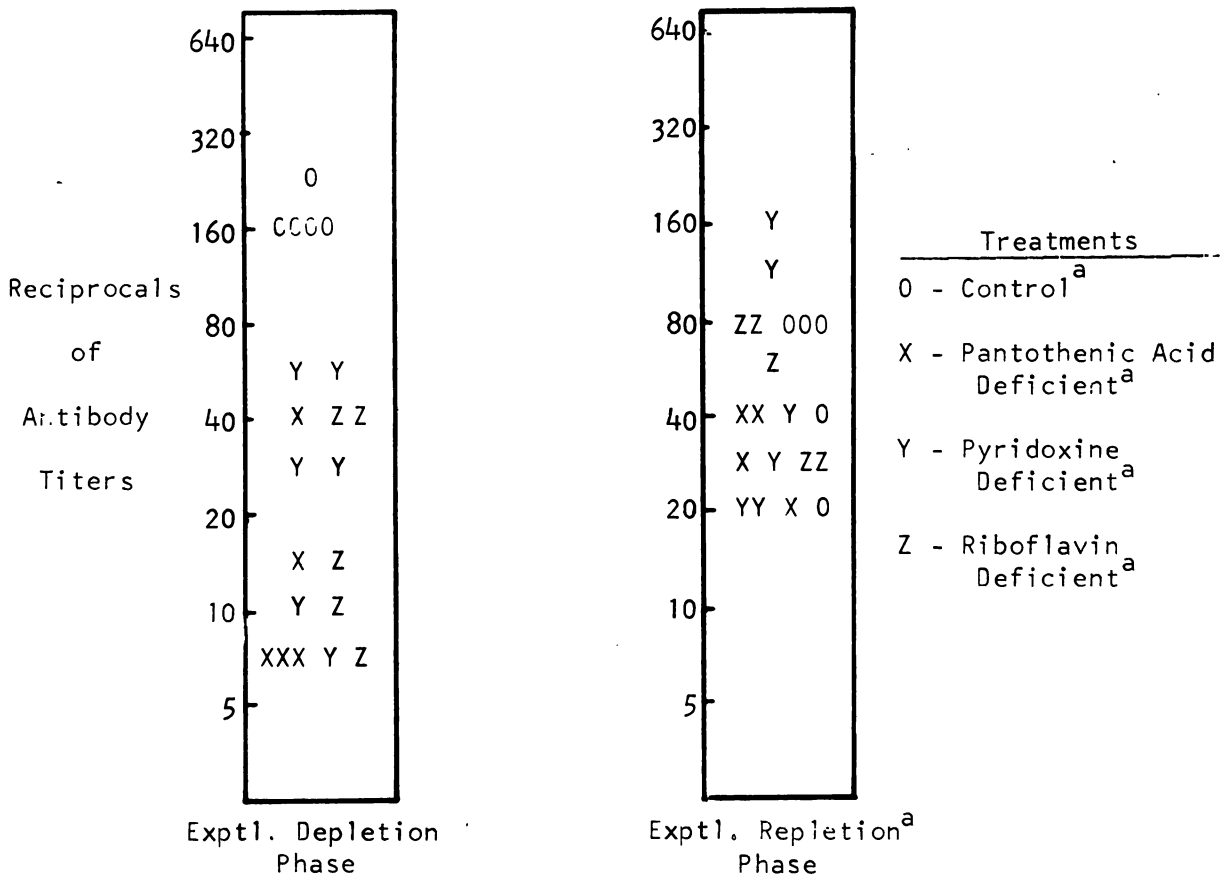

FIGURE 9 TRIAL III. INDIVIDUAL NET ANTIBODY TITERS PRODUCED AGAINST SALMONELLA PULLORUM IN THE DEPLETION PHASE AND HUMAN ERYTHROCYTES IN THE REPLETION PHASE

[a]All pigs received complete natural diet during repletion

TABLE 11

TRIAL IV. SUMMARY OF GROWTH AND SERUM PROTEIN VALUES IN B VITAMIN STUDY

	Exptl. Depletion Phase[a] Dietary Treatment				Exptl. Repletion Phase[a,b] Dietary Treatment			
		Deficiency				Deficiency		
	Complete	B_6	Ribo	P. A.	Complete	B_6	Ribo	P. A.
No. pigs	6	4	4	4	6	3	4	2
Final weight, lbs.	26.5[d] (±1.87)[c]	21.9 (±2.29)	20.8 (±2.16)	19.5 (±.63)	117.0[e] (±5.67)	105.0 (±5.18)	99.3 (±11.50)	85.0 (±1.01)
Av. daily gain, lbs.	.41[d] (±.037)	.28 (±.043)	.28 (±.020)	.20 (±.016)	1.58[e] (±.07)	1.44 (±.09)	1.35 (±.16)	1.12 (±.02)
Feed efficiency, lbs.	1.32[f] (±.06)	1.95 (±.30)	1.78 (±.08)	2.67 (±.27)				
Av. serum protein, gm. %	5.02 (±.40)	5.22 (±.33)	4.90 (±.43)	4.80 (±.34)	6.67 (±.31)	6.47 (±.13)	6.20 (±.35)	6.90 (±.10)
Av. % albumin	55.8 (±2.33)	48.4 (±4.66)	52.5 (±1.74)	46.6 (±7.42)	48.4 (±1.61)	46.5 (±.49)	43.1 (±3.01)	47.0 (±6.70)
Av. % alpha gobulin	18.9 (±.96)	25.7 (±2.53)	22.1 (±.88)	29.4[g] (±5.00)	18.1 (±.83)	19.6 (±1.78)	21.0 (±1.72)	19.6 (±1.25)
Av. % beta globulin	14.0 (±.57)	12.7 (±1.02)	13.9 (±.65)	14.1 (±1.21)	13.9 (±.87)	13.6 (±.29)	14.3 (±.46)	14.1 (±1.70)
Av. % gamma globulin	11.2 (±1.34)	13.2 (±1.77)	11.1 (±.48)	9.8 (±1.19)	19.6 (±1.00)	19.6 (±1.80)	21.6 (±1.60)	19.2 (±3.80)

[a]Diets: Depletion - Semi-synthetic diets listed in Table 2
Repletion - Natural diet listed in Table 5

[b]All pigs received complete natural diet during repletion phase

[c]Standard error of the mean in parenthesis under mean value

[d]Significantly greater ($P < 0.05$) than corresponding value for all other treatment groups

[e]Significantly greater ($P < 0.05$) than corresponding value for pantothenic acid deficient pigs

[f]Significantly less ($P < 0.05$) pounds of feed per pound of gain than all other treatment groups

[g]Significantly greater ($P < 0.05$) than corresponding value for control pigs

were significantly superior ($P<0.05$) in daily gain and feed efficiency than were the pigs deficient in pantothenic acid, pyridoxine or riboflavin.

The mortality rate among deficient pigs was much higher in this trial than in Trial III. This increased mortality may be related to the fact that Trial IV lasted one week longer than the previous trial. One pig each from the pantothenic acid and pyridoxine and two from the riboflavin deficient groups died during the last week of the depletion portion of the trial. In addition, two pantothenic acid deficient pigs and one pyridoxine

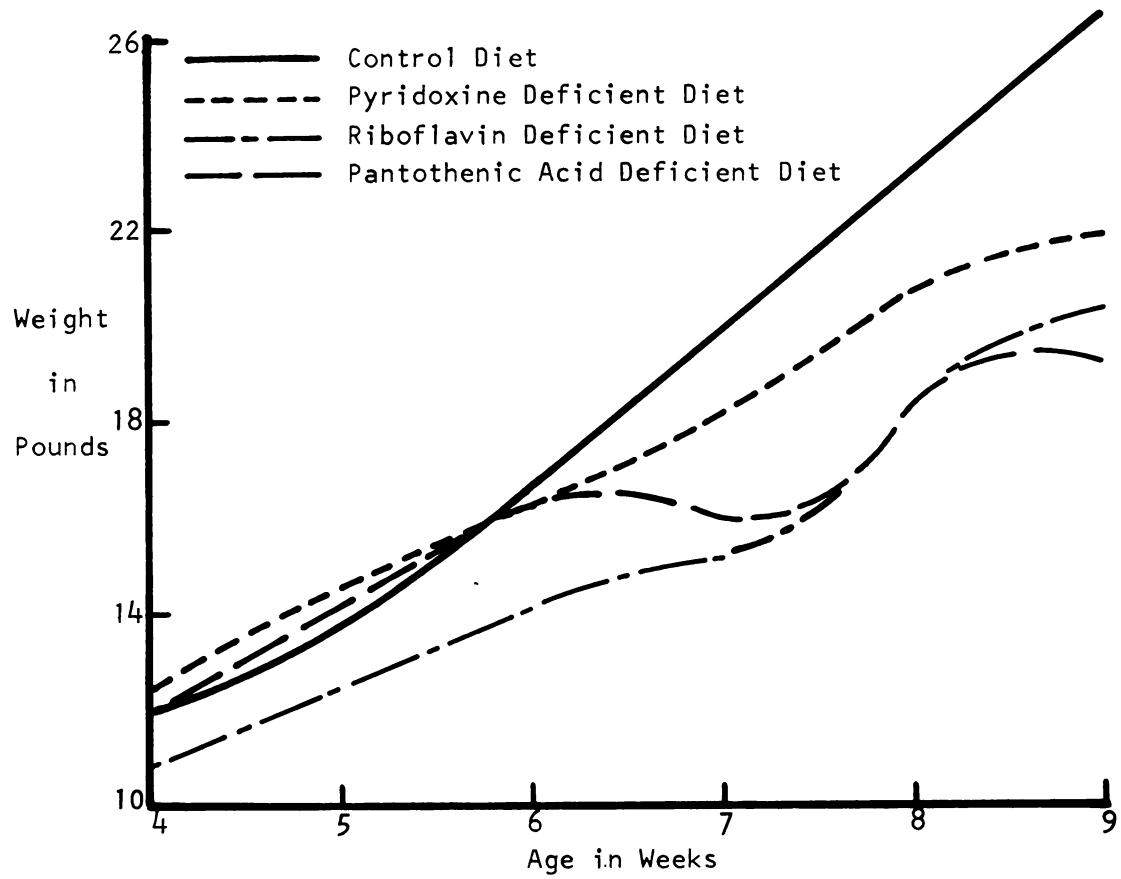

FIGURE 10 GROWTH CURVES OF PIGS ON PANTOTHENIC ACID, PYRIDOXINE, RIBOFLAVIN DEFICIENT OR CONTROL DIETS

deficient pig succumbed shortly after being placed on the complete natural diet. Since the control designated pigs remained significantly heavier than the other pigs after six weeks of repletion feeding in Trial III, the repletion feeding period was extended to seven weeks in Trial IV. However, in spite of the lengthened repletion period the pigs that had previously been on the pantothenic acid deficient diet still remained significantly lighter than the control designated pigs.

TABLE 12

TRIAL IV. BLOOD CELLULAR VALUES IN B VITAMIN STUDY

	Experimental Depletion Phase			
	Dietary Treatment[a]			
		Deficiency		
	Complete	B_6	Ribo	P. A.
Hematocrit, /	38.7 (±1.15)[b]	33.1 (±3.03)	39.5 (±2.14)	37.1 (±1.14)
Hemoglobin, gm. /	11.90 (±1.11)	9.65 (±1.22)	12.25 (±.51)	11.12 (±.50)
Erythrocytes, 10^6	7.89 (±.21)	7.58 (±.24)	8.30 (±.48)	8.01 (±.51)
Leukocytes, 10^3	18.56 (±1.87)	14.04 (±1.89)	16.30 (±2.56)	15.80 (±5.74)
Differential				
Lymphocytes, /	77.3 (±2.47)	66.6 (±8.10)	71.0 (±4.59)	69.0 (±1.40)
Neutrophils, /	17.8 (±1.85)	31.2 (±7.75)	26.7 (±4.10)	27.8 (±1.99)

[a]Diets listed in Table 2

[b]Standard error of the mean in parenthesis under mean value

The hematocrit, hemoglobin, erythrocyte and leukocyte values were lower in the pyridoxine deficient pigs than for the other treatements though the differences were not statistically significant. The percent of polymorphonuclear neutrophils was not significantly greater in the riboflavin deficient pigs over the other treatments as had been observed in Trial III. The blood cellular values collected on individual pigs in this trial are compiled in Appendix Table 20 and summarized in Table 12.

