THE B VITAMIN REQUIREMENT OF THE BABY PIG

I. RIBOFLAVIN
II. THIAMINE
III. PYRIDOXINE

by

Elwyn R. Miller

AN ABSTRACT

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

DOCTOR OF PHILOSOPHY

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The work presented is an attempt to establish the requirement of the baby pig for riboflavin, thiamine and pyridoxine and to obtain more information regarding the clinical and subclinical changes which occur when these vitamins are deficient in the diet.

In all of the experiments the baby pigs were taken from the sow at three to five days of age. The basal diet was a synthetic milk containing 15.15 percent solids of which casein composed 30 percent, lard 10 percent, cerelose 54 percent and minerals 6 percent. Adequate supplementation of all the known essential fat soluble and water soluble vitamins other than that vitamin being studied were supplied to the basal diet.

Fifty-five baby pigs were used in three trials to determine the riboflavin requirement. Following a depletion-adjustment period on a riboflavin-free, synthetic milk diet the pigs were individually or group fed various levels of riboflavin in the diet. Analysis of the data on individual growth response and dietary intake indicates that the riboflavin requirement of the baby pig for optimum growth and feed efficiency approximates 3.0 mg per kilogram of solids in the diet. External, gross and microscopic lesions were present only in those animals receiving less than 2.0 mg of riboflavin per kilogram of solids. Deficiency symptoms

could be alleviated by riboflavin supplementation.

Fifty-five baby pigs were used in a triplicated experiment to determine the thiamine requirement. Following a depletion-adjustment period on a thiamine-free, synthetic milk diet, the pigs were individually fed diets containing 0, 0.5, 1.0, 1.5 and 2.0 mg of thiamine per kilogram of solids. Analysis of the data on individual growth response and dietary intake indicates that the minimum thiamine requirement of the baby pig for optimum growth rate and feed efficiency approximates 1.5 mg per kilogram of dietary solids intake. External, gross and microscopic lesions were present in all pigs receiving less than 1.0 mg per kilogram of solids. Blood thiamine levels were positively related to dietary thiamine intake. Good gaining ability was rapidly restored to deficient animals which received thiamine treatment.

Sixty-five baby pigs were used in three trials to determine the pyridoxine requirement. An analysis of the growth and feed consumption data indicates that the pyridoxine requirement of the baby pig does not exceed 0.5 mg per kilogram of dietary solids. However, blood hemoglobin data and urine xanthurenic acid data are presented which indicate that the minimum requirement, when a more nearly total consideration of the pig's well-being is recognized, is not less than 1.0 mg of pyridoxine per kilogram of solids. Classical deficiency symptoms were observed in those pigs receiv-

ing no pyridoxine. The performance of paired fed controls indicated that the reduced blood hemoglobin level and the increased urine xanthurenic acid concentration in deficient pigs were due specifically to a lack of pyridoxine and not an effect of inanition. Therapeutically treated, pyridoxine-deficient pigs recovered rapidly.

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III. Pyridoxine

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INTRODUCTION

For generations animal husbandmen have been concerned with the proper feeding of farm animals. This concern has become increasingly greater as the science of animal nutrition has advanced and has brought about a demand for a qualitative knowledge of all the nutrient elements which are required by economically important animal species at all stages of their growth and productive life. Further, a quantitative knowledge of the required nutritional essentials is no less indispensable.

Probably in no area of animal nutrition endeavor has the determination of kind and amount of essential nutrients been more difficult than in the acquiring of such information for the very young of mammals during the suckling period. To obtain such qualitative and quantitative information the research worker must place the young animals on a practically simulated regimen which will produce normal physical and physiological development. Furthermore it requires the feeding of a diet in which exact amounts of all elementary ingredients are known to the experimenter. Such are the basic problems involved in the determination of the B-vitamin requirements of the baby pig.

An early impetus to the study was given by Wintrobe (1939a) when he was able with a synthetic diet to obtain posi-

tive, although admittedly poor growth in early weaned pigs. The composition of this diet was based on the equivalent values of nutrients in sow's milk as reported by Newlander and Jones (1935), Hughes and Hart (1935) and Abderhalden (1899) together with such pig requirement determinations as were reported previously by Thompson (1932), Newlander and Jones (1935), Dunlop (1935a,b) Aubel et al. (1936), Mitchell et al. (1937), Guilbert et al. (1937), Chick et al. (1938a,b, c) and Hughes (1938). Using this diet Wintrobe was able to determine crude requirements of a few of the B-vitamins for very young pigs.

McRoberts and Hogan (1944) obtained somewhat better gains and Anderson and Hogan (1947, 1950) good gains in pigs to weaning age in one experiment but only fair growth rate in two other trials using a simplified synthetic diet. Bustad et al. (1948) reported having no success in raising pigs from birth on a synthetic milk diet containing all known vitamins unless they received some colostrum or colostrum substitute. By providing either serum or plasma as a colostrum substitute they were able to obtain survival of the pigs for a maximum of 22 days. A serious diarrhea was present which could not be controlled by any of the then commonly used antibiotics.

Green et al. (1947) made a very practical contribution to the more satisfactory rearing of baby pigs under artificial conditions by studying the pigs eating habits and providing specifications for equipment and environmental conditions.

Johnson et al. (1948a) then were able to obtain an essentially normal growth rate in pigs to weaning age on a synthetic milk diet similar to that which they had prepared for dairy calves and reported by Wiese et al. (1947). However, these pigs remained on the sow for a minimum of four days before going on the synthetic diet. Lehrer et al. (1949), using a similar diet, successfully raised 2 day old pigs to weaning age. Urinary excretion of B-vitamins indicated that the pigs were receiving adequate amounts of these vitamins. The diets used by Johnson et al. (1948a), Wiese et al. (1947a) and Lehrer et al. (1949) then became the basis for many experimental and commercial sow milk replacers which have been much used since.

The formulation of adequate synthetic milk, baby pig diets have been abetted by the work on older pigs of Cunha and coworkers (1944, 1946a,b, 1947, 1948, 1949a,b, 1950; Ross et al. 1944; Ensminger et al. 1947; Heinemann et al. 1946), Ellis et al. (1943, 1944, VanEtten et al. 1940, Powick et al. 1947a, b, 1948, Miller and Ellis 1951), Hughes et al. (1939, 1940a, b, 1942a,b,c,d, 1943) and Luecke et al. (1947a, 1948, 1949a, b, 1950a, 1951, 1952) together with the studies of Wintrobe et al. (1939b, 1940, 1942a,b,c, 1943a,b,c, 1944, 1946) and the less apparent contribution of many others.

With an adequate synthetic milk diet established Johnson et al. (1948a,b) then studied pteroylglutamic acid and choline deficiency in the baby pig and Lehrer et al. (1951, 1952a,b)

and Wiese et al. (1951) produced experimental pyridoxine, pantothenic acid, biotin and riboflavin deficiencies. Nesheim et al. (1948) and Neumann et al. (1949a,b, 1950) determined the requirement of baby pigs for vitamin B_{12} and choline. Forbes and Haines (1952) reported a study on the riboflavin requirement of the baby pig and Stothers (1952, 1954, 1955) then made a study of the pantothenic acid and niacin requirements of the baby pig and reported on the pathology of these vitamin deficiencies. The reports of Miller et al. (1954, 1955) on the thiamine and riboflavin requirements of the baby pig comprise a portion of this thesis.

The work to be presented in this thesis is a study of the requirement of the baby pig for thiamine, riboflavin and pyridoxine. Various physiological, biochemical and pathological studies were made in addition to collecting growth and dietary intake data in order to provide more criterial bases for determining requirement levels of these B-vitamins.

REVIEW OF LITERATURE

Part I--Riboflavin

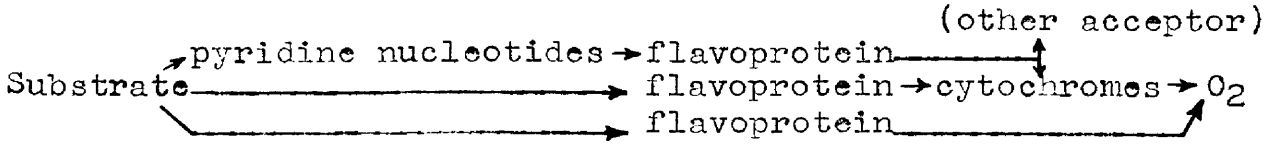
After Goldberger (1926) showed that there was a heat stable water soluble factor, an official committee in England (1927) named this component (which they assumed to be a single substance) vitamin B_2. Many workers then started working with this fraction in an attempt to identify it.

The first recognized vitamin isolated from the B_2 component was riboflavin. This was accomplished in 1933 when Kuhn, György and Wagner-Jauregg showed its growth promoting activity for rats. Warburg and Christian (1932) had discovered the "yellow enzyme" and had described lumiflavin (a degradation product of riboflavin) which was valuable in the elucidation of the chemical structure of riboflavin by Kuhn and his coworkers. The synthesis of riboflavin was accomplished in 1935 by Kuhn and coworkers in Heidelberg and Karrer and his coworkers in Zürich.

Riboflavin crystallizes into fine orange-yellow needles. Its melting and decomposition point is 278 to 282°C. It is odorless and has a bitter taste. Riboflavin is soluble in water to an extent of 13 mg percent and in ethanol to 4.5 mg percent. Its chemical name is 6,7-Dimethyl-9-(D,l'-ribityl) isoalloxazine. Its richest feed sources are dried brewers yeast and dried milk products.

Theorell (1934) demonstrated that Warburg's enzyme contained one molecule of phosphate and Kuhn et al. (1936) gave proof that the constitution of this prosthetic group was riboflavin-5-phosphate (FMN). Warburg and Christian (1938) showed that there was another riboflavin containing co-enzyme, flavin adenine dinucleotide (FAD). The enzymatic phosphorylation of riboflavin by an enzyme in yeast named flavokinase has been reported by Kearney and Englard (1951). This catalyzed reaction is: Riboflavin+ATP $\xrightarrow{\text{flavokinase}}$ riboflavin-5-phosphate+ADP. The mechanism of transformation of FMN to FAD is not known, but it has been shown to occur in human blood cells by Klein and Kohn (1940).

Both FAD and FMN act as prosthetic groups or coenzymes with apoenzymes to form flavoproteins or metalloflavoproteins and as such provide hydrogen transfer in the oxidative systems of the cell. Mahler (1954) has diagrammed their activity as shown below.



Following the successful synthesis of riboflavin and its general availability a vast amount of research has been done to determine the symptoms manifest by a riboflavin deficiency in many of the animal species. Further, work has been done to determine the requirement of riboflavin in the diet of these species, especially of the young growing animals.

Lepkovsky and Jukes (1935) found the adsorbate of liver

extract to be essential in producing normal growth in chicks. Phillips and Engel (1938) demonstrated that the presence of insufficient riboflavin in chick diets caused two types of deficiencies. The first produces a rapidly acute paralysis characterized as neuromalacia and the second, a more slowly developing form called "curled toe" paralysis. Both could be prevented by supplementing the basal diet with riboflavin.

Day et al. (1938) found that lens cataracts developed in a high percentage of rats receiving a riboflavin deficient diet. Decreased growth rate, alopecia and keratitis were other symptoms present. György (1935) and Bessey and Wolbach (1939) observed only rare cases of lens cataracts, however, the latter observed corneal vascularization in all rats kept on the riboflavin diet for 4 weeks or more. They considered vascularization of the cornea, in the absence of antecedent pathology, to be specific and the most reliable criterion of riboflavin deficiency and present the hypothesis that the vascularization is a response to asphyxia of the tunica propria. Lippincott and Morris (1942) associated riboflavin deficiency in the mouse with greatly impaired growth rate, loss of hair, an atrophic and hyperkeratotic dermatosis associated with fissuring of the skin, keratitis, infrequently cataract, as well as ill-defined myelin degeneration in the spinal cord and the sciatic nerves.

Street and Cowgill (1939) produced in dogs an acute riboflavin deficiency as evidenced by collapse after 102 to

140 days on a riboflavin deficient diet. Axelrod et al. (1940) using a more highly purified diet produced similar collapse in 6 to 8 weeks. Potter et al. (1941) ascribed loss in weight, muscular weakness, coma, and lens opacities to be results of riboflavin deficiency in the dog. Schaeffer et al. (1947) observed similar symptoms with red fox pups.

Cooperman et al. (1945) in studying riboflavin deficiency in young Rhesus monkeys found that after 6 to 8 weeks on a riboflavin deficient diet the monkeys had lost weight, a freckled type of dermatitis appeared, and hemoglobin, red cell and white cell level decreased, with a hypochromic normocytic type of anemia developing. Monkeys which died of riboflavin deficiency had developed characteristic fatty livers.

Luecke et al. (1950b) demonstrated that the pre-ruminant lamb has a definite requirement for riboflavin. Symptoms which the riboflavin deficient lambs exhibited were lowered appetite, poor growth, and finally pneumonia. Fatty degeneration of the hepatic cells of the liver and fatty degeneration of the cortex of the kidneys were apparent in the deficient animals. Similar symptoms were observed by Wiese et al. (1947) in the pre-ruminant dairy calf.

Horwitt et al. (1949) in studying the effects of dietary depletion of riboflavin in human males observed angular stomatitis, seborrheic dermatitis, scrotal skin lesions and diminution of ability to perceive flicker of light as symptoms of riboflavin deficiency.

Chick et al. (1938a) gave evidence that riboflavin might be essential in the nutrition of the weanling pig although it alone was not capable of rectifying the deficiencies produced on a maize diet. Hughes (1938) found that the addition of riboflavin as whey adsorbate to a riboflavin deficient diet of weanling pigs improved the appetite, increased the rate of gain, and improved the efficiency of food utilization. Hughes (1939) using a purified diet with 72 day old pigs concluded that riboflavin is essential to the nutrition of the growing pig. Pigs on a riboflavin deficient diet exhibited retarded growth, diarrhea, leg abnormalities and impaired walking ability. Wintrobe (1939a) noted improved growth from the addition of riboflavin to an artificial diet.

Patek et al. (1941) using a diet designed to create a chronic riboflavin deficiency rather than an acute deficiency in 7 week old pigs found the deficiency to be clinically characterized by retarded growth, corneal opacities, ulcerated skin, rough haircoat and a terminal collapse accompanied with a drop in body temperature, slow and irregular pulse and respiration. The response to injection of riboflavin following collapse was dramatic. There was no vascularization of the cornea, but cloudiness appeared in the deeper corneal stroma. The corneal surface was smooth and free from scars or ulcers and the light reflex was normal. The corneal epithelium was about 1/3 normal thickness, the parallel arrangement of cells was lost, the basal cells were swollen,

the cell nuclei were hypertrophied and contained large vacuoles and the superficial cells were cornified.

Mitchell et al. (1950) considered an increase in the concentration of neutrophilic granulocytes in the blood and in the percentage of these cells in the total leukocyte count as being the most sensitive index of riboflavin deficiency. Shukers and Day (1943) considered this change in leukocytes in rats to be due to inanition. This change occurs before the eye symptoms appear.

Miller and Ellis (1951) using practical rations with weanling pigs noted that pigs on the lowest level of riboflavin exhibited retarded growth, poor appetite, rough haircoat, rough skin sometimes caked with sebaceous exudations, infrequent periods of diarrhea and decreased efficiency of of food utilization.

Ensminger et al. (1947) reported the results of feeding bred gilts on a purified ration containing no riboflavin. The gilts lost their appetite, gained poorly and farrowed prematurely. In one case resorption of the fetus had commenced. All pigs were either dead at birth or died within 48 hours thereafter. Two hairless litters were born and in other litters pigs had enlarged front legs and showed generalized edema. The storage of riboflavin was low in both the gilts and the offspring. On post mortem all pigs showed pale kidneys, mottled with congested areas and yellow granular accumulations, yellow friable livers, red areas in the

stomach and excess body fluid. On microscopic examination hemorrhage and marked hyperemia of the kidneys was observed along with hemorrhage and edema in the lymph nodes, numerous coarse granules in the cytoplasm of the liver cells and marked subcutal edema of the legs.

In studying riboflavin deficiency in young pigs, Wintrobe et al. (1944) found growth impairment, rough, dry and thin haircoats, a mottled, erythematous eruption together with scaling and ulceration of the skin, lens opacities, normocytic anemia, and an abnormal gait to be associated with riboflavin deficiency. No vascularization of the cornea was observed. The lens cataracts observed were located in the superficial cortex, the part of the lens which is laid down after birth indicating recent origin.

Lehrer and Wiese (1952b) feeding baby pigs on a synthetic milk, riboflavin deficient diet, observed alopecia, anorexia, poor growth, rough haircoat, dermatitis, scours, ulcerative colitis, inflammation of anal mucosa, vomiting, light sensitivity, unsteady gait, and many abnormal internal complications. Necropsy showed necrosis and sloughing of the corium, the liver showed leucocytic infiltration, the kidneys showed a subacute glomerulonephritis, and the lungs showed the onset of pneumonia.

A portion of the research that has taken place to determine the requirements of the various animal species other than swine for overcoming the symptoms of riboflavin defici-

ency reveal the following:

Species	Riboflavin Requirement	Research Report
Chick	275-325 mcg/100gm ration	Bird et al.(1946)
Turkey poult	325-375 mcg/100gm ration	Bird et al.(1946)
Duckling	400 mcg/100 gm ration	Hegsted & Perry(1948)
Monkey	25-30 mcg/kg body wt.	Cooperman et al.(1945)
Dog	200-400 mcg/100gm ration	Potter et al.(1942)
Guinea pig	300 mcg/100gm ration	Slanetz (1943)
Rat	7.5 mcg per day	Czaczkes and Guggenheim (1946)
Mouse	.4-.6 mg/100 gm ration	Fenton & Cowgill(1947)
Dairy calf	1.0mcg per gm D.M.intake	Draper & Johnson(1952)
Human infant	.4 mg per day	Snyderman et al.(1948)
Human adult	1.1-1.6 mg per day	Horwitt et al. (1949)
Human adult	.5mg/1000 calorie intake	Williams et al.(1943)

We shall now consider the literature concerning the requirement of pigs for riboflavin. Hughes (1940a) using a purified diet supplemented with the B vitamins other than riboflavin found the riboflavin requirement to be between 1 and 3 mg per hundred pounds of weanling pig per day. Dyer et al. (1949) using a corn-soybean meal diet found that adding 1.5 mg of riboflavin per day to this diet for weanling pigsgreatly increased the rate of daily gain and the efficiency of feed utilization. McMillen et al. (1949) using a ration consisting of corn, soybean oil meal, meat scraps and mineral noted slow growth on rations containing 1.12 mg of riboflavin per pound of feed. Krider et al. (1949) in experiments with both purified and practical rations concluded that 1.4 mg of riboflavin per pound of ration was the practical minimum level for weanling pigs in the dry lot.

Mitchell et al. (1950) showed that the riboflavin require-

ment is higher for weanling pigs in low temperatures than for similar pigs in a warm environment, the requirements being 1.2 ppm or .55 mg per pound at 85°F and 2.3 ppm or 1.05 mg per pound at 42°F. This differs from the finding of Mills (1943) for rats in which the riboflavin requirements for optimal growth in young rats raised in environments of 90°F and 68°F were the same. Miller and Ellis (1951) found .83 mg of riboflavin per pound of diet to be adequate for growing weanling pigs on a practical ration.

Terrill and coworkers (1955) found the requirement for the growing pig to be between .4 and .65 mg per pound of diet when the mean environmental temperature was 53°F and the diet included chlortetracycline. The .4 mg per pound level was clearly suboptimal since it limited feed consumption and reduced rate and efficiency of gain resulted.

The requirements of weanling pigs for riboflavin as determined by the previously cited investigators range from the .55 mg per pound for pigs in 85°F environment determined by Mitchell et al. (1950) to 1.4 mg per pound reported by Krider et al. (1949). These differences may be explained in part by the following: 1, differences in temperature as shown by Mitchell et al. (1950), 2, inherent differences between animals as indicated by Fenton and Cowgill (1947) in which they found the riboflavin requirement for one inbred strain of mice to be .4 mg per 100 gm of feed for maximum growth while for another inbred strain the requirement was .6 mg per 100 gm of feed under

the same conditions, 3, differences in the percentage of fat or protein in the ration as demonstrated in rats by Czaczkes and Guggenheim (1946), and 4, the presence or absence of an antibiotic or vitamin B_{12}.

Miller et al. (1953) determined that a level of 1.25 mg of riboflavin per pound of feed is indicated as the practical minimum recommended allowance for breeding gilts and sows. They found that sows receiving .83 mg of riboflavin per pound of diet produced pigs of a lower birth and weaning weight and an increased mortality rate. Krider et al. (1948) had previously shown that the addition of dried fermentation solubles to a natural diet of sows and gilts in dry lot improved their gestation-lactation performance. The supplemented diet containing 2.3 mg of riboflavin per pound of feed gave significantly better results than the ration containing 1.2 mg of this vitamin per pound.

The first reported work dealing with the riboflavin requirement of baby pigs on a synthetic milk diet was that of Lehrer et al. (1952b) in which these workers cured the external symptoms of riboflavin deficiency present in baby pigs by supplementing the diet with 1 to 1.5 mg of riboflavin per day for a 16 day period. However, all deficiency symptoms were not cured. These pigs were fed the synthetic milk ration described by Lehrer et al. (1949). From the result of this study these workers felt that the requirement for riboflavin by the suckling pig was near the maximum of 3 mg per 100

pounds body weight reported by Hughes (1940a). Neither the method of reporting the requirement of riboflavin in terms of the amount required per day or the amount required per 100 pounds of body weight seem to be very acceptable since by reason of the nature of its function the riboflavin requirement should be reported in terms of total diet consumed as pointed out by Mitchell et al. (1950).

Forbes and Haines (1952) using a modified paired feeding technique concluded that the riboflavin requirement of the baby pig raised in an environment of 85°F temperature and 70% relative humidity lies between 1.5 and 2.0 mg per kilogram of feed dry matter. In contrast to the earlier report of Mitchell et al. (1950) there was no increase in the percent of neutrophiles in the blood of deficient pigs.

The riboflavin content of sows milk as determined by Davis et al. (1951) from the average composition of milk from 35 sows was 2.0 to 3.2 mcg of riboflavin per milliliter. These values are equivalent to 4.7 to 7.2 mg per pound of solids, considering sows milk to be 20% solids as Heidebrecht et al. (1951) found. They found that the riboflavin level in sow's colostrum is somewhat higher. Luecke et al. (1947b) and Braude et al. (1946) obtained similar results with colostrum.