Analyses of the serum proteins indicated that no significant differences existed between any treatments at any time during the trial for total protein, albumin, beta globulin or gamma globulin. However, the pantothenic acid deficient pigs had a significantly greater (P 0.05) alpha globulin fraction than the riboflavin deficient group after five weeks and the control group after seven weeks of feeding the deficient diets. After feeding the complete repletion diet for seven weeks, differences in alpha globulin fraction no longer existed. Appendix Tables 21, 22 and 23 contain the individual serum protein data for Trial IV.

The xanthurenic acid values collected from the urine of pyridoxine deficient and control pigs paralleled closely the concentrations reported in Trial III. The pyridoxine deficient pigs were excreting significantly larger (P<0.01) amounts of xanthurenic acid than were the control pigs before tryptophan dosage in the diet. The addition of tryptophan was followed by a three fold increase in urinary xanthurenic acid in the pyridoxine deficient pigs and no change in the control pigs. The xanthurenic acid values for Trials III and IV are contained in Appendix Table 24. Apparantly the metabolic lesion in the conversion of tryptophan to niacin was sufficiently severe to inhibit conversion of the amount of tryptophan

present in casein. Miller et al. (1957a) suggest that increased xanthurenic acid in urine is a more sensitive test for pyridoxine adequacy than is pig growth.

TABLE 13

TRIAL IV. RECIPROCALS OF NET ANTIBODY TITERS IN B VITAMIN STUDY

	Dietary Treatment[a]			
		Deficiency		
	Complete	B_6	Ribo	P. A.
Experimental depletion phase	67[c] (±9.6)[b]	4 (±2.5)	12 (±1.9)	6 (±1.6)
Experimental repletion phase	267 (±38.1)	200 (±23.1)	165 (±26.3)	200 (±40.0)

[a] Diets: Depletion - Semi-synthetic diets listed in Table 2
Repletion - Natural diet listed in Table 5

[b] Standard error of the mean in parenthesis under mean value

[c] Significantly greater ($P < 0.01$) than corresponding value for all other treatment groups

The response to type O, Rh positive, human erythrocytes (Figure 11) by the pigs in the four treatment groups followed the same pattern as was established during the depletion phase of Trial III. However, the net hemagglutinin response was always proportionately smaller than the net agglutinin response to Salmonella pullorum. The individual values are presented in Appendix Table 25 and summarized in Table 13. Background titers determined previously to the antigen injections were uniformly low measuring less than 1:10 in all treatments. The average net hemagglutination titers were significantly higher ($P < 0.01$) in the control lot than in any of the lots on deficient diets. However, at the conclusion

of the seven week repletion period the uniform antibody titers produced to Salmonella pullorum demonstrated that the formerly deficient pigs do regain the ability to produce measurable antibody titers.

The paired feeding studies which were included as a part of Trial IV clearly established that anorexia was not a predisposing factor to decreased antibody production in pigs deficient in pantothenic acid, pyridoxine or riboflavin. With equivalent feed intake the control pigs produced a hemagglutinin titer that was significantly higher ($P<0.01$) than

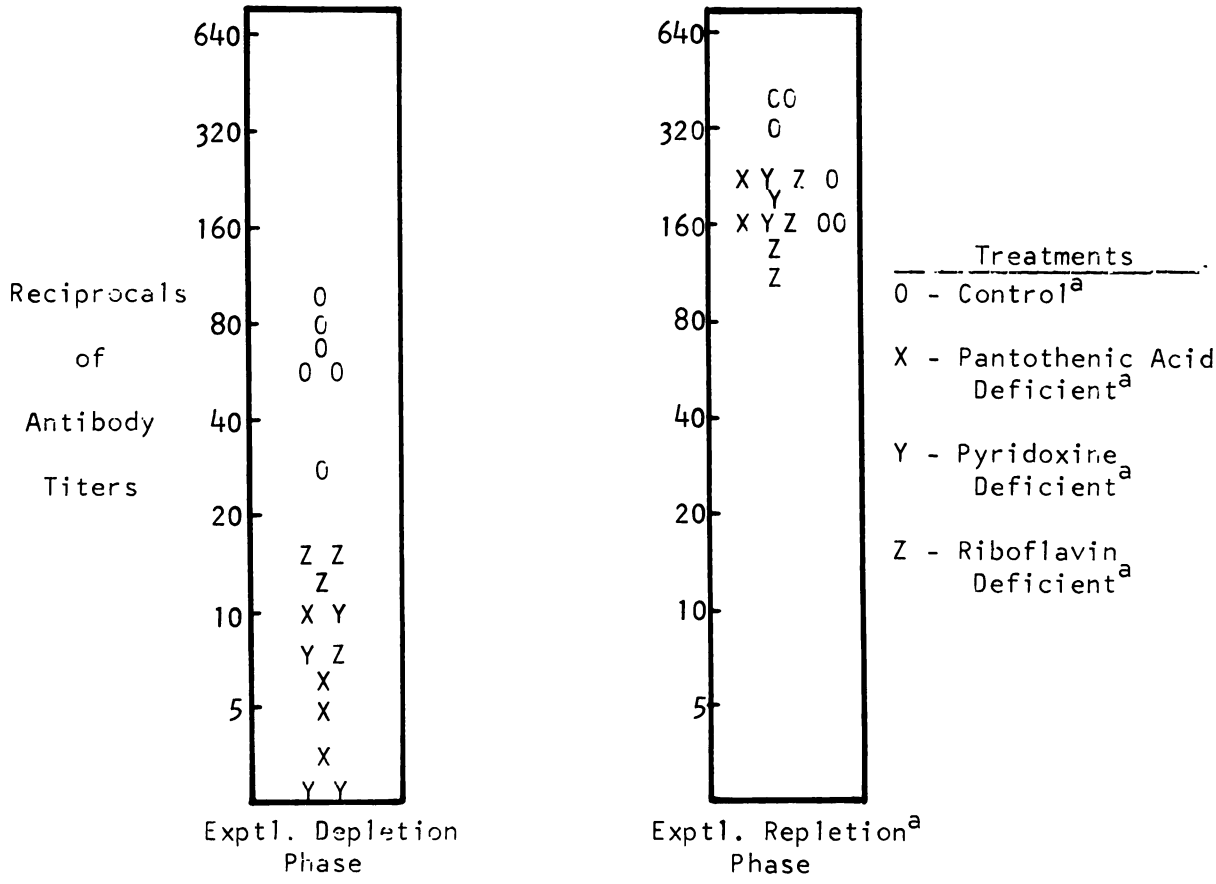

FIGURE 11 TRIAL IV. INDIVIDUAL NET ANTIBODY TITERS PRODUCED AGAINST HUMAN ERYTHROCYTES IN THE DEPLETION PHASE AND SALMONELLA PULLORUM IN THE REPLETION PHASE

[a]All pigs received complete natural diet during repletion

the titer produced by the pigs on each of the B vitamin deficiencies. Table 14 presents a summary of the data collected in the pair fed study. The total weight gain and feed efficiency of the pigs on the complete synthetic diet was significantly higher(P 0.05) than for the deficient pigs.

TABLE 14

TRIAL IV. PAIR FED - B VITAMIN STUDY

	Experimental Depletion Phase			
	Dietary Treatment[a]			
		Deficiency		
	Complete	B_6	Ribo	P. A.
No. pigs[b]	3	2	3	2
Av. total gain, lbs.	14.3[c]	8.9	11.1	7.9
Hematocrit, %	38.7	33.3	43.7	39.2
Hemoglobin, gm. %	11.7	9.9	12.3	11.8
Erythrocytes, 10^6	8.0	7.2	9.2	8.8
Antibody titer	63.0[d]	4.0	14.0	4.0

[a]Diets listed in Table 2

[b]One pigs in each the pyridoxine and pantothenic acid deficient treatments died

[c]Significantly higher ($P<0.05$) than corresponding value for all other treatments

[d]Significantly higher ($P<0.01$) than corresponding value for all other treatments

In addition to the changes already recorded, other apparent manifestations of pantothenic acid, pyridoxine or riboflavin deficiencies were observed in both Trials III and IV. Figure 12 shows the unthrifty condition of examples from each of the deficient treatments as compared

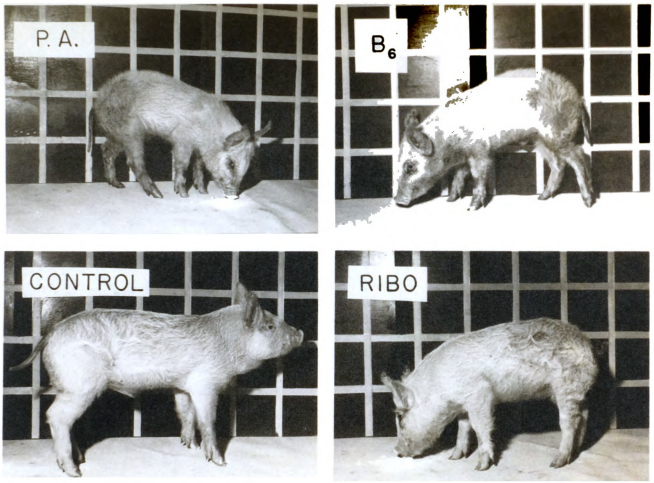

FIGURE 12. EXAMPLES OF A CONTROL PIG AND PIGS DEFICIENT IN PANTOTHENIC ACID, PYRIDOXINE OR RIBOFLAVIN AFTER BEING ALLOTTED TO THE RESPECTIVE TREATMENTS FOR FIVE WEEKS

to the controls. The hair coats became quite rough in many of the deficient pigs. Intermittent diarrhea posed a real problem in each of the three deficient treatment groups. Withholding feed from the diarrhetic pigs was the most consistently effective treatment for the condition. Prolapse of the rectum occurred in at least two pigs from each deficient treatment in both Trials III and IV. Stothers et al. (1955) reported finding this condition in severely pantothenic acid deficient pigs.

Wintrobe et al. (1943), Luecke et al. (1949,1950) have reported that pigs deficient in pantothenic acid develop an unthrifty appearance, severe diarrhea and locomotor paralysis. No instances of locomotor paralysis were observed in any of the pantothenic acid deficient pigs in these trials. However, Luecke et al. (1949) maintained a deficiency feeding period of seven weeks prior to observing the locomotor paralysis.

Two of the pyridoxine deficient pigs in Trial IV underwent epileptiform seizures during the last 10 days of the deficient diet feeding period. The seizures occurred in periods of excitement such as feeding or weighing. Miller et al. (1957a) reported that epileptiform seizures were observed frequently in pyridoxine deficient pigs. Blood studies in both trials revealed that oligocythemia and oligochromemia developed in the pyridoxine deficient pigs though the differences from other treatments were only significant in Trial III.

The results of the agglutination and hemagglutination studies in Trials III and IV coincide closely with the findings of Axelrod et al. (1947) and Pruzansky and Axelrod (1955). Axelrod and Pruzansky (1955a) have reviewed the research relating nutrient deficiencies and antibody production in rats, mice and chicks. They have ascribed a major role in

antibody production to pantothenic acid and pyridoxine. To riboflavin they have designated a lesser relationship. In the present study, separate deficiencies of all three B vitamins significantly lowered the measurable antibody production.

Ludovici et al. (1951a) have shown that deficiencies of pantothenic acid or pyridoxine do not increase antibody destruction or delay release of formed antibodies. Axelrod and Pruzansky (1955b) also postulated that gamma globulin and serum antibodies originate from a common precursor. Axelrod and Pruzansky (1955b) suggest that in the vitamin deficiencies they have worked with, the conversion of the precursor to serum antibodies is probably blocked while the formation of gamma globulin is uninhibited. To support this hypothesis they failed to find a change in the percent serum gamma globulin in vitamin deficient animals.

The results obtained in Trials III and IV and reported here would support the conclusions of Axelrod and Pruzansky (1955b). However, the vitamin A deficient animals (Trials I and II) actually had an increased percent gamma globulin which would not necessarily be in disagreement with the hypothesis by Axelrod and Pruzansky (1955b). Pauling (1940) has suggested that serum antibodies arise from normal gamma globulin under the influence of an antigen. Pauling reported that the normal gamma globulin and serum antibodies differ only in the manner in which the second peptide coils. Stavitsky (1961) claimed the theory by Pauling is untenable and reported as support that radioactive amino acids were incorporated into antibodies both in vivo and in vitro. This finding, however, would not necessarily preclude the passage thru a common precursor. Stavitsky (1961) also found that immunized splenic cells from pantothenic acid deficient

rats failed to produce antibodies *in vitro*, while the control animals maintained the capability of antibody production *in vitro*.

V. SUMMARY

Four trials were conducted to study and evaluate the relationship of specific deficiencies of vitamin A, pantothenic acid, pyridoxine or riboflavin to antibody production in swine. Each of the trials involved pigs which were weaned to semi-synthetic diets at two weeks of age or less. The first two trials in which 30 pigs were employed were designed to study the antibody response of positive control pigs and pigs deficient in vitamin A. Additional data obtained were weight gain, feed efficiency, serum vitamin A level, serum protein concentration and the electrophoretic components of serum protein.

The pigs receiving the vitamin A free diet exhibited significantly lower ($P<0.01$) serum vitamin A levels at six weeks of age after having been weaned to the experimental diet at ages of six days and 12 hours respectively in Trials I and II. The pigs in a marginal vitamin A condition (less than 20 micrograms per 100 milliliters of serum) produced significantly lower ($P<0.01$) antibody titers to experimental intraperitoneal introduction of killed cultures of Salmonella pullorum than did the control pigs. In Trial I the average daily gain and feed efficiency values of the vitamin A deficient and control pigs were not statistically significantly different. However, in Trial II the daily gain was significantly greater and more efficient ($P<0.01$) in the control pigs. Analysis of the electrophoretic components of serum protein at the time the antibody production was measured disclosed that the deficient pigs had significantly higher ($P<0.01$) percentages of alpha and gamma globulin than did the control pigs. This was accompanied by a significant decrease ($P<0.01$) in the percent albumin in the deficient pigs.

Following a repletion phase during which all pigs received a complete natural diet the immunological response was measured with human erythrocytes. All pigs from both previous treatments responded with similar hemagglutination titers. In the second trial the control pigs continued to gain significantly faster ($P<0.01$) during the repletion phase than did the pigs which were previously vitamin A deficient. Serum vitamin A and protein values were similar in all pigs following the repletion phase of each trial.

Trials III and IV, which involved 48 pigs, were designed to study the antibody response of positive control pigs and pigs deficient in pantothenic acid, pyridoxine or riboflavin. Additional data collected were measures of weight gain, feed efficiency, blood cellular components, serum protein concentration and electrophoretic components of serum protein. Also, urinary xanthurenic acid values were obtained from the pyridoxine deficient and control pigs. The pigs in both trials were placed on one of the four experimental diets at four weeks of age after having been weaned to a semi-synthetic diet deficient in the three B vitamins under consideration at two weeks of age. In Trial III Salmonella pullorum antigen was injected after the pigs on experimental treatment had been deprived of the particular vitamin for four weeks. In Trial IV the experimental feeding period was extended one week and human erythrocytes were employed as the antigen. The agglutination titers in Trial III and hemagglutination titers in Trial IV were significantly greater ($P<0.01$) in control pigs than for any of the deficient groups. A series of equated feeding groups (a group included a pig from each of the four treatments) established that inanition was not responsible for decreased antibody pro-

duction in the deficient pigs since the control pigs on limited feed produced significantly greater ($P < 0.01$) antibody titers.

At the conclusion of the depletion phase of the trials the weight gain of the control pigs was significantly greater and more efficient ($P<0.01$) than that of any deficient group. Pyridoxine deficient pigs had lower hematocrit, hemoglobin, total erythrocytes and total leukocytes than did the other pigs. The pyridoxine deficient pigs also had significantly higher concentrations ($P<0.05$) of urinary xanthurenic acid than did the controls, both before as well as after additions of tryptophan to the regular diet.

Analyses of the serum proteins at the conclusion of the depletion feeding phase of the trials established that the alpha globulin was significantly greater ($P<0.05$) in the pantothenic acid deficient pigs than in the controls.

Following repletion periods of six to seven weeks all pigs responded with similar antibody titers to human erythrocytes in Trial III and Salmonella pullorum in Trial IV. Also at this time, similar serum protein values were measured in all treatments. In Trial III the weight gain of the control pigs was significantly greater ($P<0.05$) after six weeks on a complete natural diet than all the pigs formerly fed the deficient diets. However, in Trial IV after extending the repletion feeding period one week, the control pigs remained only significantly heavier ($P<0.05$) than the pigs formerly receiving the pantothenic acid deficient diet.

VI. CONCLUSIONS

The results obtained in the four trials conducted in this study indicate that specific nutrient deficiencies of vitamins A, pantothenic acid, pyridoxine or riboflavin result in significantly lower ($P<0.01$) serum levels of antibodies produced in response to experimentally introduced antigens. On the basis of paired feeding studies, the decreased serum antibody titers measured in the pigs deficient in pantothenic acid, pyridoxine or riboflavin were not brought about by low feed intake, since with equalized feed intake the control pigs had significantly higher ($P<0.01$) net antibody titers than did any of the deficient treatment groups.

Following a restoration feeding period on a complete natural diet all pigs formerly deficient in vitamins A, pantothenic acid, pyridoxine or riboflavin were once again capable of producing measurable antibodies to specific antigens. The antibody production was comparable to that of the control pigs.

In both vitamin A trials the reciprocals of the antibody titers were highly correlated with serum vitamin A levels (.60 and .67 respectively) during the depletion phase of the trials. However, the values included in this observation covered a rather narrow range of serum vitamin A levels.

The inhibition of serum antibody titers became evident when serum vitamin A levels fell below 20 micrograms per 100 milliliters. It became exceedingly difficult to recover a pig when the serum vitamin A levels descended below 10 micrograms per 100 milliliters. Daily gains and feed efficiency values were not consistently affected by the feeding of a vi-

tamin A free diet.

Pigs fed diets deficient in pantothenic acid, pyridoxine or riboflavin gained at a significantly slower rate and required significantly more feed to put on a pound of gain than did the control pigs.

The total serum protein values were not significantly altered from the controls during any of the four trials. However, significantly different means values were found for the serum protein electrophoretic components. The percent alpha and gamma globulins were significantly increased in vitamin A deficient pigs over the control pigs with a concomitantly significant decrease in the percent albumin. The alpha globulin fraction in the pantothenic acid deficient pigs was greater than in the pyridoxine or riboflavin deficient or the control pigs. The differences, however, were statistically significant only between the control and pantothenic acid deficient pigs and then only in Trial III. A multiple variance analysis of the two trials demonstrated that the alpha globulin fraction of the pantothenic acid deficient pigs was significantly greater than the same fraction in the control pigs.

The pigs which were deficient in pyridoxine had lower mean values for hematocrit, hemoglobin, erythrocytes and leukocytes, than did the pantothenic acid deficient, riboflavin deficient or control pigs. However, the differences were not significant in both trials.

Pyridoxine deficient pigs excreted significantly more xanthurenic acid than did the control pigs regardless of whether additional tryptophan was superimposed upon the regular diet.

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APPENDIX TABLE 1

TRIAL I. PIG WEIGHTS IN VITAMIN A STUDY(LB.)

Pig no.	Initial[b] wt.	2 wk.	4 wk.	6 wk.	7 wk.	8 wk.	9 wk.	10 wk.
				Experimental Depletion Phase[a]				
				Positive Control				
78-1	6.0	12.0	15.9	20.5	25.8	30.5	36.5	
78-5	7.5	10.0	15.8	21.8	27.8	33.8	37.5	
78-6	7.8	11.6	16.4	23.1	28.6	34.8	40.0	
78-8	4.8	9.1	12.3	17.1	23.0	25.2	31.5	
79-1	9.4	14.8	20.6	32.8	40.0	44.0	50.0	55.0
79-4	9.8	16.5	21.8	32.4	42.5	43.0	48.0	53.0
81-5	5.5	7.9	11.1	20.0	25.5	30.5		
Av.	7.26 (±.73)[c]	11.7 (±1.17)	16.3 (±1.48)	24.0 (±2.34)	30.5 (±2.88)	34.5 (±2.59)	40.6 (±2.65)	54.0 (±1.0)
				Vitamin A Deficient				
78-2	6.9	10.0	15.6	20.6	20.6	17.5	24.5	
78-7	7.5	13.1	19.3	23.9	22.0	18.8	d	
78-10	4.0	6.8	12.0	16.0	19.5	13.8	d	
78-11	7.6	11.3	16.0	21.5	27.5	35.3	d	
78-12	5.6	11.3	16.3	21.6	28.0	34.3	36.0	
79-2	9.3	15.4	21.3	30.9	39.0	37.0	46.0	52.5
79-3	10.3	18.3	23.1	34.4	44.3	46.0	52.0	60.5
79-5	9.5	14.8	19.8	31.4	40.3	46.0	50.5	57.5
81-2	6.0	8.5	11.8	13.0	16.5	21.0		
Av.	7.41 (±.68)[c]	12.2 (±1.20)	17.2 (±1.31)	23.7 (±2.41)	28.6 (±3.39)	30.0 (±4.14)	41.8 (±5.15)	56.8 (±2.33)

[a]Depletion phase terminated after 9, 10, and 8 weeks in litters 78, 79, and 81 respectively

[b]Pigs allotted to treatment at two weeks of age

[c]Standard error of the mean in parenthesis under mean value

[d]Died

APPENDIX TABLE 2

TRIAL I. PIG WEIGHTS, GAIN AND FEED EFFICIENCY IN VITAMIN A STUDY(LB.)

	Exptl. Depletion Phase[a]		Exptl. Repletion Phase[a,b]	
Pig no.	Daily gain 2 - 8 wk.	Feed efficiency	Final weight	Daily gain
		Positive Control		
78-1	0.44	1.77	82	0.81
78-5	0.57	1.32	76	0.69
78-6	0.55	1.31	103	1.12
78-8	0.34	1.67	62	0.68
79-1	0.70	1.11	132	1.79
79-4	0.63	1.32	125	1.67
81-5	0.53	1.61	75	1.03
Av.	0.54 (±.04)[c]	1.44 (±.09)	93.6 (±10.2)	1.11 (±.17)
		Vitamin A Deficient		
78-2	0.25	1.44	63	0.69
78-7	0.26	1.37	d	
78-10	0.30	1.27	d	
78-11	0.57	1.43	d	
78-12	0.55	1.42	85	0.88
79-2	0.51	1.29	120	1.57
79-3	0.65	1.28	127	1.55
79-5	0.74	1.14	119	1.43
81-2	0.30	2.65	42	0.48
Av.	0.46 (±.04)[c]	1.48 (±.15)	92.7 (±14.3)	1.10 (±.19)

[a]Diets: Depletion - Semi-synthetic diet listed in Table 2
Repletion - Natural diet listed in Table 5

[b]All pigs received complete natural diet during repletion phase

[c]Standard error of the mean in parenthesis under mean value

[d]Died

APPENDIX TABLE 3

TRIAL I. SERUM VITAMIN A LEVELS(mcg./100 ml.)