The riboflavin requirements listed by the National Research Council (Beeson et al. 1953) is 1.0 to 1.2 mg per pound of diet for growing pigs and 1.2 mg per pound of diet for breeding stock. The latter is equivalent to 2.64 mg per

kilogram of diet.

A summary of the reported riboflavin requirements of swine is given in the table below.

Riboflavin requirement of swine

Reported requirement	Equivalent in mg/kg diet	Investigator
A. Weanling pigs:		
1-3 mg per cwt. daily	1.3*	Hughes (1940a)
1.4 mg per pound ration	3.1	Krider et al. (1949)
1.2-2.3 ppm ration	2.3	Mitchell et al. (1950)
.83 mg per pound ration	1.8	Miller & Ellis (1951)
.4-.65 mg per pound ration	1.4	Terrill et al. (1955)
B. Sows for reproduction:		
1.2-2.3 mg per pound ration	5.0	Krider et al. (1948)
1.25 mg per pound ration	2.8	Miller et al. (1953)
C. Baby pigs:		
3 mg per cwt. daily	1.3*	Lehrer & Wiese (1952b)
1.5-2.0 mcg/gm diet	2.0	Forbes & Haines (1952)

*In translating the reported requirements in terms of mg riboflavin per kilogram of diet, it has been assumed that the animals consume daily an amount of diet constituting 5% of their body weight. Where a range in requirement has been given, the higher requirement value has been chosen in this translation.

Part II--Thiamine

Takaki (1885) reported curing beriberi among seamen in the Japanese Navy by making certain changes in the men's diet. He concluded that the cure was achieved as a result of increase in protein intake.

Eijkman (1890, 1892) demonstrated that fowl fed polished rice developed a polyneuritis which soon ended fatally. It could be cured by feeding raw rice. Later (1896) he proved that beriberi was caused by eating polished rice as the chief food. He believed, however, that polished rice contained some toxin which was causing the disease. Eijkman's collaborator, Grijns (1901) was the first to state clearly that beriberi was due solely to a dietary deficiency. He called this dietary deficient substance "protective substance".

Funk (1911) extracted and partially purified the anti-neuritic factor in rice bran to the extent that 50 mg of the extract rapidly cured a severely polyneuritic pigeon. In 1912 he used the term 'vitamine' in refering to this and other factors needed in small amounts in the diet. Hopkins (1912) noting growth factors from small amounts of milk called them "accessory factors of the diet". McCollum and Davis (1915 a, b) found essential growth factors in both the water soluble and the fat soluble portions of the diet. They refered to these as "fat-soluble A" and "water-soluble B".

Since no evidence had been forthcoming to support Funk's original idea that these indispensable constituents were amines,

Drummond (1920) suggested that the terminal "-e" be dropped. He further suggested that McCollum's (fat-soluble A and water-soluble B) be dropped and these substances be refered to as vitamin A, B, etc. Both of these suggestions were adopted. Later Jansen and Donath (1926) succeeded in isolating the anti-neuritic or antiberiberi vitamin in the pure crystalline state.

In 1926 Goldberger and coworkers showed that pellagra was associated with the lack of a vitamin which had a distribution similar to that of the antiberiberi factor but was more heat stable. In 1927 an official committee in England revised the nomenclature and the antiberiberi or antineuritic vitamin was named vitamin B_1. Jansen (1935) proposed the name "aneurine" for this substance, a name extensively adopted in Europe while in America the name "thiamine" has been consistently used.

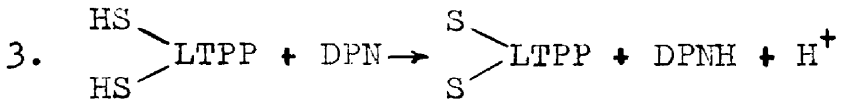
The structural formula of thiamine then became established and the synthesis was accomplished by Williams and Cline (1936), Grewe (1936), Todd and Bergel (1937) and Andersag and Westphal (1937). Thiamine is composed of both a pyrimidine and a thiazole portion and its chemical name is 3-(4-amino-2-methylpyrimidyl-5-methyl)-4-methyl-5- b eta-hydroxyethyl thiazolium chloride hydrochloride. Thiamine chloride hydrochloride is a white crystalline substance which melts and decomposes at 248-250°C., is soluble in water and alcohol and is stable in acid solution. Thiamine is very labile to heat and in alkaline solutions. Thiamine has a widespread distribu-

tion in human foods and animal feeds. Brewer's yeast is an especially rich source.

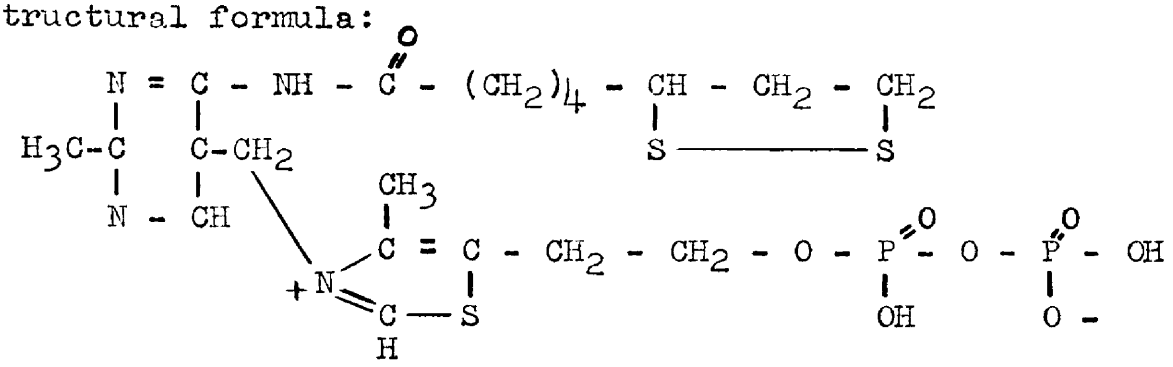
Lohman and Schuster (1937) succeeded in isolating cocarboxylase in a pure, crystalline state and showed that it was the pyrophosphoric ester of thiamine. This coenzyme plus yeast carboxylase plus magnesium ions decarboxylated pyruvic acid. Thiamine is present in the animal body mainly in the pyrophosphate form. Jansen (1949, 1954) ably reviewed the results of many studies and lists 25 separate reactions in which thiamine pyrophosphate has been shown to act in a coenzymal capacity. Later reviews by Cheldelin and King (1954), Johnson (1955) and Weinhouse (1954) have clarified the dual action of thiamine in oxidative decarboxylation reactions. Gunsalus (1953) and Seaman (1953) have shown that while lipoic acid is not necessary for the direct decarboxylation of pyruvate to acetate, it is required for the oxidative decarboxylation reaction in which pyruvate enters into acetyl CoA formation. Reed (1953) and DeBusk (1953) presented evidence that the overall conversion of pyruvate to acetyl CoA may be expressed in these three steps:

1. $$\text{Pyruvate} + \begin{matrix} S \\ | \\ S \end{matrix}\!\!>\text{LTPP} \rightarrow \begin{matrix} \text{Acetyl-S} \\ \\ \text{HS} \end{matrix}\!\!>\text{LTPP} + Co_2$$

2. $$\begin{matrix} \text{Acetyl-S} \\ \\ \text{HS} \end{matrix}\!\!>\text{LTPP} + \text{CoASH} \rightarrow \begin{matrix} \text{HS} \\ \\ \text{HS} \end{matrix}\!\!>\text{LTPP} + \text{Acetyl S CoA}$$



in which LTPP is lipothiamide pyrophosphate and has this structural formula:



Soon after the isolation, identification and synthesis of thiamine, riboflavin and niacin and their recognition as B-vitamin components, Chick (1938a), Hughes (1938) and Wintrobe (1938) demonstrated thiamine to be beneficial in the growth of weanling pigs on purified diets.

Hughes (1939) found thiamine deficient pigs had greatly reduced appetites and leg weakness. Two deficient pigs died suddenly but flabby hearts were not in evidence in these pigs. Supplementation of other thiamine deficient pigs with crystalline thiamine rapidly restored their appetite and activity. This experiment gave evidence that the requirement of the weanling pig for thiamine was less than 6 mg per 100 pounds of pig daily. The following year, Hughes (1940) reported an experiment with weanling pigs on a purified diet in which rate of growth and muscle thiamine assay were the criteria for adequacy of thiamine supplementation. The results indicated the

requirement to be about 1 mg per 100 pounds of growing pig daily.

VanEtten and coworkers (1940) reported a study with young pigs. Three week-old pigs were placed on a sodium sulfite-sulfur dioxide treated diet containing 10% fat and supplemented with different levels of thiamine. The pigs receiving no thiamine in the diet consistently developed symptoms as follows: almost complete refusal of food with occasional vomiting, extreme emaciation and marked lowering of body temperature. When thiamine was not added, death usually occurred within 5 weeks. Upon autopsy of these animals very flabby hearts were noted. The intestinal tract was always almost completely empty but pathological changes were not always noted. In some cases liver damage was noted. Thiamine administration rapidly alleviated the anorexic and growth depressed condition of the deficient pigs. For pigs under these conditions the requirement for thiamine appeared to be 106 to 120 mcg per 100 gm of carbohydrate and protein consumed in the diet.

Wintrobe *et al.* (1942c) studied thiamine deficiency in young pigs on a purified diet in which crystalline vitamins supplied the chief source of the B-vitamins. The chief symptoms of thiamine deficiency again were anorexia, vomiting, dyspnea, cyanosis and great weakness. The symptoms of cardiac failure appeared suddenly and unless thiamine was given resulted promptly in death. Impairment of growth was not a prominent sign in pigs dying early of acute thiamine deficiency.

In all animals dying of thiamine deficiency, focal necroses of the myocardium were observed. No neurological symptoms were observed nor were any degenerative changes in the nervous system found even in animals in which thiamine deficiency of long duration was produced.

These authors found that blood pyruvic acid level was found to be positively correlated with the onset and severity of symptoms of thiamine deficiency and is a useful test. More useful than a single determination of blood pyruvate, however, was comparison of the value before and after the administration of a measured amount of glucose.

Follis and coworkers (1943) found that cardiac dilatation without hypertrophy, and focal and difuse myocardial necrosis were the characteristic heart findings in thiamine deficient pigs. In most of these pigs necrotic lesions were found in both the auricles and the ventricles. These lesions were infiltrated with polymorphonuclear and mononuclear cells. In older lesions there were connective tissue cells as well. The initial change seemed to be a loss of striation accompanied by vacuolization and hyalinization of the fiber. In older lesions no intact muscle fibers could be found. The interventricular septum was more severely damaged than the walls of the ventricles. Fat stains revealed foci of fatty infiltration of the muscle fibers in the fresh lesions. The increase of heart weight as a percent of total body weight was due more to loss of body weight rather than to cardiac hypertrophy.

There was usually lung edema present with fluid and red blood cells in the pulmonary alveoli. Histological examination of the nervous tissues revealed no lesions in any of the pigs. None of the cardiac or the pulmonary lesions were found in any of the animals in which inanition alone was produced.

Miller et al. (1943) found that weanling pigs receiving 3.5 mg thiamine per pound of feed did not gain any more rapidly or efficiently than pigs receiving 1.3 mg thiamine per pound of feed but that these pigs receiving the higher level deposited twice as much thiamine in the muscle. This may be of significance since pork has long been considered (Hughes, 1941 and Waisman and Elvehjem, 1941) a good source of thiamine in the human diet.