Pig no.	Experimental Depletion Phase[a] 4[c] wk.	5 wk.	6 wk.	7 wk.	8 wk.	10 wk.	Experimental Repletion Phase[a,b] Final
			Positive Control				
78-1	25.6	23.1	22.4	20.9	27.9		33.6
78-5	25.0	27.5	23.2	21.2	22.7		36.0
78-6	24.6	28.2	23.6	21.5	24.8		32.5
78-8	22.8	23.8	26.3	26.9	28.6		28.9
79-1	26.3	26.6	30.7	26.7	33.0	33.4	40.3
79-4	27.5	27.3	36.8	37.1	39.4	36.8	39.9
81-5	25.2	29.9	27.2	27.5	28.4		36.1
Av.	24.6[e] (±.55)[d]	26.6[e] (±.91)	27.2[e] (±.85)	26.0[e] (±2.15)	29.3[e] (±2.08)	35.1[e] (±1.70)	35.3 (±1.53)
			Vitamin A Deficient				
78-2	21.3	16.4	8.7	7.6	7.8		34.8
78-7	17.2	15.0	10.0	8.6	8.3		f
78-10	13.0	11.3	7.6	6.6	9.4		f
78-11	16.4	15.2	9.8	8.1	10.2		f
78-12	15.8	14.4	10.5	9.1	12.3		41.5
79-2	22.4	21.9	22.3	22.9	21.0	20.6	39.2
79-3	23.0	23.1	21.2	22.0	17.2	16.1	40.4
79-5	20.4	19.8	20.6	21.7	18.8	16.4	39.0
81-2	20.3	21.7	21.0	20.1	19.6		43.9
Av.	17.3 (±.36)[d]	17.6 (±1.36)	14.6 (±2.13)	14.1 (±2.43)	13.8 (±1.76)	17.7 (±1.45)	39.8 (±1.54)

[a]Diets: Depletion - Semi-synthetic diet listed in Table 2
Repletion - Natural diet listed in Table 5

[b]All pigs received complete natural diet during repletion phase

[c]Weeks after allotted to treatments

[d]Standard error of the mean in parenthesis under mean value

[e]Significantly greater (P 0.01) than corresponding value for deficient pigs

[f]Died

APPENDIX TABLE 4

TRIAL I. SERUM PROTEIN VALUES IN VITAMIN A STUDY

Pig no.	Total protein gm. %	Albumin %	Alpha globulin %	Beta globulin %	Gamma globulin %
		Experimental Depletion Phase[b]			
		At Time of Antigen Injection			
		Positive Control			
78-1	5.1	46.7	25.7	19.0	8.5
78-5	4.5	48.6	24.8	17.4	9.2
78-6	3.9	39.9	32.2	16.1	11.8
78-8	4.8	53.8	22.5	15.6	9.3
79-1	4.0	34.5	33.3	20.7	11.5
79-4	5.6	41.9	30.8	16.2	11.8
81-5	4.6	38.5	31.1	20.1	10.1
Av.	4.65 (±.23)[a]	43.4 (±2.50)	28.6 (±1.59)	17.8 (±.78)	10.3 (±.52)
		Vitamin A Deficient			
78-2	5.0	45.9	26.6	14.7	12.8
78-7	4.8	40.2	30.4	17.4	12.0
78-10	4.7	34.2	30.3	17.3	18.2
78-11	4.7	50.6	23.7	16.5	9.2
78-12	5.8	48.9	21.3	18.3	11.5
79-2	5.8	46.0	25.8	16.5	11.0
79-3	4.8	43.1	27.2	17.8	11.6
79-5	3.9	43.6	30.7	18.8	7.6
81-2	5.6	32.2	34.7	18.2	14.9
Av.	5.03 (±.21)[a]	43.8 (±1.98)	27.6 (±1.51)	17.3 (±.22)	12.7 (±1.46)

[a] Standard error of the mean in parenthesis under mean value

[b] Semi-synthetic diets listed in Table 2

APPENDIX TABLE 4 (CONTINUED)

TRIAL I. SERUM PROTEIN VALUES IN VITAMIN A STUDY

Experimental Depletion Phase[b]

At Time of Agglutination Determination

Pig no.	Total protein gm. /	Albumin %	Alpha globulin %	Beta globulin %	Gamma globulin %
		Positive Control			
78-1	4.6	46.1	28.1	19.5	6.3
78-5	4.0	45.1	30.3	14.1	10.6
78-6	5.0	49.7	27.0	16.4	6.8
78-8	4.5	47.6	26.9	15.1	10.3
79-1	5.8	46.7	21.6	23.3	8.4
79-4	6.0	34.8	28.9	20.4	15.8
81-5	5.9	47.8	24.5	13.3	10.1
Av.	5.10 (±.28)[a]	45.4[c] (±1.85)	26.8 (±1.21)	18.1 (±1.20)	9.8 (±1.20)
		Vitamin A Deficient			
78-2	5.9	26.4	36.8	14.2	22.6
78-7	6.0	26.4	36.8	15.7	21.1
78-10	3.1	32.3	38.4	14.1	15.2
78-11	2.5	28.2	37.0	22.8	12.0
78-12	3.1	27.5	34.1	24.2	14.3
79-2	6.0	46.0	25.8	16.5	13.3
79-3	5.3	43.1	27.2	17.8	21.0
79-5	5.3	43.6	30.7	18.8	12.5
81-2	5.3	32.2	34.7	18.2	20.3
Av.	4.72 (±.45)[a]	31.6 (±2.37)	34.6[c] (±1.51)	19.0 (±1.66)	16.9[c] (±1.41)

[a]Standard error of the mean in parenthesis under mean value

[b]Diets listed in Table 2

[c]Significantly greater (P 0.01) than corresponding value for other treatment

APPENDIX TABLE 5

TRIAL I. SERUM PROTEIN VALUES IN VITAMIN A STUDY

Pig no.	Total protein gm. %	Albumin %	Alpha globulin %	Beta globulin %	Gamma globulin %
		Experimental Repletion Phase[a,b]			
		Previously Positive Control			
78-1	6.2	34.4	28.9	13.3	23.4
78-5	5.2	28.5	30.0	15.8	25.9
78-6	5.0	39.6	27.2	16.8	16.3
78-8	5.6	39.1	21.2	13.6	27.7
79-1	4.6	48.8	20.6	12.3	18.5
79-4	4.9	32.1	24.4	17.0	26.5
81-5	4.9	36.5	25.6	14.8	23.1
Av.	5.20 (±.20)[c]	37.0 (±2.45)	25.4 (±1.37)	14.8 (±.68)	23.1 (±1.61)
		Previously Vitamin A Deficient			
78-2	5.1	32.2	24.3	14.9	29.0
78-7	d				
78-10	d				
78-11	d				
78-12	6.4	35.9	21.1	10.9	32.1
79-2	5.2	43.7	22.2	13.2	20.9
79-3	4.9	40.4	21.5	15.1	23.0
79-5	4.9	37.0	24.1	17.3	21.6
81-2	5.1	36.5	25.6	14.8	23.1
Av.	5.27 (±.23)[c]	37.6 (±1.62)	23.1 (±.74)	14.4 (±.87)	25.0 (±2.02)

[a]Repletion diet listed in Table 5

[b]All pigs received complete natural diet during repletion phase

[c]Standard error of the mean in parenthesis under mean value

[d]Died

APPENDIX TABLE 6

TRIAL I. RECIPROCALS OF ANTIBODY TITERS IN VITAMIN A STUDY

Pig no.	Experimental Depletion Phase[a] Pre injection	Post injection	Net titer	Experimental Repletion Phase[a,b] Pre injection	Post injection	Net titer
			Positive Control			
78-1	20	5120	640	40	960	60
78-5	10	640	160	80	2560	80
78-6	0	640	640	20	1280	160
78-8	0	80	80	10	320	80
79-1	5	320	160	20	320	40
79-4	0	640	640	5	160	80
81-5	0	320	320	10	160	40
Av.	5 (±2.93)[c]	1109[d] (±213)	377.1[d] (±96.7)	26.4 (±9.99)	823 (±105)	77.1 (±19.4)
			Vitamin A Deficient			
78-2	10	40	10	20	1280	160
78-7	0	15	15	[e]		
78-10	5	10	5	[e]		
78-11	5	20	10	[e]		
78-12	0	20	20	40	320	20
79-2	0	80	80	10	80	20
79-3	5	80	40	20	160	40
79-5	5	40	20	20	160	40
81-2	0	80	80	5	80	40
Av.	3.3 (±1.18)[c]	42.8 (±9.9)	31.1 (±9.8)	19.2 (±4.9)	347 (±190)	53.3 (±27.8)

[a]Diets: Depletion - Semi-synthetic diet listed in Table 2
Repletion - Natural diet listed in Table 5

[b]All pigs received complete natural diet during repletion phase

[c]Standard error of the mean in parenthesis under mean value

[d]Significantly greater ($P<0.01$) than corresponding value for deficient pigs

[e]Died

APPENDIX TABLE 7

TRIAL II. SERUM VITAMIN A LEVELS(mcg./100 ml.)

Pig no.	Experimental Depletion Phase[a] 4[c] wk.	5 wk.	6 wk.	Experimental Repletion Phase[a,b] Final
		Positive Control		
125-2	26.6	28.8	24.9	27.5
125-3	27.6	25.5	26.4	36.1
125-9	27.0	25.2	28.9	32.3
126-1	25.7	25.5	26.5	35.2
126-3	21.5	22.1	21.0	29.9
127-1	27.6	27.0	29.8	g
127-2	27.9	26.8	28.4	33.4
Av.	26.3[e] (±.84)[d]	25.7[e] (±.72)	26.6[e] (±1.12)	32.4 (±1.33)
		Vitamin A Deficient		
125-1	15.2	15.6	14.0	f
125-8	14.6	16.3	13.8	34.2
125-10	15.0	13.4	9.6	31.7
126-4	13.7	14.0	11.8	29.2
126-5	10.6	7.1	8.0	f
127-3	10.1	11.9	12.7	28.6
127-4	8.8	8.2	6.6	f
Av.	12.6 (±1.00)[d]	12.4 (±1.34)	10.9 (±1.11)	30.9 (±1.28)

[a]Diets: Depletion - Semi-synthetic diet listed in Table 2
Repletion - Natural diet listed in Table 5

[b]All pigs received complete natural diet during repletion phase

[c]Weeks after allotted to treatments

[d]Standard error of the mean in parenthesis under mean value

[e]Significantly greater ($P<0.01$) than corresponding value for deficient pigs

[f]Died

[g]Sample lost

APPENDIX TABLE 8

TRIAL II. PIG WEIGHTS IN VITAMIN A STUDY(LB.)

Pig no.	Initial[b] wt.	Experimental Depletion Phase[a] 1 wk.	2 wk.	3 wk.	4 wk.	5 wk.
			Positive Control			
125-2	3.5	4.9	7.6	10.1	11.8	14.3
125-3	3.8	5.8	9.1	11.5	12.9	15.8
125-9	4.5	6.3	9.8	12.3	13.4	17.3
126-1	5.3	7.4	10.0	11.3	15.3	18.5
126-3	6.4	9.3	11.6	12.8	16.3	19.4
127-1	6.5	8.1	11.0	13.3	16.5	19.6
127-2	5.0	6.3	6.3	10.4	13.5	14.6
Av.	5.0 (±.44)[c]	6.9 (±.56)	9.3 (±.70)	11.7 (±.45)	14.2 (±.68)	17.1 (±.83)
			Vitamin A Deficient			
125-1	3.8	5.5	9.1	12.1	13.5	16.3
125-8	4.0	5.5	9.3	11.3	12.8	15.3
125-10	4.1	5.9	9.5	12.4	14.8	15.0
126-4	5.5	8.3	11.0	13.0	13.8	16.6
126-5	6.1	8.8	10.5	12.9	14.3	13.6
127-3	5.5	8.1	9.6	12.5	12.5	10.1
127-4	5.9	10.5	12.6	15.5	15.5	14.7
Av.	5.0 (±.37)[c]	7.5 (±.73)	10.2 (±.47)	12.8 (±.49)	13.9 (±.40)	14.5 (±.82)

[a]Diets listed in Table 2

[b]Pigs allotted to treatment at two weeks of age

[c]Standard error of the mean in parenthesis under mean value

APPENDIX TABLE 9

TRIAL II. PIG WEIGHTS, GAIN AND FEED EFFICIENCY IN VITAMIN A STUDY(LB.)

Pig no.	6[c] wk.	Experimental Depletion Phase[a] Daily gain 2 - 6 wk.	Feed efficiency	Experimental Repletion Phase[a,b] Final weight	Daily gain
		Positive Control			
125-2	18.8	0.40	1.23	82.5	1.27
125-3	20.5	0.41	1.22	82.0	1.23
125-9	21.6	0.42	1.19	91.0	1.39
126-1	22.5	0.45	1.31	74.5	1.21
126-3	22.5	0.39	1.43	84.0	1.43
127-1	23.7	0.45	1.18	67.0	1.49
127-2	18.5	0.44	1.12	53.0	1.19
Av.	21.2[e] (±.74)[d]	0.42[e] (±.009)	1.24 (±.037)	76.28[f] (±4.80)	1.32[f] (±.04)
		Vitamin A Deficient			
125-1	18.4	0.33	1.38	h	
125-8	15.0	0.21	1.90	50.5	0.71
125-10	17.0	0.20	1.83	65.0	0.96
126-4	19.6	0.31	1.58	66.0	1.08
126-5	17.0	0.23	1.61	h	
127-3	11.3	0.10	3.79	41.0	1.02
127-4	15.8	0.12	2.15	h	
Av.	16.4 (±1.01)[d]	0.21 (±.029)	2.01[g] (±.360)	55.6 (±6.02)	0.94 (±.08)

[a]Diets: Depletion - Semi-synthetic diet listed in Table 2
Repletion - Natural diet listed in Table 5

[b]All pigs received complete natural diet during repletion phase

[c]Weeks after allotted to treatment

[d]Standard error of the mean in parenthesis under mean value

[e]Significantly greater (P 0.01) than corresponding value for deficient pigs

[f]Significantly greater (P<0.05) than corresponding value for deficient pigs

[g]Significantly more pounds of feed per pound of gain (P<0.05)

[h]Died

APPENDIX TABLE 10

TRIAL II. SERUM PROTEIN VALUES IN VITAMIN A STUDY

Experimental Depletion Phase[a]

At Time of Antigen Injection

Pig no.	Total protein gm. %	Albumin %	Alpha globulin %	Beta globulin %	Gamma globulin %
			Positive Control		
125-2	4.8	50.8	24.1	17.9	7.4
125-3	4.9	e	e	e	e
125-9	4.8	49.8	23.2	16.7	11.3
126-1	5.0	50.0	22.3	16.1	11.6
126-3	4.7	51.3	24.8	14.2	9.7
127-1	4.8	41.6	26.4	25.6	6.4
127-2	5.0	51.4	23.7	18.6	6.2
Av.	4.86 (±.04)[b]	49.15[c] (±1.54)	24.08 (±.58)	18.18 (±1.60)	8.77 (±.99)
			Vitamin A Deficient		
125-1	4.4	43.7	26.9	18.3	11.1
125-8	4.4	45.2	27.2	17.8	9.8
125-10	4.9	e	e	e	e
126-4	4.7	42.6	28.4	17.0	12.0
126-5	4.0	39.8	25.8	21.9	12.5
127-3	4.6	46.5	29.5	15.5	8.5
127-4	4.8	45.8	26.3	16.8	11.0
Av.	4.54 (±.09)[b]	43.93 (±1.01)	27.35[c] (±.55)	17.89 (±.89)	10.82 (±.59)

[a]Diets listed in Table 2

[b]Standard error of the mean in parenthesis under mean value

[c]Significantly greater ($P<0.01$) than corresponding value for other treatment

[d]Significantly greater ($P<0.05$) than corresponding value for other treatment

[e]Sample lost

APPENDIX TABLE 10 (CONTINUED)

TRIAL II. SERUM PROTEIN VALUES IN VITAMIN A STUDY

Experimental Depletion Phase[a]

At Time of Agglutination Determination

Pig no.	Total protein gm. %	Albumin %	Alpha globulin %	Beta globulin %	Gamma globulin %
			Positive Control		
125-2	4.2	43.2	26.0	21.9	8.9
125-3	4.0	47.7	28.5	12.2	11.6
125-9	4.4	42.0	28.4	16.5	13.1
126-1	5.0	47.5	26.6	15.7	11.1
126-3	5.0	48.2	26.2	16.2	9.3
127-1	5.2	42.8	31.6	12.3	13.3
127-2	5.3	47.6	26.2	20.6	5.7
Av.	4.73 (±.19)[b]	45.57[c] (±1.04)	27.64 (±.78)	16.49 (±1.40)	10.43 (±1.03)
			Vitamin A Deficient		
125-1	4.4	38.2	28.4	20.4	12.9
125-8	5.1	36.3	35.4	15.1	13.2
125-10	4.8	36.9	31.8	15.3	15.9
126-4	4.2	33.9	30.6	20.8	14.8
126-5	5.2	43.3	28.3	17.4	10.9
127-3	6.4	30.5	42.3	15.4	11.8
127-4	6.6	25.0	38.5	20.4	15.3
Av.	5.24 (±.11)[b]	34.87 (±2.21)	33.61[c] (±2.01)	17.83 (±1.00)	13.56[d] (±.70)

[a]Diets listed in Table 2

[b]Standard error of the mean in parenthesis under mean value

[c]Significantly greater (P 0.01) than corresponding value for other treatment

[d]Significantly greater (P 0.05) than corresponding value for other treatment

APPENDIX TABLE 11

TRIAL II. SERUM PROTEIN VALUES IN VITAMIN A STUDY

Pig no.	Total protein gm. %	Albumin %	Alpha globulin %	Beta globulin %	Gamma globulin %
		Experimental Repletion Phase[a,b]			
		Previously Positive Control			
125-2	5.4	43.6	25.4	24.9	6.1
125-3	5.8	53.4	19.2	14.4	13.0
125-9	5.7	57.1	20.5	12.1	10.3
126-1	5.8	52.8	17.2	19.4	10.6
126-3	5.2	56.2	18.5	18.2	7.1
127-1	6.0	47.1	22.0	20.3	10.6
127-2	6.0	50.0	20.0	18.4	11.6
Av.	5.70 (±.11)[c]	51.46 (±1.85)	20.40 (±.88)	18.24 (±1.57)	9.90 (±.92)
		Previously Vitamin A Deficient			
125-1	d				
125-8	5.2	49.7	17.8	18.9	13.6
125-10	5.4	49.8	20.1	14.8	15.3
126-4	6.0	46.1	20.8	20.1	13.0
126-5	d				
127-3	6.5	44.5	23.1	17.2	15.2
127-4	d				
Av.	5.78 (±.10)[c]	47.52 (±1.32)	20.45 (±1.09)	17.75 (±1.15)	14.28 (±.58)

[a]Repletion diet listed in Table 5

[b]All pigs received complete natural diet during repletion

[c]Standard error of the mean in parenthesis under mean value

[d]Died

APPENDIX TABLE 12

TRIAL II. RECIPROCALS OF ANTIBODY TITERS IN VITAMIN A STUDY

Pig no.	Experimental Depletion Phase[a] Pre injection	Post injection	Net titer	Experimental Repletion Phase[a,b] Pre injection	Post injection	Net titer
			Positive Control			
125-2	20	1280	160	40	960	60
125-3	10	640	160	20	960	80
125-9	5	1280	640	80	1280	40
126-1	10	640	160	30	320	60
126-3	0	120	120	10	320	80
127-1	8	320	120	40	1280	80
127-2	5	640	320	5	240	120
Av.	8.3 (±2.35)[c]	703.0[d] (±170.2)	240.0[d] (±71.4)	32.1 (±9.5)	766.0 (±174.5)	74.3 (±9.5)
			Vitamin A Deficient			
125-1	20	80	10	e		
125-8	0	40	40	20	640	80
125-10	10	20	5	15	160	30
126-4	0	10	10	20	640	80
126-5	10	60	15	e		
127-3	5	40	20	30	960	160
127-4	0	15	15	e		
Av.	6.4 (±2.83)[c]	25.4 (±9.6)	16.4 (±4.3)	21.2 (±4.4)	587.0 (±232.0)	87.5 (±12.0)

[a]Diets: Depletion - Semi-synthetic diet listed in Table 2
Repletion - Natural diet listed in Table 5

[b]All pigs received complete natural diet during repletion

[c]Standard error of the mean in parenthesis under mean value

[d]Significantly greater ($P<0.01$) than corresponding value for deficient pigs

[e]Died

APPENDIX TABLE 13

TRIAL III. PIG WEIGHTS GAIN AND FEED EFFICIENCY IN B VITAMIN STUDY(LB.)

Pig no.	Initial[c] wt.	Experimental Depletion Phase[a] 2 wk.	3 wk.	4 wk.	Daily gain	Feed efficiency	Experimental Repletion Phase[a,b] Final weight	Daily gain
			Positive Control					
43-4	15.6	21.8	26.1	29.4	0.49	1.47	94	1.54
43-8	15.3	22.9	26.9	29.9	0.52	1.42	94	1.53
44-4	14.2	21.2	23.9	30.1	0.57	1.31	86	1.33
45-4	12.4	15.8	18.4	20.2	0.28	2.01	79	1.40
45-10	13.7	19.4	20.3	24.2	0.38	1.78	83	1.40
Av.	14.2 (±.58)[d]	20.2[g] (±1.24)	23.1[g] (±1.64)	26.8[g] (±1.97)	0.45[g] (±.08)	1.58 (±.11)	87.2[g] (±3.0)	1.46[g] (±.04)
			Pantothenic Acid Deficient					
43-3	14.6	19.4	18.0	19.5	0.18	2.82	69	1.18
43-5	15.3	16.4	12.6	13.4	0.06	6.13	71	1.37
43-10	14.8	17.0	17.6	20.8	0.21	2.27	76	1.31
44-2	13.3	16.8	15.0	17.9	0.16	2.74	65	1.12
44-9	14.2	18.8	14.8	e				
45-2	12.4	16.8	15.7	12.5	0.16	2.76	e	
Av.	14.1 (±.43)[d]	17.5 (±.51)	15.6 (±.81)	16.8 (±1.65)	0.15 (±.04)	3.34 (±.70)	70.2 (±2.3)	1.24 (±.05)

[a]Diets: Depletion - Semi-synthetic diets listed in Table 2
Repletion - Natural diet listed in Table 5

[b]All pigs received complete natural diet during repletion phase

[c]Pigs allotted to treatment four weeks of age

[d]Standard error of the mean in parenthesis under mean value

[e]Died

[f]Removed from trial

[g]Significantly greater than all other treatments (P 0.05)

[h]Significantly more pounds of feed per pound of gain than pyridoxine deficient or control pigs (P 0.05)

APPENDIX TABLE 13 (CONTINUED)

TRIAL III. PIG WEIGHTS, GAIN AND FEED EFFICIENCY IN B VITAMIN STUDY(LB.)