The workers at Washington State, Ensminger et al. (1943) and Heinemann et al. (1946) also reported that a positive relation exists between thiamine intake and the deposition of this vitamin. Symptoms observed on the thiamine deficient pigs were vomiting, anorexia, slight staggering, cyanosis and a reduction in rectal temperature, heart rate and respiratory rate late in the experiment. Enlarged hearts were obtained from the pigs on the thiamine deficient ration. These authors showed that the pig can store thiamine and can utilize stored thiamine over a considerable period of time. In addition, it was found that .19 mg thiamine daily per kg live weight supported normal rate of gain and feed efficiency of pigs on a purified diet.

Ellis and Madsen (1944) showed that the thiamine requirement of pigs is reduced by increasing the level of fat in the diet. They fed pigs on three levels of fat (2%, 11% and 28%) in the diet. As indicated by failure in appetite and cessation of growth, the animals on the low level of fat showed evidence of thiamine depletion on the average in 25 days, those on the medium level in 28 days and those on the high level in 33 days. Lack of thiamine resulted in weakening of the heart, decrease in body temperature and emaciation. Thiamine administration rapidly restored appetite, growth and general well being. It was found that the level of thiamine required to produce a maximum rate of growth and otherwise maintain good health fell within the range of 125 to 141 mcg per 100 gm carbohydrates and protein in the diet on the medium level of fat. However, these amounts were insufficient to promote the normal amount of storage of thiamine in the meat tissue such as is found in commercial pork cuts. Such a sparing action of fat on thiamine as noted in this experiment had previously been reported by Evans and Lepkovsky (1929) in the rat and by Arnold and Elvehjem (1939) in the dog. Electrocardiograms (ECG) were taken on four pigs late in the experiment.

Electrocardiographic studies with pigs have been extremely few and limited from the standpoint of both normal animals and animals which have been subjected to nutrient deficiencies. Fortunately, some of the electrocardiographic studies that have been made in swine have been concerned with thiamine de-

ficiency also.

Lepeschkin (1951) reports that the ECG of the normal pig usually shows marked S_{II} and S_{III} and often an inverted T_1, however. T_{II} and T_{III} are usually positive and rarely diphasic. The apex lead almost invariably had a positive T wave according to the work of Wintrobe et al. (1943c). The QRS voltage usually decreased with the heart rate. The rhythm was sino-auricular with slight or moderate sinus arrhythmia. Normal heart rates of pigs used in Wintrobe's work were between 130 and 150 beats per minute (bpm), averaging 140. In these experiments electrocardiograms were not taken until the pigs were 3½ to 4 months of age. The P-R interval ranged between .06 and .12 seconds, usually .09 seconds. The P waves were usually .04 to .06 seconds in duration. The duration of QRS was .04 to .06 seconds.

Ellis and Madsen (1944) took electrocardiograms on only one normal pig. The pig was 71 days old when the ECG study was made. The heart rate was 138 bpm, the P-R interval was .10 seconds, the Q-T interval was .21 seconds and the constant for relating systolic duration to length of cycle ($K=\frac{QT}{\sqrt{RR}}$) was .34.

Moustgaard (1953) reporting electrocardiogram data from two normal pigs in his thiamine work found heart rate to be 160 bpm, P-R interval .06 seconds and S-T interval .08 seconds.

Thiamine deficiency produces greatly different electro-

cardiographic effects in different species. In thiamine deficient dogs Swank et al. (1941) found tachycardia, elevation of P, inversion of T and increase of relative Q-T duration reversible after thiamine injection. Dogs showing an elevated S-T also had myocardial necroses. In thiamine-deficient cats Toman et al. (1945) reported that bradycardia was more common. There was increase in QRS duration and the systolic portion of the cycle was relatively increased. T_{II} was often inverted. In thiamine-deficient pigeons, bradycardia, increase of P-R and inversion of T was found by Swank and Bessey (1942). Bradycardia did not appear if starvation were prevented by tube feeding. Thiamine injection rapidly restored the birds' heart manifestations to normal. In rice-fed pigeons Carter and Drury (1929) noted bradycardia and heart-block. In thiamine-deficient rats, many investigators, VanHeerswynghels (1945), Weiss et al. (1938), Zoll and Weiss (1936), Haynes and Weiss (1940) and Hundley et al. (1945) have observed bradycardia, lengthened P-R, QRS and relative Q-T duration and T wave changes all of which could be normalized by thiamine administration. Weiss et al. (1938) was not able to abolish cardiac slowing or electrocardiographic changes by the administration of atropine or by vagal section. In addition, Hundley et al. (1945) noted sinus arrhythmia, auricular fibrillation, A-V nodal rhythms, sinus arrest, shifting pacemaker, first degree A-V block and auricular, A-V nodal and ventricular ectopic beats. These were partially or completely

corrected by thiamine therapy. The heart lesion was essentially a necrosis of muscle fiber followed by cellular infiltration, fibroblast proliferation and varying degrees of fibrosis. King and Sebrell (1946) found similar symptoms. In the latter study, heart rates in thiamine-deficient rats dropped from a normal of 456 to 369 beats per minute.

The accumulation of pyruvic acid in thiamine deficiency has been shown by Haynes and Weiss (1940) and Wilkins et al. (1939) to cause bradycardia and increase of Q-T in rats. However, VanHeerswynghels (1945) found that this only occurred when the concentration of pyruvic acid became extremely high. Randles et al. (1947) reported that T wave changes appeared in dogs only when pyruvic acid began to be liberated by the heart.

In human beriberi, Weiss and Wilkins (1937) found tachycardia, bounding arterial pulsation, arterial pistol sounds, engorged veins, increased venous pressure, increased velocity of blood flow and decreased peripheral utilization of arterial oxygen.

Sturkie (1954) reported that chickens fed a diet completely devoid of thiamine developed abnormal electrocardiograms in 7 to 10 days. The abnormalities included bradycardia, depression of S-T segment, sinus arrythmia, sinus arrest, and auriculoventricular dissociation with nodal rhythm.

The most extensive work on electrocardiographic changes in thiamine-deficient pigs has been reported by Wintrobe et al.

(1943c). Their experimental animals were 36 to 38 days of age when placed on a diet low in thiamine and were maintained in a chronic to severe thiamine deficiency for up to 258 days before death occurred. The thiamine deficiency changes manifested by the electrocardiograms were bradycardia, prolonged P-R interval, second degree auriculo-ventricular block, slight to pronounced sinus arrhythmia, nodal and ventricular premature beats, auriculo-ventricular dissociation with variable ventricular foci, complete block with ectopic ventricular rhythm as well as bigeminal rhythm and auricular fibrillation. P_{II} became abnormally high and broad and was sometimes notched. One noted a great similarity between these changes and those later reported by Hundley et al. (1945) in thiamine-deficient rats.

The injection of atropine sulfate into two pigs soon resulted in increased heart rate and shortening of P-R interval. The QRS complex was no longer dropped and there was a decrease in the frequency of premature ventricular beats. This would tend to indicate that the bradycardia was due to vagal overaction since atropine is inhibitory to vagal action. These workers found that thiamine deficiency produced in pigs a much more intense bradycardia than that observed in pigs suffering from inanition due to riboflavin, niacin, pyridoxine or pantothenic acid deficiency. Jakobsen et al. (1950) concluded that the thiamine deficiency causes partly a disturbance of the regulation of the transmission frequency of the sino-auricular node as a consequence of imbalance of the vagus action

and partly a disturbance of the normal course of the impulse through the cardiac neuromuscular system.

Both Ellis and Madsen (1944) and Moustgaard (1953) observed bradycardia and increased P-R and Q-T or S-T intervals. In the former work an increase in the relative systolic portion of the cycle was reported. Both studies dealt with pigs beyond weaning age and dealt with a minimum of experimental animals.

Moustgaard (1953) reported deficiency symptoms similar to those previously reported except that no signs of disturbances of movements or intestinal disorders occurred. Furthermore, (1953, 1954) he reported that no rise in blood pyruvate level occurs if the animals have been trained to lie still for 40 to 60 minutes before taking the blood sample. If excited, normal animals showed elevated blood pyruvate and lactate. The elevated blood pyruvates found at times (Wintrobe, 1943c) in thiamine-deficient pigs are possibly attributable to their greater excitability. He reported a thiamine requirement of 75 mcg per kilogram of body weight daily to support maximum growth..

When Ensminger (1947) deleted thiamine from the ration of gilts a low storage of thiamine occurred in both the gilts and the offspring. Premature parturition occurred in several of the gilts and there was a high birth mortality in litters farrowed. The pigs which survived were usually weak and unthrifty and attained subnormal weaning weights.

The thiamine level in sow's colostrum has been reported by Luecke et al. (1947b), Braude et al. (1946) and Davis et al. (1951) as being .5 to 1.0 mcg per milliliter. Davis et al. (1951) reported that the thiamine concentration remains quite constant throughout the lactation period. Their reported mean value for sows in advanced lactation was .6 mcg per milliliter. This is equivalent to approximately 3 mg of thiamine per kilogram of solids in the milk.

In reviewing the literature one notes a great diversity in the reported thiamine requirements. These are condensed and presented in the following table.

Thiamine requirement of the growing pig

Reported requirement	Equivalent in mg/kg diet*	Investigator
1 mg/100 lb. body wt./day	0.44	Hughes (1940)
106 to 120mcg/100gm carbohydrate+protein in diet.	0.96	VanEtten et al.(1940)
125 to 141mcg/100gm carbohydrate+protein in diet.	1.12	Ellis & Madsen (1944)
75 mcg/kg body wt./day.	1.50	Moustgaard (1953)

*In translating the reported requirements in terms of mg B_1 per kilogram of diet, it has been assumed that the animals consume daily an amount of diet constituting 5% of their body weight and that the carbohydrate and protein portion constitutes 80% of the diet. Where a range in requirement has been given, the higher requirement value has been chosen in this translation.

The thiamine requirement listed by the National Research Council (Beeson et al. 1953) is .5 mg per pound of diet or 1.1 mg per kilogram of diet.