Pig no.	Initial[c] wt.	Experimental Depletion Phase[a] 2 wk.	3 wk.	4 wk.	Daily gain	Feed efficiency	Experimental Repletion Phase[a,b] Final weight	Daily gain
			Pyridoxine Deficient					
43-1	13.3	17.1	19.5	20.5	0.25	2.69	75	1.30
43-7	16.3	21.7	25.0	29.3	0.46	3.90	93	1.52
44-5	10.4	14.2	16.0	16.8	0.23	2.83	45	0.67
44-13	14.3	18.4	20.1	21.8	0.27	2.45	75	1.27
45-1	13.3	16.1	15.5	18.7	0.19	2.83	70	1.22
45-3	11.9	13.2	14.9	15.2	0.12	3.59	65	1.19
Av.	13.2 (±.82)[d]	16.8 (±1.25)	18.5 (±1.57)	20.4 (±2.03)	0.25 (±0.10)	2.61 (±.23)	70.5 (±6.4)	1.20 (±.12)
			Riboflavin Deficient					
43-2	15.0	14.7	16.3	17.4	0.09	7.18	70	1.25
43-9	10.6	17.9	22.1	25.2	0.52	1.19	76	1.21
44-1	13.7	f						
44-3	15.0	17.1	15.3	19.2	0.15	3.06	71	1.23
44-8	12.6	14.3	12.7	13.7	0.06	6.14	58	1.05
45-5	11.2	11.1	13.0	12.4	0.04	8.68	54	0.99
Av.	13.0 (±.76)[d]	15.0 (±1.20)	15.8 (±1.70)	17.6 (±2.26)	0.17 (±.09)	5.25[h] (±1.37)	65.8 (±4.2)	1.15 (±.05)

[a]Diets: Depletion - Semi-Synthetic diets listed in Table 2
Repletion - Natural diet listed in Table 5

[b]All pigs received complete natural diet during repletion phase

[c]Pigs allotted to treatment at four weeks of age

[d]Standard error of the mean in parenthesis under mean value

[e]Died

[f]Removed from trial

[g]Significantly greater than all other treatments ($P<0.05$)

[h]Significantly more pounds of feed per pound of gain than pyridoxine deficient or control pigs ($P<0.05$)

APPENDIX TABLE 14

TRIAL III. BLOOD CELLULAR DATA IN B VITAMIN STUDY

Experimental Depletion Phase[a]

Pig no.	Hematocrit %	Hemoglobin gm. %	Erythrocytes 10^6	Leukocytes 10^3	Differentials: Lymphocytes %	Differentials: Neutrophils %
Positive Control						
43-4	46.8	13.62	8.16	22.72	45	53
43-8	44.8	13.87	7.02	30.30	44	52
44-4	40.1	11.28	5.75	22.52	53	45
45-4	42.8	12.78	6.98	30.50	46	50
45-10	46.0	13.09	7.75	23.25	57	41
Av.	44.1 (±1.20)[b]	12.93 (±1.00)	7.13 (±.42)	25.86 (±1.86)	49.0 (±2.60)	48.2 (±2.05)
Pantothenic Acid Deficient						
43-3	47.2	15.62	11.21	30.62	51	47
43-5	41.9	13.68	10.75	12.28	53	39
43-10	37.1	11.47	8.38	38.70	45	54
44-7	37.5	16.33	8.00	28.52	55	43
45-2	25.0	8.10	4.70	10.35	57	39
Av.	37.7 (±3.67)[b]	13.04 (±1.34)	8.61 (±1.05)	24.09 (±5.50)	52.2 (±2.06)	44.4 (±2.82)

[a]Diets listed in Table 2

[b]Standard error of the mean in parenthesis under mean value

APPENDIX TABLE 14 (CONTINUED)

TRIAL III. BLOOD CELLULAR DATA IN B VITAMIN STUDY

Pig no.	Hemato-crit %	Hemo-globin gm. %	Erythro-cytes 10^6	Leuko-cytes 10^3	Differentials: Lympho-cytes %	Differentials: Neutro-phils %
			Experimental Depletion Phase[a]			
			Pyridoxine Deficient			
43-1	37.8	10.47	6.91	18.20	72	28
43-7	38.4	10.29	8.90	26.77	62	37
44-5	23.8	7.26	5.49	20.25	55	45
44-13	37.9	11.78	7.22	16.12	75	25
45-1	24.6	7.11	3.81	22.50	71	29
45-2	41.4	13.71	6.00	26.12	32	61
Av.	34.00[c] (±3.14)[b]	10.10 (±1.05)	6.39 (±.70)	18.57[d] (±1.91)	61.2 (±6.60)	37.5 (±5.55)
			Riboflavin Deficient			
43-2	42.9	13.43	7.56	27.42	22	77
43-9	46.1	13.18	10.70	19.70	39	60
44-3	45.0	13.71	8.56	34.88	35	64
44-8	39.1	11.22	8.64	25.30	37	62
45-5	50.8	14.28	7.99	25.60	56	42
Av.	44.78 (±1.72)[b]	13.16 (±.51)	8.69 (±.54)	26.58 (±2.44)	37.8[e] (±5.44)	61.0[f] (±5.60)

[a] Diets listed in Table 2

[b] Standard error of the mean in parenthesis under mean value

[c] Significantly lower ($P < 0.05$) than corresponding value for riboflavin deficient or control pigs

[d] Significantly lower ($P < 0.05$) than corresponding value for pantothenic acid or riboflavin deficient pigs

[e] Significantly lower ($P < 0.05$) than corresponding value for pyridoxine deficient pigs

[f] Significantly greater ($P < 0.05$) than corresponding value for pantothenic acid or pyridoxine deficient pigs

APPENDIX TABLE 15

TRIAL III. SERUM PROTEIN VALUES IN B VITAMIN STUDY

Experimental Depletion Phase[a]

At Time of Antigen Injection

Pig no.	Total protein gm. %	Albumin %	Alpha globulin %	Beta globulin %	Gamma globulin %
		Positive Control			
43-4	5.8	53.6	17.8	12.9	15.7
43-9	5.4	52.9	24.4	14.0	8.6
44-4	6.8	42.2	25.6	17.7	14.1
45-4	6.0	49.7	21.4	15.3	13.6
45-10	5.9	50.2	22.6	17.6	9.6
Av.	5.98 (±.24)[b]	49.7 (±2.02)	22 4 (±1.34)	15.1 (±.96)	12.3 (±1.38)
		Pantothenic Acid Deficient			
43-3	6.9	41.2	18.9	15.7	24.3
43-5	8.5	49.2	22.5	15.3	13.3
43-10	6.0	45.4	22.7	13.0	18.9
44-2	5.9	55.0	20.5	15.8	8.7
45-2	5.6	54.2	20.8	16.2	9.9
Av.	6.58 (±.52)[b]	49.0 (±2.62)	21.3 (±.63)	15.2 (±.57)	15.8 (±2.69)

[a]Diets listed in Table 2

[b]Standard error of the mean in parenthesis under mean value

APPENDIX TABLE 15 (CONTINUED)

TRIAL III. SERUM PROTEIN VALUES IN B VITAMIN STUDY

Experimental Depletion Phase[a]

At Time of Antigen Injection

Pig no.	Total protein gm. %	Albumin %	Alpha globulin %	Beta globulin %	Gamma globulin %
		Pyridoxine Deficient			
43-1	6.4	42.9	20.0	15.1	22.0
43-7	5.8	45.5	24.1	15.8	15.1
44-5	5.6	46.7	30.7	14.8	7.8
44-13	6.3	44.9	25.2	15.9	12.8
45-1	5.8	51.2	23.8	15.1	10.0
45-3	5.5	45.3	26.6	18.6	9.3
Av.	5.90 (±.15)[b]	46.1 (±1.14)	25.1 (±1.41)	15.9 (±.57)	12.8 (±2.12)
		Riboflavin Deficient			
43-2	4.2	51.9	21.3	11.6	15.2
43-9	6.3	43.9	21.1	15.7	19.3
44-3	5.6	57.6	22.4	13.6	6.5
44-8	6.2	53.1	26.2	14.5	6.2
45-5	6.6	41.6	29.2	17.1	12.1
Av.	5.78 (±.43)[b]	49.6 (±1.12)	24.0 (±1.58)	14.5 (±1.04)	11.8 (±2.52)

[a]Diets listed in Table 2

[b]Standard error of the mean in parenthesis under mean value

APPENDIX TABLE 16

TRIAL III. SERUM PROTEIN VALUES IN B VITAMIN STUDY

Experimental Depletion Phase[a]

At Time of Antibody Determination

Pig no.	Total protein gm. %	Albumin %	Alpha globulin %	Beta globulin %	Gamma globulin %
			Positive Control		
43-4	6.3	37.[illegible]	22.1	1[illegible].5	21.6
43-8	6.8	41.4	24.7	18.7	15.2
44-4	5.4	45.4	21.9	19.8	13.0
45-4	6.9	37.8	22.8	20.6	18.7
45-10	6.3	43.6	23.4	18.0	15.0
Av.	6.34 (±.06)[b]	41.20 (±1.52)	22.98 (±.50)	19.12[c] (±.46)	16.70 (±1.52)
			Pantothenic Acid Deficient		
43-3	6.8	38.5	21.1	16.1	24.3
43-5	6.3	51.3	15.4	16.3	17.0
43-10	5.5	47.9	18.8	12.4	20.9
44-2	5.6	44.6	26.5	18.6	10.3
45.2	6.4	26.4	43.6	18.7	11.3
Av.	6.01 (±.31)[b]	41.74 (±4.38)	25.08 (±4.97)	16.40 (±1.15)	16.80 (±2.70)

[a]Diets listed in Table 2

[b]Standard error of the mean in parenthesis under mean value

[c]Significantly greater than corresponding value for all other treatments ($P < 0.05$)

APPENDIX TABLE 16 (CONTINUED)

TRIAL III. SERUM PROTEIN VALUES IN B VITAMIN STUDY

Experimental Depletion Phase[a]

At Time of Antibody Determination

Pig no.	Total protein gm. %	Albumin %	Alpha globulin %	Beta globulin %	Gamma globulin %
		Pyridoxine Deficient			
43-1	7.5	42.1	18.5	14.8	24.6
43-7	6.2	45.4	21.0	14.2	19.4
44-5	6.4	55.3	21.8	15.1	7.8
44-13	7.0	48.4	17.0	11.8	22.8
45-1	6.4	39.4	32.1	15.6	12.9
45-3	6.0	47.7	23.2	13.0	16.2
Av.	6.58 (±.25)[b]	46.38 (±2.26)	22.26 (±2.17)	14.11 (±.58)	17.28 (±2.57)
		Riboflavin Deficient			
43-2	6.0	43.8	24.2	14.6	12.4
43-9	5.8	42.6	22.5	15.9	19.0
44-3	6.3	52.2	23.6	13.7	10.6
44-8	5.7	52.7	24.8	14.8	7.7
45-5	6.3	40.2	24.8	17.0	17.9
Av.	6.02 (±.12)[b]	46.30 (±2.58)	23.98 (±.43)	15.20 (±.57)	13.52 (±1.93)

[a] Diets listed in Table 2

[b] Standard error of the mean in parenthesis under mean value

[c] Significantly greater than corresponding value for all other treatments ($P \sim 0.05$)

APPENDIX TABLE 17

TRIAL III. SERUM PROTEIN VALUES IN B VITAMIN STUDY

Pig no.	Total protein gm. %	Albumin %	Alpha globulin %	Beta globulin %	Gamma globulin %
		Experimental Repletion Phase[a,b]			
		Previously Positive Control			
43-4	6.6	49.6	18.0	14.2	18.2
43-8	7.3	47.9	17.0	13.3	21.8
44-4	6.4	51.9	21.6	11.1	15.4
45-4	7.7	48.4	20.8	12.1	18.6
45-10	7.0	44.8	20.2	14.6	20.4
Av.	7.00 (±.24)[c]	48.52 (±1.16)	19.52 (±.81)	13.06 (±.65)	18.88 (±.70)
		Previously Pantothenic Acid Deficient			
43-3	6.8	47.6	16.2	13.9	22.3
43-5	6.4	44.2	20.8	13.5	21.5
43-10	7.2	50.2	17.1	11.4	21.3
44-2	6.4	53.6	20.1	11.0	15.2
45-2	[d]				
Av.	6.70 (±.19)[c]	48.90 (±1.99)	18.55 (±1.12)	12.45 (±.73)	20.07 (±1.64)

[a]Repletion diet listed in Table 5

[b]All pigs received complete natural diet during repletion phase

[c]Standard error of the mean in parenthesis under mean value

[d]Died

APPENDIX TABLE 17 (CONTINUED)

TRIAL III. SERUM PROTEIN VALUES IN B VITAMIN STUDY

Experimental Repletion Phase[a,b]