Part III--Pyridoxine

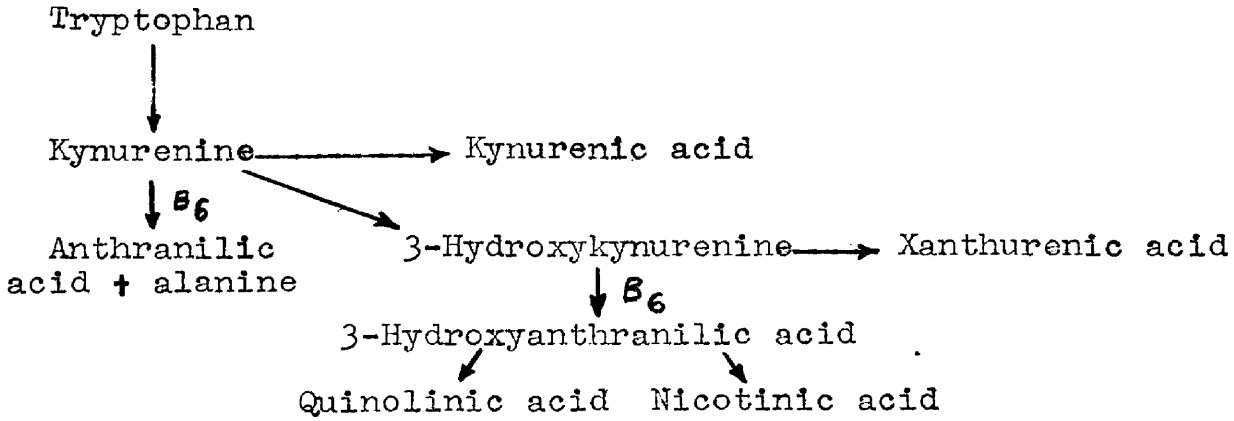
Soon after riboflavin became recognized as a vitamin, György (1934) identified a second component of the vitamin B_2 complex in an experiment with rats. This was first called vitamin B_6, later renamed adermin and now is generally known as pyridoxine and the related amine and aldehyde. Birch and György (1936) definitely established the apparent and specific chemical properties of vitamin B_6 as present in crude concentrates. Lepkovsky (1938), Keresztesy and Stevens (1938), György (1938), Kuhn and Wendt (1938) and Ichiba and Michi (1938) succeeded in isolating pyridoxine from various natural materials. The structure of pyridoxine was established by Stiller et al. (1939) and Kuhn et al. (1939). In the same year Kuhn et al. (1939) and Harris and Folkers (1939a,b,c) completed snythesis of the vitamin.

Vitamin B_6 is now used as a class name and includes pyridoxine: 2-methyl-3-hydroxy-4, 5-dihydroxymethylpyridine, pyridoxal: 2-methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine and pyridoxamine: 2-methyl-3-hydroxy-4-aminomethyl-5-hydroxymethylpyridine. Snell and coworkers (1942, 1944) and Harris et al. (1944a,b) were responsible for the recognition, isolation and synthesis of these active components of B_6 other than pyridoxine. The three compounds are all water-soluble, fairly stable to heat, but unstable to light. Vitamin B_6 is

widely distributed in animal feeds. Yeast, milk and cereal by-products are excellent sources.

Pyridoxine, like thiamine and riboflavin, functions in the form of a coenzyme. This coenzyme, pyridoxal-5-phosphate, functions with both decarboxylase and transaminase enzymes. The structure of codecarboxylase and its synthesis were determined and accomplished by Harris and his associates Heyl et al. (1951) and Wilson (1951). Baddiley and Gale (1945) had previously indicated its function.

Umbreit (1954) has reviewed the literature and summarized the activity of pyridoxal phosphate with amino acid decarboxylases, transaminases and racemases. He has shown the scheme whereby tryptophan is metabolized in the animal body. Pyridoxal-5-phosphate has been shown by Dalgliesh et al. (1951) to be the coenzyme of the reaction converting kynurenine to anthranilic acid and alanine and also the reaction converting 3-hydroxykynurenine to 3-hydroxyanthranilic acid.



Sherman (1950) has made a review of the apparent inter-

relationships of pyridoxine and fat. He has cited studies in which pyridoxine plays a role in both the synthesis and the deposition of essential fatty acids, particularly linoleic and arachidonic. Witten and Holman (1952) have supplied further evidence of this.

Later noteworthy reviews are those of Sebrell and Harris (1954), Merck & Co. (1954), Cheldelin and King (1954), Snell (1953) and Fried and Lardy (1955). All are in accord that B_6 functions as a metal chelate formed from the amino acid and pyridoxal and refered to as the Schiff base. The scheme is particularly well presented by the last cited reviewers.

Since Sherman (1950) and Sebrell and Harris (1954) have so ably reviewed the literature anent the effects of pyridoxine deficiency and the requirements for pyridoxine in various species, the present writer will limit the remainder of the review to include predominantly only that which appertains to swine.

Hughes (1938) fed a semi-purified diet to weanling pigs and found that supplying thiamine, riboflavin and nicotinic acid did not support as rapid growth as when the filtrate factor in the form of rice bran filtrate was supplied. This factor included both pyridoxine and pantothenic acid.

Chick and her coworkers (1938c) found that optimum growth could be obtained in pigs on a purified diet if 4% yeast were added. Replacing yeast with thiamine, riboflavin and nicotinic acid did not support growth. When the eluate

fraction was absent from the diet the pigs showed slow growth, epileptiform seizures, and microcytic hypochromic anemia. Administration of the eluate, which was known to contain pyridoxine, relieved the anemia and caused the fits to cease. Wintrobe (1939a) fed early weaned pigs on a synthetic milk diet supplied with yeast as a source of B-vitamins. When the yeast was withdrawn, prostration developed and convulsions occurred. Again, these could not be corrected by supplying thiamine, riboflavin and nicotinic acid but could be avoided by feeding yeast as .3% of the diet.

Wintrobe et al. (1939b) had also observed anemia in pigs receiving thiamine, riboflavin and nicotinic acid but no yeast. Further, these authors (1940) showed that degenerative changes in the nervous system of the pig occurs when only these three B vitamins were supplied. Wintrobe and coworkers (1942a) also showed that anti-pernicious anemia liver extract did not prevent convulsions and anemia from developing on this diet.

Wintrobe et al. (1942b) when feeding a diet more completely supplied with B vitamins found that whenever either pyridoxine or pantothenic acid was omitted from the diet, the pigs developed an abnormal gait and showed degenerative changes in the peripheral nerves, the posterior root ganglion, the posterior roots and the posterior funiculi of the spinal cord. When pyridoxine was absent epileptiform fits were observed and anemia appeared. These promptly disappeared with administration of pyridoxine. Fatty infiltration of the liver occurred

when either choline or pyridoxine was omitted from the diet for a prolonged period.

Wintrobe and his coworkers (1943a) studied uncomplicated pyridoxine deficiency and found that a severe anemia developed which differed from pernicious anemia and iron-deficient anemia. This anemia was characterized by microcytosis, an increase of polychromatophilia, reticulocytes and nucleated red cells in the blood, a rise in serum iron, bone marrow hyperplasia and hemosiderosis in the spleen, liver and bone marrow. Administration of pyridoxine orally at a level of 40 mcg per kilogram body weight daily was followed by a maximal reticulocyte response and rapid regeneration of blood with restoration of the normal size of the red corpuscles. Mobilization of iron from the tissues and its utilization in blood formation was indicated by the disappearance of hemosiderosis and a fall in the serum iron. This indicated that pyridoxine was related to the utilization of iron. Epileptiform seizures were also observed in this study and fatty infiltration of the liver was apparent when prolonged pyridoxine deficiency had taken place.

Follis and Wintrobe (1945) observed that pyridoxine deficient pigs showed an abnormal gait characterized by swaying and twisting of the legs. This gait differed from the 'goose stepping' characteristic of pantothenic acid deficiency.

In a further study on the anemia which develops in pyridoxine-deficient pigs, Cartwright et al. (1944) found that the condition was not due to an increase in hemolysis but due to a

diminution in hemoglobin formation. Vitamin B6 anemia was found to be similar to pernicious anemia in that both conditions were characterized by increased serum iron, hemosiderosis of the tissues, hyperplastic bone marrow and neurological lesions. Both differed from iron-deficient anemia in which neither hemosiderosis nor ferremia occurred. Limiting the iron intake in pyridoxine anemia, while abolishing hemosiderosis and ferremia, did not, however, prevent the development of ataxia, convulsions, neurological lesions and fatty livers. Evidence presented indicated that the continued absorption or decreased excretion of iron prevailed even when the hemoglobin formation was at a minimum and when the tissues had an abundant content of iron. These workers (1945) found that the anemia produced in pigs caused by a lack of dietary tryptophan was quite different from vitamin B6 anemia. In tryptophan-deficiency anemia there was no hemosiderosis. The serum iron level was low. It was essentially a normocytic, normochromic anemia. A reduction of tryptophan in the diet diminished the severity of B6 deficiency.

Cartwright and Wintrobe (1948a) suggested that the fundamental disturbance in pyridoxine-deficient anemia in swine is a failure to synthesize protoporphyrin. They determined erythrocyte protoporphyrin level in normal and pyridoxine deficient pigs and found that the mean for pyridoxine-deficient pigs was 47±13.6 mcg per 100 ml of red cells as compared to 118± 43.4 mcg per 100 ml of red cells in normal pigs. Plasma iron

levels at the same time rose from a normal of 169±38.8 mcg percent to 468±166.6 mcg percent in the B6-deficient pigs. On the otherhand, plasma copper levels dropped from a normal of 206±26.3 mcg percent to 160±38.8 mcg percent. Urinary coproporphyrin levels appeared unaffected.

Hughes and Squibb (1942d) used two groups of weanling pigs fed a purified diet in which the positive controls received 5 mg of pyridoxine per 100 pounds daily while the other group received no supplemental pyridoxine. The pyridoxine-deficient pigs became unsteady on their feet and showed reduced appetites within a month after the start of the experiment. Later the pigs ceased to gain and occasionally an epileptic-like fit was observed. The author's description of a typical epileptiform seizure is presented as follows: "A pig would be walking about the pen when suddenly he would stop, become unsteady on his feet and fall on his side, struggling violently, and screaming with a high-pitched voice, with mouth open and legs kicking out in all directions, then his body would stiffen with head back and with open starry eyes. This would be followed by a comatose stage when the pig appeared dead. Respiration was first deep, then shallow, then deep again. In about a minute or two, the pig would regain partial consciousness, would arise and stagger in any direction. Finally, in a few seconds, he would become conscious and would walk to the trough and drink water and would appear normal though somewhat weakened". Blood studies showed the characteristic microcytic

hypochromic anemia in the deficient pigs. Administration of pyridoxine to these pigs rapidly corrected all of the observed symptoms. None of these symptoms were observed in the control pigs.

Emsbo and associates (1949) in Denmark observed similar B_6 deficiency symptoms in pigs. In their trials reduction of appetite occurred during the second week of deficiency and growth stopped after 3 weeks. Pigs died after two months on the diet lacking pyridoxine. Convulsions were frequent late in the experiment. Nitrogen balance studies showed a reduction in nitrogen retention in B_6 deficiency. This was apparent before the external manifestations and the reduction increased as the severity of B_6 deficiency advanced.

Møller (1951) performing complete balance studies with human babies and with pigs on all known B_6 compounds found that elimination of B_6 was greater than the intake. This suggested limited intestinal bacterial synthesis which, however, was inadequate to meet the growing pigs' needs.

Lehrer et al. (1951) studied pyridoxine deficiency in baby pigs taken from the sow at 2 days of age and placed on a synthetic milk diet (Lehrer et al., 1949). In these experiments pyridoxine deficiency was characterized by poor appetite, vomiting, inco-ordination of the muscles, spastic gait, poor growth, epileptiform fits, comas, rough haircoat, brown exudate around the eyes and impairment of eyesight. All deficiency symptoms with the exception of impaired eyesight could be

cured by adequate pyridoxine supplementation. The excretion of xanthurenic acid was detected in the B_6-deficient animals and these animals excreted low levels of pyridoxine.

Xanthurenic acid was first identified in the urine of pyridoxine-deficient pigs by Cartwright et al. (1944) although a green pigment-producing substance was earlier reported by Wintrobe et al. (1943). The presence of this green pigment-producing substance was first reported in the urine of pyridoxine-deficient rats by Lepkovsky and Nielsen (1942). Later Fouts and Lepkovsky (1942) found this substance in the urine of pyridoxine-deficient dogs. Still later Lepkovsky et al. (1943) showed this substance to be xanthuranic acid, a metabolic product of tryptophan.

Moustgaard and coworkers (1952, 1953, 1954) have made the most recent study of the requirement of the pig for pyridoxine. At the time these studies were made the function of pyridoxine was more fully understood and a greater number of criterial measures could be employed in determining this requirement. Their studies showed that the daily requirement value of 40 mcg of pyridoxine per kilogram of body weight found by Wintrobe et al. (1943a), while adequate to prevent the occurrence of clinical deficiency symptoms, was inadequate to support maximum growth and nitrogen deposition in weanling pigs. To acquire maximum protein utilization it was necessary that they supply twice this amount of pyridoxine.

Several determinations reported by Moustgaard (1953) have

added to the evidence of poor protein utilization in B_6-deficient pigs in addition to reduced growth rate and nitrogen retention. Urine studies showed a greatly increased excretion of xanthurenic acid and kynurenic acid in the urine of these pigs which paralleled the fall in protein utilization. When these pigs were given an oral dose of L-tryptophan, the disturbance of the enzymatic transformation of tryptophan caused by the pyridoxine deficiency was revealed through an increased urinary xanthurenic acid excretion long before any clinical symptoms of pyridoxine deficiency could be demonstrated. The simultaneous reduction of blood hemoglobin and the increase of serum iron probably results from its liberation from the ferritin iron of the tissues as hemoglobin formation is reduced.

Moustgaard (1953) explains the occurrences of epileptiform seizures in pyridoxine-deficient pigs as an excitation release. The pigs are on the verge of seizures and their threshold of excitation is lowered. The disturbances of the protein metabolism also gave rise to impairment of the pig's ability to produce the antibody-carrying serum protein, gamma globulin. The gamma globulin fraction of total serum protein in the pyridoxine-deficient pigs constituted only 5 percent. This fraction normally comprises 15 to 20 percent of the serum protein. Moustgaard (1953) feels that since this fraction is especially rich in tryptophan, this may account for a reduced defense mechanism. It should be stated here that pyridoxine has been shown to function in enzyme systems concerned with most if not

now all of the essential amino acids (viz. reviews cited).

It would seem wise to consider this pyridoxine-antibody response relation further as applied to other experimental animals. Stoerk (1946a) and Agnew and Cook (1949) found a greatly reduced thymus weight in pyridoxine-deficient rats. The lymph nodes were greatly depleted of lymphocytes and there was a general atrophy of all lymphoid tissue. Dougherty et al. (1944) had previously demonstrated in mice the presence of antibodies in lymphocytes and substantiated the production of antibodies in lymph nodes. A significant portion of the antibody protein was contained in lymphocyte elements of immunized animals. These authors later (1945) showed that adrenal cortical mediation is essential for control of the release of antibodies from lymphocytes.

Stoerck and Eisen (1946) and Agnew and Cook (1949) immunized pyridoxine-deficient rats with washed sheep erythrocytes. The development of antibody response in the serum of these animals was greatly reduced as compared to that developed in either inanition controls or full controls. Axelrod et al. (1947) made similar observations in hemagglutinin production in response to human erythrocyte inoculation in both pyridoxine and pantothenic acid-deficient rats. Ludovici et al. (1951) observed, however, that if the immunization were made while the rats were receiving a complete diet, the serum antibody titers remained high even during protracted periods of either pantothenic acid or pyridoxine deficiency. Bosse and

Axelrod (1948) noted marked impairment of rate and quality of healing in pyridoxine or riboflavin-deficient rats. Axelrod (1952a,b) has written two reviews on the role of the B vitamins in antibody synthesis.

Olsen and Martindale (1954b) have reported that there was an increase in the wet weight of the adrenal, ventricular mass, liver and kidney of pyridoxine-deficient rats. There was a 30% decrease in the oxygen consumption of the broken-cell preparations of hepatic tissue as studied in the Warburg apparatus. Renal tissue preparations showed a 20% decrease in O_2 consumption. Agnew (1955) also found heavier wet and dry weights of heart, liver and kidneys in pyridoxine-deficient rats. Olsen and Martindale (1954a) observed an elevation in systolic blood pressure in B6-deficient rats. No such observations as these have been reported for the pyridoxine-deficient pigs.

This writer believes that while many enlightening and detailed studies have been made in relation to various clinical and subclinical conditions arising in a pyridoxine deficiency in the pig, the determination studies on the requirement of the pig for this vitamin have been inadequate, especially so for the baby pig. The pyridoxine requirement listed by the National Research Council (Beeson et al., 1953) for the weanling pig is .6 mg per pound of diet. This is equivalent to 1.3 mg per kilogram of diet. The few reported requirements are presented in the table below.

Pyridoxine requirement of the growing pig

Reported requirement	Equivalent in mg/kg diet*	Investigator
40 mcg per kg body wt. daily	.8	Wintrobe et al. (1943a)
5 mg per 100 lb. body wt. daily	2.2	Hughes and Squibb (1942d)
80 mcg per kg body wt. daily	1.6	Moustgaard (1953)

*In translating the reported requirements in terms of mg pyridoxine per kilogram of diet, it has been assumed that the animals consume daily an amount of diet constituting 5% of their body weight.

Experimental Procedure

I. Riboflavin

The riboflavin experiments were conducted first. This consisted of three trials involving 55 baby pigs. The first of these trials was a group feeding trial in which 15 purebred Duroc Jersey pigs were used. These pigs were taken from the sow when they were three days old and were lotted as uniformly as possible according to sex, size and litter into five groups of three pigs each. They were reared in 'terralactors'. These were commercially made metal cages with wire-mesh bottoms that were designed for the battery raising of early weaned baby pigs on sow milk replacers.

The diet fed was basically that used by Lehrer et al. (1949), Wiese et al. (1947a) and Johnson et al. (1948a) and identical to that used by Stothers (1952) with the exceptions that the pantothenic acid was supplied at a level of 2 mg per kilogram of synthetic milk and riboflavin was added at graded levels of 0, 2, 4, 6 and 8 mg per kilogram of solids in the milk. Stothers (1952) has adequately described the preparation of the milk.

The baby pigs readily learned to drink the milk from a shallow trough when it was witheld from them for the first eight hours. To encourage its consumption milk was warmed somewhat for the first few days. The pigs were fed ad libitum. Feedings were made three or four times daily to prevent sour-

ing of the milk in the trough. The amount of milk was carefully measured. Troughs were scrubbed and rinsed once daily.

Room temperature averaged about 72°F. and heating units within the cages supplied additional heat as needed during the first two weeks of the experiment. Pig weights were taken and recorded every fourth day.

The pigs remained on the experimental feeding period for 32 days. The deficient pigs remained on their diet until death or until autopsy was made when death appeared imminent. Post mortem studies were made on the three pigs receiving no riboflavin. A measure of the hemoglobin and white blood cell levels was made on one of these pigs.

Samples of the diet of each lot were assayed microbiologically for riboflavin at frequent intervals to verify the prepared concentration. Determinations were made using the method of Snell and Strong (1939) and samples were prepared according to the directions of Strong and Carpenter (1942).

In the second riboflavin trial the pigs were fed individually. This was made possible by inserting two removable partitions into each cage. This provided for the isolation of each of three pigs within a cage. Accurate records of individual pig consumption were then possible. A 4-day depletion-adjustment period was also introduced into this trial and was followed in subsequent trials. In this 4-day period, all pigs in the trial were fed a riboflavin-free diet for the purpose of reducing riboflavin stores within the pigs and to

allow for an adjustment by the pigs to the cages, to the diet and to the feeding procedure. At the end of this depletion-adjustment period, the pigs were assigned to lots on the bases of sex, size, litter and general appearance. The assigned lots then were placed on different levels of dietary riboflavin intake. These levels were 0, 1, 2, 3, 4 and 5 mg of riboflavin per kilogram of solids in the milk consumed. Otherwise, the feeding procedures and the environmental conditions were similar to those described for the first trial. All pigs used in this trial were purebred Yorkshires.

Work by Mitchell et al. (1950) suggested that blood studies might reveal a more sensitive test for adequacy of riboflavin intake. Consequently, blood samples were taken from a large ear vein bi-weekly to determine blood hemoglobin levels, white cell counts and white cell differentiation. Blood samples were taken from the anterior vena cava during the late stages of the trial for blood riboflavin assay. The pigs in this trial were on the experimental diets for 28 days. Again, as in the former trial, post mortem examinations were made on all pigs which died or were sacrificed.

During the second trial three other pigs were placed on a commercial sow milk replacer to which in addition to the B-vitamins which it contained was added the same supplementation of B-vitamins used in the synthetic milk. These three pigs were all males and were heavier than most of the other pigs started. Further, they were placed in newly designed individ-

ual cages which became available at this time. Microbiological assay revealed that the diet of these pigs contained 14 mg of riboflavin per kilogram of solids. This diet contained about ten percent solids.

From the experience gained from these two trials and from a related study on the bacterial count in the milk as affected by frequency of feeding and cleaning of troughs and from some of the experiences of Stothers (1952), it became apparent that a standardization of experimental procedures for future experimental work of this nature was necessary in order to obtain more valid results with a lesser degree of individual variation within lots (experimental error). These procedures are listed below.

Standard procedures for experimentation with baby pigs on synthetic milk diet

1. Selection - Be able to select from 150% of the number of pigs required in the experiment. Since the pigs are being individually fed it is not necessary to start the pigs all at once. This is a practical aid in selection.

2. Starting age and weight - Minimum of 3 days of age and 3 pounds body weight and maximum of 5 days of age and 5 pounds body weight.

3. Starvation period - 8 hours.

4. Depletion-adjustment period - 4 days.

5. Temperature of milk fed to pigs -

(a) 80° to 100°F for first week.

(b) 50° to 70°F for second week.

(c) temperature of stored milk thereafter.

6. Milk storage temperature - 3° to 4°C.

7. Temperature of water for making milk - 65° to 70°C.

8. Amount of water per can of milk - 28 liters.

9. Amount of total solids per can of milk - 5 kilograms.

(a) casein - 30% - 1500 gm.

(b) lard - 10% - 500 gm.

(c) salts - 6% - 300 gm.

(d) cerelose - 54% - 2700 gm.

This makes a total of 33 kilograms of milk per can. The milk contains 15.15% solids.

10. Amount of vitamins per kilogram of milk -

Thiamine	0.65 mg
Riboflavin	0.65 "
Niacin	2.50 "
Pyridoxine	0.65 "
Calcium pantothenate	3.00 "
Para-aminobenzoic acid	2.60 "
Inositol	26.00 "
Choline	260.00 "
Biotin	0.01 "
Pteroylglutamic acid	0.052"
Ascorbic acid	16.00 "
Alpha-tocopherol acetate	1.00 "
2-methyl-1, 4-naphthoquinone	0.28 mg
Vitamin A	2,000 I.U.
Vitamin D	200 I.U.

Samples of the diet should be assayed periodically for the particular vitamin in study to verify prepared levels.

11. Feeding schedule -

(a) First 2 weeks - 6 times daily

(b) Thereafter - 5 times daily

12. Cleaning schedule -

(a) Troughs - Rinse with hot water after each feeding.

(b) Cages - Scrub and rinse 2 or 3 times weekly.

13. Environmental temperature - Room temperature of 70°F. plus infra-red heat lamps to give proper temperature for the pig.