Pig no.	Total protein gm. %	Albumin %	Alpha globulin %	Beta globulin %	Gamma globulin %
		Previously Pyridoxine Deficient			
43-1	7.0	48.1	16.1	10.8	25.0
43-7	6.9	43.7	21.4	15.4	19.4
44-5	6.1	65.1	14.6	11.1	9.2
44-13	6.7	53.6	14.9	10.6	20.9
45-1	5.8	53.4	15.3	11.7	19.6
45-3	7.2	47.7	19.1	11.7	21.5
Av.	6.62 (±.22)[c]	51.93 (±3.05)	16.90 (±1.11)	11.88 (±.73)	19.26 (±2.17)
		Previously Riboflavin Deficient			
43-2	6.4	53.2	17.9	13.1	15.8
43-9	7.1	44.9	18.1	14.1	23.0
44-3	6.2	57.1	17.9	10.6	14.4
44-8	6.0	57.3	17.7	10.6	14.4
45-5	5.9	44.7	19.9	12.0	23.4
Av.	6.32 (±.21)[c]	51.44 (±2.81)	18.30 (±.40)	12.08 (±.69)	18.20 (±2.06)

[a] Repletion diet listed in Table 5

[b] All pigs received complete natural diet during repletion phase

[c] Standard error of the mean in parenthesis under mean value

APPENDIX TABLE 18

TRIAL III. RECIPROCALS OF ANTIBODY TITERS IN B VITAMIN STUDY

Pig no.	Experimental Depletion Phase[a] Pre injection	Post injection	Net titer	Experimental Repletion Phase[a,b] Pre injection	Post injection	Net titer
			Positive Control			
43-4	0	160	160	5	160	80
43-8	2	240	160	5	80	40
44-4	5	320	160	5	40	20
45-4	0	240	240	10	320	80
45-10	0	160	160	5	160	80
Av.	1.4 (±.98)[c]	224[e] (±29 9)	176[e] (±16.0)	6.0 (±.79)	152 (±51.0)	60 (±12.6)
			Pantothenic Acid Deficient			
43-3	10	30	7	5	80	40
43-5	7	40	15	10	160	40
43-10	5	80	40	5	40	20
44-2	10	30	7	5	60	30
44-9	[d]					
45-2	2	10	7	[d]		
Av.	7.0 (±1.48)[c]	38.0 (±11.6)	15.2 (±6.3)	6.2 (±1.30)	84.0 (±26.4)	32.5 (±4.8)

[a]Diet: Depletion - Semi-synthetic diets listed in Table 2
Repletion - Natural diet listed in Table 5

[b]All pigs received complete natural diet during repletion phase

[c]Standard error of the mean in parenthesis under mean value

[d]Died

[e]Significantly greater than corresponding value for all other treatments ($P<0.01$)

APPENDIX TABLE 1[illegible] (CONTINUED)

TRIAL III. RECIPROCALS OF ANTIBODY TITERS IN B VITAMIN STUDY

Pig no.	Experimental Depletion Phase[a] Pre injection	Post injection	Net titer	Experimental Repletion Phase[a,b] Pre injection	Post injection	Net titer
			Pyridoxine Deficient			
43-1	7	30	10	5	240	120
43-7	0	60	60	0	30	30
44-5	0	30	30	5	40	20
44-13	0	60	60	5	320	160
45-1	10	30	7	5	80	40
45-3	0	30	30	10	80	20
Av.	2.83 (±1.80)[c]	40.0 (±6.3)	32.8 (±9.5)	5.0 (±1.29)	127.0 (±48.6)	65.0 (±24.5)
			Riboflavin Deficient			
43-2	2	10	7	5	160	80
43-9	7	40	15	7	80	30
44-3	0	40	40	5	60	30
44-[illegible]	2	60	40	10	320	80
45-5	7	30	10	7	120	60
Av.	3.60 (±1.67)[c]	32.0 (±8.0)	22.6 (±7.2)	6.8 (±1.20)	155.0 (±59.0)	56.0 (±14.4)

[a] Diets: Depletion - Semi-synthetic diets listed in Table 2
Repletion - Natural diet listed in Table 5

[b] All pigs received complete natural diet during repletion phase

[c] Standard error of the mean in parenthesis under mean value

[d] Died

APPENDIX TABLE 19

TRIAL IV. PIG WEIGHTS, GAIN AND FEED EFFICIENCY IN B VITAMIN STUDY(LB.)

		Experimental Depletion Phase[a]					Experimental Repletion Phase[a,b]	
Pig no.	Initial[c] wt.	2 wk.	3 wk.	4 wk.	5 wk.	Daily gain	Feed efficiency	Final weight	Daily gain
				Positive Control					
84-2	14.6	22.3	24.2	27.9	32.1	0.50	1.16	139	1.84
84-5	11.5	16.1	21.0	23.3	25.7	0.41	1.26	122	1.66
84-9	12.2	14.8	15.9	18.7	22.3	0.29	1.55	115	1.60
85-1	14.2	19.0	23.7	26.4	28.9	0.42	1.35	120	1.57
85-10	8.5	9.5	11.3	16.6	20.2	0.33	1.36	98	1.34
87-3	11.5	17.8	22.4	27.8	29.7	0.52	1.25	108	1.45
Av.	12.1 (±.90)[d]	16.6 (±1.77)	19.8 (±2.08)	23.4 (±1.97)	26.5[g] (±1.87)	0.41[g] (±.04)	1.32[h] (±.06)	117[i] (±5.7)	1.58[i] (±.07)
				Pantothenic Acid Deficient					
84-1	13.1	16.1	[e]						
84-6	12.7	16.6	16.8	17.2	19.4	0.19	2.36	[f]	
84-10	6.2	10.6	9.3	[f]					
85-6	17.2	21.6	20.6	24.3	21.0	0.20	2.84	86	1.12
85-12	11.2	15.3	15.3	16.2	19.6	0.24	2.13	84	1.11
87-1	10.0	[e]							
87-2	12.2	18.5	17.6	16.2	17.9	0.16	3.34	[f]	
Av.	11.8 (±1.26)[d]	16.4 (±1.49)	15.9 (±1.87)	18.5 (±1.95)	19.5 (±.63)	0.20 (±.02)	2.67 (±.27)	85 (±1.0)	1.12 (±.02)

[a]Diets: Depletion - Semi-synthetic diets listed in Table 2
Repletion - Natural diet listed in Table 5

[b]All pigs received complete natural diet during repletion phase

[c]Pigs allotted to treatments at four weeks of age

[d]Standard error of the mean in parenthesis under mean value

[e]Removed from trial

[f]Died

[g]Significantly greater (P 0.05) than corresponding value for all other treatments

[h]Significantly less pounds of feed per pound of gain than all other treatments (P 0.05)

[i]Significantly greater (P<0.05) than corresponding value for pantothenic acid deficient pigs

APPENDIX TABLE 19 (CONTINUED)

TRIAL IV. PIG WEIGHTS, GAIN AND FEED EFFICIENCY IN B VITAMIN STUDY(LB.)

Pig no.	Initial[c] wt.	Experimental Depletion Phase[a] 2 wk.	3 wk.	4 wk.	5 wk.	Daily gain	Feed efficiency	Experimental Repletion Phase[a,b] Final weight	Daily gain
				Pyridoxine Deficient					
84-4	13.4	13.3	14.5	18.0	19.6	0.18	2.55	111	1.58
84-8	9.0	12.2	13.5	18.3	16.7	0.23	1.89	[f]	
85-2	12.1	15.6	16.8	17.8	[f]				
85-4	13.9	19.4	22.6	24.2	24.8	0.31	1.81	110	1.47
85-9	13.7	19.9	[e]						
87-5	12.4	17.9	24.0	25.5	26.6	0.41	1.55	95	1.27
Av.	12.4 (±.74)[d]	16.3 (±1.31)	18.2 (±2.13)	20.7 (±1.68)	21.9 (±2.29)	0.28 (±.04)	1.95 (±.30)	105 (±5.2)	1.44 (±.09)
				Riboflavin Deficient					
84-3	12.0	14.8	17.5	20.6	22.0	0.29	1.59	106	1.45
84-7	11.4	14.9	15.8	17.6	20.5	0.26	1.96	106	1.47
85-7	12.1	15.0	15.7	[f]					
85-8	13.5	18.8	20.4	24.3	25.5	0.34	1.69	119	1.61
85-11	7.0	9.6	10.4	13.3	15.1	0.23	1.87	66	0.88
87-9	9.3	11.3	11.4	16.2	[f]				
Av.	10.9 (±.96)[d]	14.1 (±1.32)	15.2 (±1.53)	18.4 (±1.89)	20.8 (±2.16)	0.28 (±.02)	1.78 (±.08)	99.3 (±11.5)	1.35 (±.16)

[a]Diets: Depletion - Semi-synthetic diets listed in Table 2
Repletion - Natural diet listed in Table 5

[b]All pigs received complete natural diet during repletion phase

[c]Pigs allotted to treatment at four weeks of age

[d]Standard error of the mean in parenthesis under mean value

[e]Removed from trial

[f]Died

APPENDIX TABLE 20

TRIAL IV. BLOOD CELLULAR DATA IN B VITAMIN STUDY

Pig no.	Hematocrit %	Hemoglobin gm. %	Erythrocytes 10^6	Leukocytes 10^3	Differentials: Lymphocytes %	Differentials: Neutrophils %
Experimental Depletion Phase[a]						
Positive Control						
84-2	38.1	11.42	8.19	15.92	81	15
84-5	35.0	10.78	7.22	20.55	75	20
84-9	36.1	11.66	8.60	22.60	78	19
85-1	42.0	12.83	8.13	21.15	73	26
85-10	39.5	11.86	7.34	12.58	86	12
87-3	41.5	12.86	7.85	8.58	71	15
Av.	38.7 (±1.15)[b]	11.90 (±1.11)	7.89 (±.21)	18.56 (±1.87)	77.3 (±2.47)	17.8 (±1.85)
Pantothenic Acid Deficient						
84-6	36.9	10.66	9.00	6.40	67	29
84-10	33.8	9.88	6.67	37.95	69	21
85-6	41.5	12.89	8.58	14.12	74	25
85-12	36.2	11.36	8.95	7.73	66	34
87-2	37.0	10.81	6.85	12.80	69	30
Av.	37.1 (±1.14)[b]	11.12 (±.50)	8.01 (±.51)	15.80 (±5.74)	69.0 (±1.40)	27.8 (±1.99)

[a]Diets listed in Table 2

[b]Standard error of the mean in parenthesis under mean value

APPENDIX TABLE 20 (CONTINUED)

TRIAL IV. BLOOD CELLULAR DATA IN B VITAMIN STUDY

Experimental Depletion Phase[a]

Pig no.	Hemato-crit %	Hemo-globin gm. %	Erythro-cytes 10^6	Leuko-cytes 10^3	Differentials: Lympho-cytes %	Differentials: Neutro-phils %
Pyridoxine Deficient						
84-4	28.7	8.74	7.13	15.52	53	45
84-8	38.0	10.96	7.26	20.42	75	24
85-2	29.1	8.98	8.16	12.12	42	54
85-4	29.1	7.78	8.18	9.20	82	16
87-5	43.6	12.92	7.16	12.90	80	17
Av.	33.1 (±3.03)[b]	9.65 (±1.22)	7.58 (±.24)	14.04 (±1.89)	66.6 (±8.10)	31.2 (±7.75)
Riboflavin Deficient						
84-3	42.1	12.62	9.88	26.30	82	18
84-7	35.5	10.48	7.19	20.32	64	30
85-7	37.2	11.54	8.23	9.95	70	28
85-8	47.8	14.12	9.34	11.72	82	18
85-11	41.1	12.89	8.14	12.08	75	22
87-9	33.3	11.86	7.04	17.40	53	44
Av.	39.5 (±2.14)[b]	12.25 (±.51)	8.30 (±.48)	16.30 (±2.56)	71.0 (±4.59)	26.7 (±4.10)

[a]Diets listed in Table 2

[b]Standard error of the mean in parenthesis under mean value

APPENDIX TABLE 21

TRIAL IV. SERUM PROTEIN VALUES IN B VITAMIN STUDY

Experimental Depletion Phase[a]

At Time of Antigen Injeciton

Pig no.	Total protein gm. %	Albumin %	Alpha globulin %	Beta globulin %	Gamma globulin %
			Positive Control		
84-2	5.6	57.0	19.5	13.0	10.4
84-5	3.6	48.0	25.4	14.7	11.9
84-9	5.0	52.1	19.6	13.2	15.6
85-1	5.3	60.1	18.4	13.6	7.8
85-10	5.0	62.8	16.0	13.8	7.4
87-3	5.6	50.8	23.3	14.9	10.9
Av.	5.02 (±.30)[b]	55.13 (±2.35)	20.37 (±1.38)	13.83 (±1.01)	10.67 (±1.22)
			Pantothenic Acid Deficient		
84-6	4.5	52.6	22.3	15.2	9.8
84-10	5.7	48.0	23.2	17.5	11.3
85-6	5.0	58.5	22.3	12.5	6.7
85-12	5.4	53.9	27.2	12.3	6.7
87-2	5.3	60.0	19.8	12.2	8.1
Av.	5.18 (±.20)[b]	54.60 (±2.15)	22.96[c] (±1.20)	13.94 (±1.04)	8.52 (±.89)

[a]Diets listed in Table 2

[b]Standard error of the mean in parenthesis under mean value

[c]Significantly greater than corresponding value for riboflavin deficient pigs ($P<0.05$)

APPENDIX TABLE 21 (CONTINUED)

TRIAL IV. SERUM PROTEIN VALUES IN B VITAMIN STUDY

Experimental Depletion Phase[a]

At Time of Antigen Injection

Pig no.	Total protein gm. %	Albumin %	Alpha globulin %	Beta globulin %	Gamma globulin %
		Pyridoxine Deficient			
84-4	5.2	48.6	23.7	12.3	15.3
84-8	4.7	56.3	19.8	13.1	10.8
85-2	5.0	58.7	18.3	10.0	13.0
85-4	4.0	55.8	23.2	13.0	8.0
87-5	4.8	64.8	17.0	12.4	5.8
Av.	4.73 (±.21)[b]	56.84 (±2.60)	20.40 (±1.22)	12.16 (±.56)	10.58 (±1.70)
		Riboflavin Deficient			
84-3	5.6	59.2	16.3	13.8	10.7
84-7	4.6	56.0	18.7	13.5	11.8
85-8	5.6	60.1	12.8	17.3	9.9
85-11	5.1	57.0	21.3	12.8	8.9
Av.	5.23 (±.24)[b]	58.08 (±.95)	17.28 (±1.81)	14.35 (±1.01)	10.33 (±.62)

[a]Diets listed in Table 2

[b]Standard error of the mean in parenthesis under mean value

[c]Significantly greater than corresponding value for riboflavin deficient pigs ($P<0.05$)

APPENDIX TABLE 22

TRIAL IV. SERUM PROTEIN VALUES IN B VITAMIN STUDY

Experimental Depletion Phase[a]

At Time of Antibody Determination

Pig no.	Total protein gm. %	Albumin %	Alpha globulin %	Beta globulin %	Gamma globulin %
			Positive Control		
84-2	5.4	56.0	17.4	16.2	10.4
84-5	3.8	48.1	22.7	15.2	14.0
84-9	5.1	49.9	19.8	13.8	16.5
85-1	5.0	58.2	20.0	12.5	9.2
85-10	4.2	61.3	17.3	13.0	8.4
87-3	6.6	61.5	16.3	13.5	8.7
Av.	5.02 (±.40)[b]	55.83 (±2.33)	18.92 (±.96)	14.03 (±.57)	11.20 (±1.34)
			Pantothenic Acid Deficient		
84-6	4.6	54.6	24.2	13.0	8.2
84-10	d				
85-6	4.0	56.2	22.5	12.9	8.4
85-12	5.0	51.0	26.9	12.8	9.3
87-2	5.6	24.7	44.2	17.8	13.3
Av.	4.80 (±.34)[b]	46.62 (±7.42)	29.45[c] (±5.00)	14.12 (±1.21)	9.80 (±1.19)

[a]Diets listed in Table 2

[b]Standard error of the mean in parenthesis under mean value

[c]Significantly greater than corresponding value for the control pigs ($P<0.05$)

[d]Died

APPENDIX TABLE 22 (CONTINUED)

TRIAL IV. SERUM PROTEIN VALUES IN B VITAMIN STUDY

Experimental Depletion Phase[a]

At Time of Antibody Determination

Pig no.	Total protein gm. %	Albumin %	Alpha globulin %	Beta globulin %	Gamma globulin %
		Pyridoxine Deficient			
84-4	4.3	40.0	27.9	14.8	17.2
84-8	5.4	55.8	19.6	11.5	13.1
85-2	d				
85-4	5.8	57.0	23.9	10.5	8.6
87-5	5.4	40.6	31.3	14.1	14.0
Av.	5.22 (±.33)[b]	48.35 (±4.66)	25.68 (±2.53)	12.72 (±1.02)	13.23 (±1.77)
		Riboflavin Deficient			
84-3	4.7	54.0	20.9	14.0	11.1
84-7	5.3	53.8	21.1	14.6	10.5
85-8	5.8	56.1	21.7	12.0	10.3
85-11	3.8	48.0	24.7	14.9	12.4
Av.	4.90 (±.43)[b]	52.48 (±1.74)	22.10 (±.88)	13.88 (±.65)	11.08 (±.48)

[a] Diets listed in Table 2

[b] Standard error of the mean in parenthesis under mean value

[c] Significantly greater than corresponding value for the control pigs ($P<0.05$)

[d] Died

APPENDIX TABLE 23

TRIAL IV. SERUM PROTEIN VALUES IN B VITAMIN STUDY

Experimental Repletion Phase[a,b]

Pig no.	Total protein gm. %	Albumin %	Alpha globulin %	Beta globulin %	Gamma globulin %
		Previously Positive Control			
84-2	6.2	46.9	17.6	16.5	19.0
84-5	6.9	46.1	20.1	14.8	19.0
84-9	7.9	42.4	19.1	13.5	25.1
85-1	6.9	50.6	20.0	11.2	18.2
85-10	6.5	52.9	17.2	11.7	18.2
87-3	5.6	51.2	14.8	15.7	18.2
Av.	6.67 (±.31)[c]	48.36 (±1.61)	18.13 (±.83)	13.88 (±.87)	19.64 (±1.00)
		Previously Pantothenic Acid Deficient			
84-6	d				
84-10	d				
85-6	6.8	40.3	20.9	15.8	23.0
85-12	7.0	53.7	18.4	12.4	15.4
87-2	d				
Av.	6.90 (±.10)[c]	47.00 (±6.70)	19.65 (±1.25)	14.10 (±1.70)	19.20 (±3.80)

[a]Repletion diet listed in Table 5

[b]All pigs received complete natural diet during repletion phase

[c]Standard error of the mean in parenthesis under mean value

[d]Died

APPENDIX TABLE 23 (CONTINUED)

TRIAL IV. SERUM PROTEIN VALUES IN B VITAMIN STUDY

Pig no.	Total protein gm. %	Albumin %	Alpha globulin %	Beta globulin %	Gamma globulin %
		Experimental Repletion Phase[a,b]			
		Previously Pyridoxine Deficient			
84-4	6.6	46.7	20.6	13.7	16.9
84-8	d				
85-2	d				
85-4	6.2	45.6	22.0	13.4	19.0
87-5	6.6	47.3	16.1	13.6	23.0
Av.	6.47 (±.13)[c]	46.53 (±.49)	19.60 (±1.78)	13.57 (±.29)	19.6 (±1.80)
		Previously Riboflavin Deficient			
84-3	6.6	44.8	18.4	14.4	22.3
84-7	6.4	43.2	22.3	15.5	19.0
85-7	d				
85-8	6.6	49.5	18.0	13.3	19.2
85-11	5.2	35.0	25.3	13.9	25.8
87-9	d				
Av.	6.20 (±.35)[c]	43.14 (±3.01)	21.01 (±1.72)	14.28 (±.46)	21.58 (±1.60)

[a] Repletion diet listed in Table 5

[b] All pigs received complete natural diet during repletion phase

[c] Standard error of the mean in parenthesis under mean value

[d] Died

APPENDIX TABLE 24

URINARY XANTHURENIC ACID VALUES(mcg./ml.)

Experimental Depletion Phase[a]

Pig no.	Trial III Pre tryptophan	Trial III Post tryptophan	Pig no.	Trial IV Pre tryptophan	Trial IV Post tryptophan
		Positive Control			
43-4	23.0	35.0	84-9	35.5	32.5
44-4	14.0	6.0	85-1	25.0	22.5
45-4	24.0	23.0	87-3	22.5	28.5
45-10	8.0	16.5			
Av.	17.25 (±3.81)[b]	20.13 (±6.06)	Av.	27.67 (±3.98)	27.83 (±2.91)
		Pyridoxine Deficient			
43-1	62.0	112.0	84-4	63.5	111.5
43-7	31.5	38.5	85-4	109.5	359.0
44-5	127.5	423.0	87-5	63.5	165.0
44-13	94.5	409.0			
45-1	112.0	524.0			
45-3	87.5	16.4			
Av.	85.80[c] (±14.10)[b]	278.42[d] (±80.90)	Av.	78.83[c] (±15.32)	211.83[d] (±75.19)

[a]Diet listed in Table 2

[b]Standard error of the mean in parenthesis under mean value

[c]Significantly greater ($P < 0.01$) than corresponding value for other treatment

[d]Significantly greater ($P < 0.05$) than corresponding value for other treatment

APPENDIX TABLE 25

TRIAL IV. RECIPROCALS OF ANTIBODY TITERS IN B VITAMIN STUDY

Pig no.	Experimental Depletion Phase[a] Pre injection	Post injection	Net titer	Experimental Repletion Phase[a,b] Pre injection	Post injection	Net titer
			Positive Control			
84-2	10	400	100	0	320	320
84-5	10	280	70	5	560	360
84-9	10	240	60	0	160	160
85-1	10	240	60	0	160	160
85-10	5	60	30	0	240	240
87-3	5	160	80	0	360	360
Av.	8.3 (±1.06)[c]	230[d] (±46.7)	66.7 (±9.55)	0.8 (±.26)	300 (±62)	266.7 (±38.1)
			Pantothenic Acid Deficient			
84-6	10	27	6	e		
84-10	e					
85-6	5	7	2	5	480	240
85-12	10	20	5	0	160	160
87-2	10	40	10	e		
Av.	8. (±.37)[c]	23.5 (±6.8)	5.6 (±1.56)	2.5 (±2.50)	320 (±160)	200 (±40)

[a]Diets: Depletion - Semi-synthetic diets listed in Table 2
Repletion - Natural diet listed in Table 5

[b]All pigs received complete natural diet during repletion phase

[c]Standard error of the mean in parenthesis under mean value

[d]Significantly greater than corresponding value for all other treatments ($P<0.01$)

[e]Died

APPENDIX TABLE 25 (CONTINUED)

TRIAL IV. RECIPROCALS OF ANTIBODY TITERS IN B VITAMIN STUDY

Pig no.	Experimental Depletion Phase[a] Pre injection	Post injection	Net titer	Experimental Repletion Phase[a,b] Pre injection	Post injection	Net titer
			Pyridoxine Deficient			
84-4	15	40	7	5	400	200
84-8	7	7	0	e		
85-2	e					
85-4	7	7	0	0	160	160
87-5	5	20	10	5	480	240
Av.	8.5 (±.68)[c]	18.5 (±7.7)	4.2 (±2.53)	3.3 (±1.68)	347 (±96)	200 (±23.1)
			Riboflavin Deficient			
84-3	10	60	15	5	320	160
84-7	10	35	7	0	240	240
85-7	e					
85-8	5	30	15	0	120	120
85-11	5	25	12	0	140	140
87-9	e					
Av.	7.5 (±1.41)[c]	37.5 (±7.8)	12.2 (±1.89)	1.2 (±.71)	205 (±46)	165 (±26.3)

[a] Diets: Depletion - Semi-synthetic diets listed in Table 2
Repletion - Natural diet listed in Table 5

[b] All pigs received complete natural diet during repletion phase

[c] Standard error of the mean in parenthesis under mean value

[d] Significantly greater than corresponding value for all other treatments ($P<0.01$)

[e] Died

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