14. Hemoglobin level precaution - Give several drops of saturated ferrous sulfate solution to pigs when they are placed on experiment; thereafter give an Fe-Cu-Co tablet to each pig weekly.

15. Blood studies - Make bi-weekly cell count and hemoglobin determinations.

16. Relative humidity - Keep as uniform as possible; measure and record occasionally.

17. Recording data - Keep accurate individual records of all measures and observations.

18. Closing the experiment -

(a) Make arrangements for blood assays.

(b) Make arrangements for autopsies.

(c) Make arrangements for pictures.

(d) Thoroughly clean all cages and utensils.

(e) Thoroughly clean and steam the room twice annually.

These procedures have been followed with but few modifications in subsequent trials.

In the third riboflavin trial seven Chester White-Duroc Jersey crossbred pigs and 12 Chester White-Yorkshire Crossbred pigs from two litters were used. It was quite clear from the two previous experiments as well as from reports of other workers under a variety of conditions that the minimum riboflavin requirement of the baby pig for normal growth was not in excess of 4 mg of riboflavin per kilogram of solids consumed. Consequently, this level was chosen as the positive control level in this experiment. Three pigs were placed on the basal diet containing no riboflavin and 4 pigs were placed on each of 4 diets containing the following levels of riboflavin, in mg per kilogram of solids: 1.0, 2.0, 3.0 and 4.0. All pigs were fed regularly 6 times a day an amount of diet which they would consume in the 4-hour period. The amount of diet was limited only when scouring appeared. Scouring was not a particular problem after the first week of experimental feeding. The experimental feeding period was 28 days in duration.

II. Thiamine

The thiamine work was embodied in a triplicated experiment involving 55 baby pigs of which 25 were Chester White-Yorkshire crossbred pigs, 13 were purebred Chester White pigs, 7 were Berkshire-Yorkshire crossbred pigs and 10 were purebred Duroc Jersey pigs. The previously stated standard procedures were followed.

On the basis of the reported work with older pigs, the minimum thiamine requirement of the baby pig was judged not to exceed 2 mg of thiamine per kilogram of dietary solids intake. Consequently, this level was chosen as the positive control level in this experiment and fed concurrently with other lots receiving levels of 0, 0.5, 1.0 and 1.5 mg of thiamine per kilogram of solids consumed. Samples of each diet were assayed frequently by the thiochrome method as described in the methods of vitamin assay of the A. V. C. (1951) to verify the prepared concentration of thiamine in the diet.

Electrocardiographic studies were made weekly on all pigs throughout the second and third replications. In order to obtain valid electrocardiographic data the pigs were suspended in a canvas carrier device shown in figure 1. With some training the pigs learned to lie still for a considerable period of time. No recordings were made until the pigs had remained still for sometime.

The recording machine used was a 4 channel, model 67 Sanborn. Five leads were connected to electrodes situated on the four legs and the chest. The leg electrodes were situated just above the knees in the fore legs and a little above the hocks in the hind legs. The chest electrode was situated slightly to the left of the sternum. The hair was clipped from these areas and electrode paste was applied in order to establish intimate contact between electrode and skin.

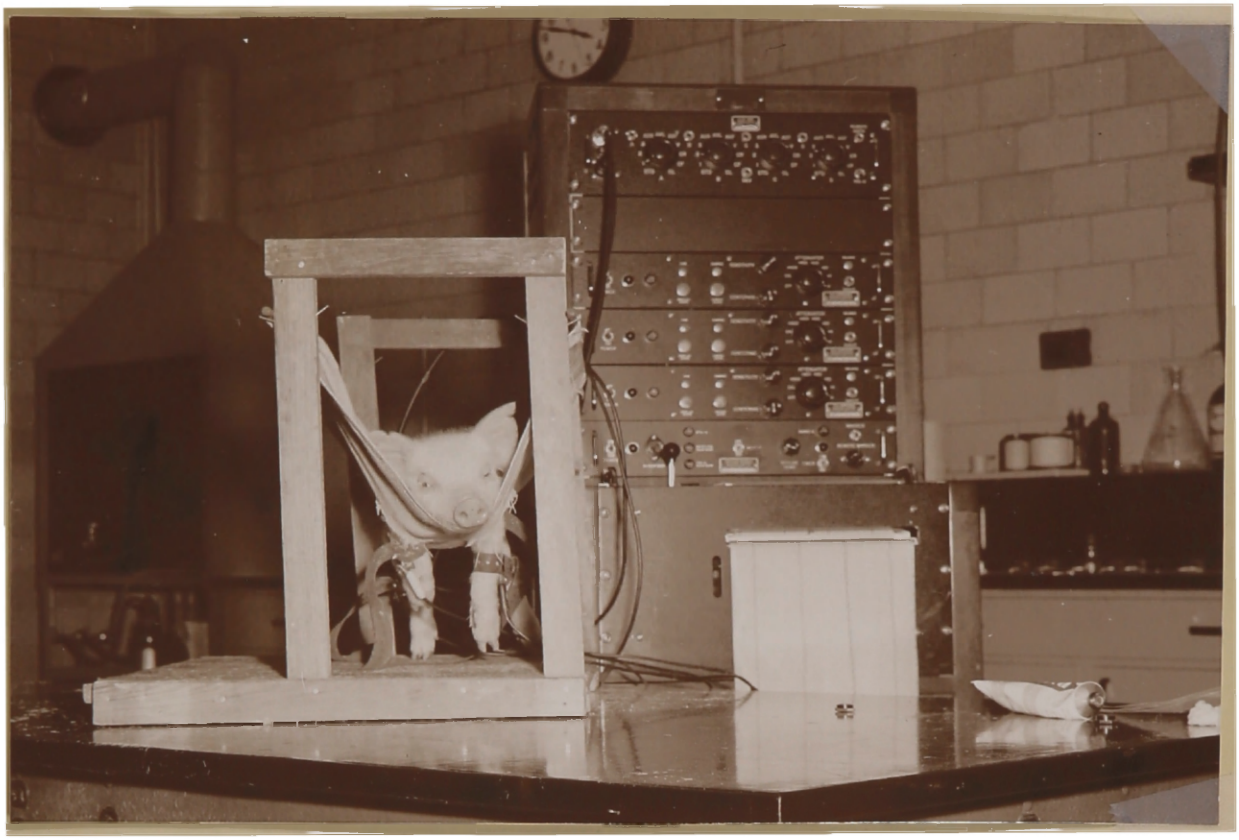


Fig. 1 - Apparatus used in obtaining electrocardiograms. The pig is suspended in a canvas sling in which holes were cut to allow the legs to hang freely. The recording device is a 4 channel, model 67 Sanborn.

Blood samples were taken from the anterior vena cava during the late stages of the experimental period for blood thiamine assay. The experimental feeding period lasted 32 days.

Post mortem examinations were made on all pigs that died during the experiment and on those pigs which were sacrificed while in a semimoribund condition. Several positive control pigs were sacrificed at the end of the experiment to serve as comparative standards in these examinations. Heart, adrenal

and thyroid weights were taken and recorded. Blocks of tissues were taken and fixed in formal saline. Hematoxylin-eosin was used on all sections and myelin sheaths were stained using Weil's (1945) method. Nissl bodies were stained by a thionin technique described by Fletcher (1947).

III. Pyridoxine

Three pyridoxine trials have been conducted involving the use of 65 experimental animals. In the first trial, 4 lots of 4 pigs each were individually fed diets containing 0, 1.0, 2.0 and 4.0 mg of pyridoxine per kilogram of solids in the diet. In addition, two other pigs received the positive control diet but were pair fed with two pigs in the negative control lot. The experimental feeding period lasted 32 days and all 18 pigs used were Chester White-Yorkshire crossbreds. Blood samples were taken weekly for red and white cell counts and hemoglobin determination.

Twenty-four purebred Duroc Jersey pigs were used in the second trial. Five levels of pyridoxine supplementation were used in this trial. These levels were 0, 0.5, 0.75, 1.0 and 2.0 mg of pyridoxine per kilogram of solids in the diet. Four pigs were individually fed on each diet and in addition four other pigs were fed the positive control diet but were pair fed with pigs in the lot receiving no pyridoxine. Complications developed in this experiment which rendered a consideration of the data obtained inadvisable. A description of some

of the results of this experiment, however, will appear later in the thesis.

Twenty-three pigs in all were used in the third trial. Fourteen of these were purebred Chester White pigs, 4 were Chester White-Yorkshire crossbred pigs and 5 were Chester White-Duroc crossbred pigs. These pigs were placed on the same levels of pyridoxine supplementation used in the second trial. Complicating factors unrelated to level of pyridoxine supplementation developed in the case of three pigs whereby it was deemed inadvisable to use the data obtained from them.

Blood samples again were taken weekly from an ear vein to determine R B C and W B C counts and hemoglobin level. Urine collections were taken at weekly intervals and xanthurenic acid excretion determinations were made. Prior to the urine collection, the pigs received an oral dose of DL-tryptophan amounting to 100 mg per pound of body weight. Urine xanthurenic acid concentrations were determined using the method of Wachstein and Gudaitis (1952). Blood samples were taken from the anterior vena cava during the last week of the experiment to determine the serum euglobulin fraction. As in previous experiments, post mortem studies were made and various organ weights recorded.

RESULTS

Part I--Riboflavin Studies

Experiment I

The results of the first trial pertaining to pig growth and feed consumption are presented in table 1. Statistical treatment was given to these data and to the data of subsequent trials, unless otherwise specified, using the method of Snedecor (1946) for analyzing single classification variance. These results show that all lots receiving riboflavin gained significantly faster at the 10% level than the lot receiving no riboflavin. Pigs from the lots receiving riboflavin also gained 25% to 34% more efficiently than pigs from the lot receiving no riboflavin.

The rate of pig growth was not particularly good in any of the lots and there was a great deal of individual variation within all of the lots. However, all of the pigs receiving riboflavin appeared much thriftier after about two weeks of the experiment and throughout the remainder of the trial than the deficient pigs. Near the end of the trial the haircoats of the deficient pigs became quite rough, a heavy sebaceous exudate accumulated about the eye and a dried somewhat caked exudate collected on the skin. There was a considerable amount of loss of pigment from the skin of these pigs

Table 1 - Response of group fed baby pigs to different dietary levels of riboflavin[1]

	Level of riboflavin in diet, in mg/kg solids				
	0	2	4	6	8
Number of pigs	3	3	3	3	3
Days on test	32	32	32	32	32
Ave. initial weight (lb.)	3.25±0.29[2]	3.06±0.25	2.96±0.21	2.96±0.21	3.11±0.41
Ave. final weight (lb.)	8.73±0.21	12.63±0.81	13.04±1.90	12.90±3.12	14.52±3.35
Ave. daily gain (lb.)[3]	0.17±0.03	0.30±0.02	0.32±0.06	0.31±0.09	0.36±0.09
Ave. daily feed consumed (lb.)	0.31	0.40	0.43	0.42	0.43
Solids per lb. gain (lb.)	1.82	1.36	1.34	1.35	1.20

[1]Pigs taken from sow when 72 hours old and placed on experiment.
[2]Standard error of mean.
[3]All lots gained significantly faster at the 10% level than the lot receiving no riboflavin.

and diarrhea was more frequently a problem in these pigs than in those pigs receiving riboflavin in the diet. No differences were apparent between any of the lots receiving riboflavin.

All three of the pigs receiving no riboflavin died or were sacrificed within a few days after the close of the experimental feeding period. Extensive fatty degeneration was apparent in the livers of all these pigs and pneumonia was observed in one animal.

Experiment II

In the second trial considerable growth variation was observed within lots. The growth rate and feed consumption data are presented in table 2. Analysis of the data indicated that maximum growth rate and greatest feed efficiency were obtained when the pig's diet contained 3 or 4 mg of riboflavin per kilogram of solids.

The blood studies made during this trial indicate that the level of blood hemoglobin was not affected by the level of riboflavin in the diet. This is shown in table 3. There appeared to be an increase in the total leucocyte count and the percentage of these which are polymorphonuclear when dietary riboflavin was reduced. However, the individual variation within lots was so extensive that these differences at the end of the experiment were not statistically significant. Mitchell et al. (1950) reported that the percent of neutro-

Table 2 - Response of baby pigs to different dietary levels of riboflavin[1]

	Level of riboflavin in diet, in mg/kg solids					
	0	1	2	3	4	5
Lot number	1	2	3	4	5	6
Number of pigs	3	3	3	3	3	3
Days on test	28	28	28	28	28	28
Ave. initial weight (lb.)	4.77±0.65[2]	4.83±0.65	5.02±0.63	5.09±0.58	5.05±0.58	4.90±0.59
Ave. final weight (lb.)	8.21±1.75	10.48±1.53	11.19±2.51	13.83±3.35	14.23±3.00	12.06±2.28
Ave. daily gain (lb.)[3]	0.12±0.04	0.20±0.03	0.22±0.07	0.31±0.09	0.33±0.09	0.26±0.09
Ave. daily feed consumed (lb.)	0.26±0.03	0.33±0.44	0.40±0.07	0.42±0.10	0.48±0.12	0.38±0.08
Solids per lb. gain (lb.)[4]	2.38±0.42	1.68±0.18	2.02±0.29	1.48±0.20	1.51±0.13	1.48±0.11

[1]Pigs were taken from sow when 72 hours old and placed on depletion diet containing no riboflavin for 4 days. Pigs were then assigned to lots and started on feeding trial.

[2]Standard error of mean.

[3]Lots 4 and 5 gained significantly faster at the 5% level than lot 1.

[4]Lots 4, 5 and 6 were significantly more efficient in food utilization at the 5% level than lot 1.

Table 3 - Levels of certain blood components of baby pigs receiving different dietary levels of riboflavin

	Level of riboflavin in diet, in mg/kg solids					
	0	1	2	3	4	5
Hemoglobin[1]						
1st. week	10.8±1.8[2]	10.2±1.1	8.3±0.8	10.2±1.7	9.6±0.6	9.9±1.5
3rd. week	12.4±1.7	12.2±1.2	11.9±0.6	11.5±1.2	10.8±1.0	13.6±0.4
5th. week	11.8±0.4	9.7±0.7	10.8±0.7	11.9±1.4	12.0±0.9	11.1±1.8
WBC count[3]						
1st. week	14.6±4.0	9.9±2.1	10.8±3.4	16.6±6.3	18.6±5.9	10.7±2.9
3rd. week	22.6±7.3	13.1±1.0	16.2±3.8	24.4±4.1	21.8±4.9	19.6±2.5
5th. week	27.7±3.7	20.4±5.5	16.6±1.3	15.5±3.8	19.7±1.4	18.6±1.3
Neutrophiles[4]						
1st. week	40±9	47±4	35±5	41±12	49±12	46±3
3rd. week	59±10	44±5	60±5	45±9	56±13	55±2
5th. week	55±8	63±7	39±2	44±10	50±16	48±2

[1]Hemoglobin values expressed in grams percent.
[2]Standard error of mean.
[3]WBC count expressed in thousands per cubic millimeter.
[4]Neutrophiles expressed as percentage of total leukocyte count.

philes in the blood was a sensitive index of the riboflavin adequacy of the diet, increasing with the severity of the deficiency. However, Forbes and Haines (1952) found no such relation. The average percentage of neutrophiles for all pigs in the latter work was 56% at one week of age and 53% at 9 weeks of age. The average percentage neutrophiles for all pigs reported in table 3 was 43% at one week of age and 50% at 5 weeks of age.

The mean values for blood riboflavin level determined for some of the pigs at the close of the experiment did indicate that these values were related to dietary riboflavin intake. These mean values expressed in micrograms per 100 milliliters of blood for lots 1 through 6 were 13.0, 21.0, 16.5, 34.0, 27.0 and 38.0 respectively.

Experiment III

The most important results of the third trial are presented in table 4. Growth curves showing the average lot weights throughout the feeding period are shown in figure 2.

All of the pigs on the basal diet containing no riboflavin and all of the pigs receiving 1 mg of riboflavin per kilogram of solids showed gross external symptoms of riboflavin deficiency. These pigs exhibited a rough haircoat, a heavy sebaceous exudate about the eye and ear and a dried, somewhat caked exudate on the skin (fig. 3). These pigs became weak and thin and had a higher incidence of diarrhea

Table 4 - Response of baby pigs to synthetic milk diets containing different levels of riboflavin

	Level of riboflavin in diet, in mg/kg solids				
	0	1	2	3	4
Lot number	1	2	3	4	5
Number of pigs	3	4	4	4	4
Days on test	28	28	28	28	28
Ave. initial weight (lb.)	6.46±0.75	6.30±0.75	6.28±0.27	6.27±0.44	6.07±0.39
Ave. final weight (lb.)	6.86±0.69	10.24±1.20	14.31±0.68	18.21±0.70	17.31±0.79
Ave. daily gain (lb.)[1]	0.01±0.004	0.14±0.03	0.29±0.03	0.43±0.01	0.40±0.03
Ave. daily feed consumed (lb.)[2]	0.22±0.005	0.33±0.02	0.45±0.02	0.57±0.01	0.53±0.02
Solids per lb. gain (lb.)[3]	17.91±3.93	2.48±0.31	1.59±0.12	1.33±0.03	1.32±0.04

[1]There was a significant difference in daily gain at the 1% level between all lots except lots 4 and 5.

[2]There was a significant difference in daily feed consumption at the 5% level between all lots except lots 4 and 5.

[3]All lots were significantly more efficient in food utilization at the 1% level than the lot receiving no riboflavin. Lot 3 was significantly more efficient at the 5% level than lot 2 . Lots 4 and 5 were significantly more efficient at the 1% level than lot 2.

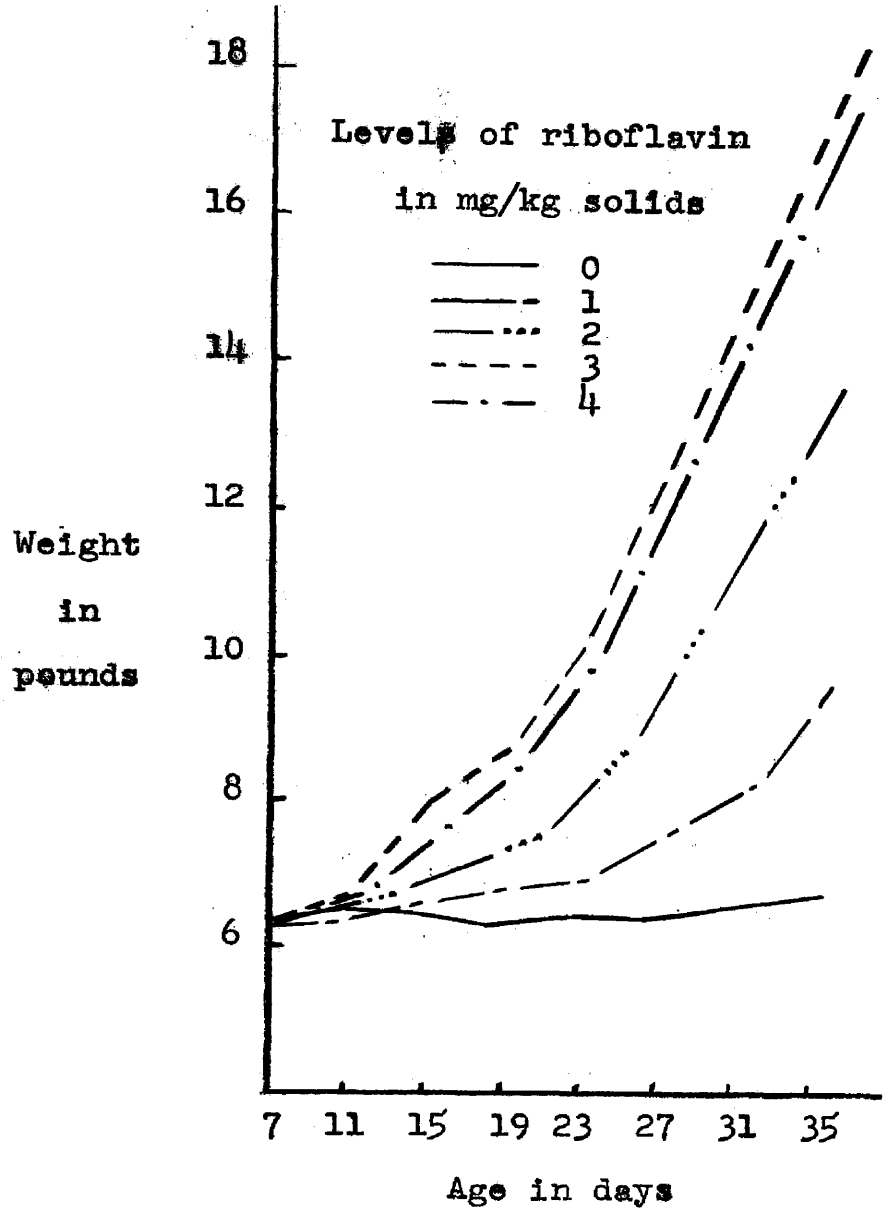


Fig. 2 - Growth curves of baby pigs receiving different levels of dietary riboflavin intake.

than the pigs on the other diets. The dietary intake of the pigs receiving no riboflavin gradually decreased, until at the end of the experiment they were consuming less than 75 gm of solids per pig per day, or an amount approximating only 2% of the animal's body weight. Thus, the pig in this state of

acute anorexia is undoubtedly influenced by a multiple nutrient deficiency.

Evidence of the existence of a multiple nutrient deficiency was provided by the treatment and subsequent response of one of the pigs in lot 2 of this experiment. This pig, after 21 days of experimental feeding, had a greatly reduced appetite, was very thin and weak and lay in a comatose state near death. An intraperitoneal injection of 10 ml of a riboflavin-free B vitamin preparation was given, containing (in mg per milliliter): nicotinamide 5; calcium pantothenate 5; thiamine 2; pyridoxine 1; folic acid 0.25: p-aminobenzoic acid 0.25; inositol 1; and vitamin B_{12} 0.005. Within 12 hours the pig was able to walk, became much more alert and showed an improved appetite. When a similar intraperitoneal injection was given three days later, the pig continued to respond to treatment. However, during the 4-week experimental period this animal had gained less than three pounds.

In order to determine whether the deficiency symptoms exhibited by this pig could be alleviated, the animal was fed the diet of the positive control lot containing 4 mg of riboflavin per kilogram of solids. In addition, a 10 ml injection of the previously described multiple vitamin preparation was given on the first and second days, followed by an intraperitoneal injection of 100 mg of riboflavin every 4th day of the 19-day recovery period. The animal's appetite improved rapidly and during the recovery period he gained 0.5 lb. per

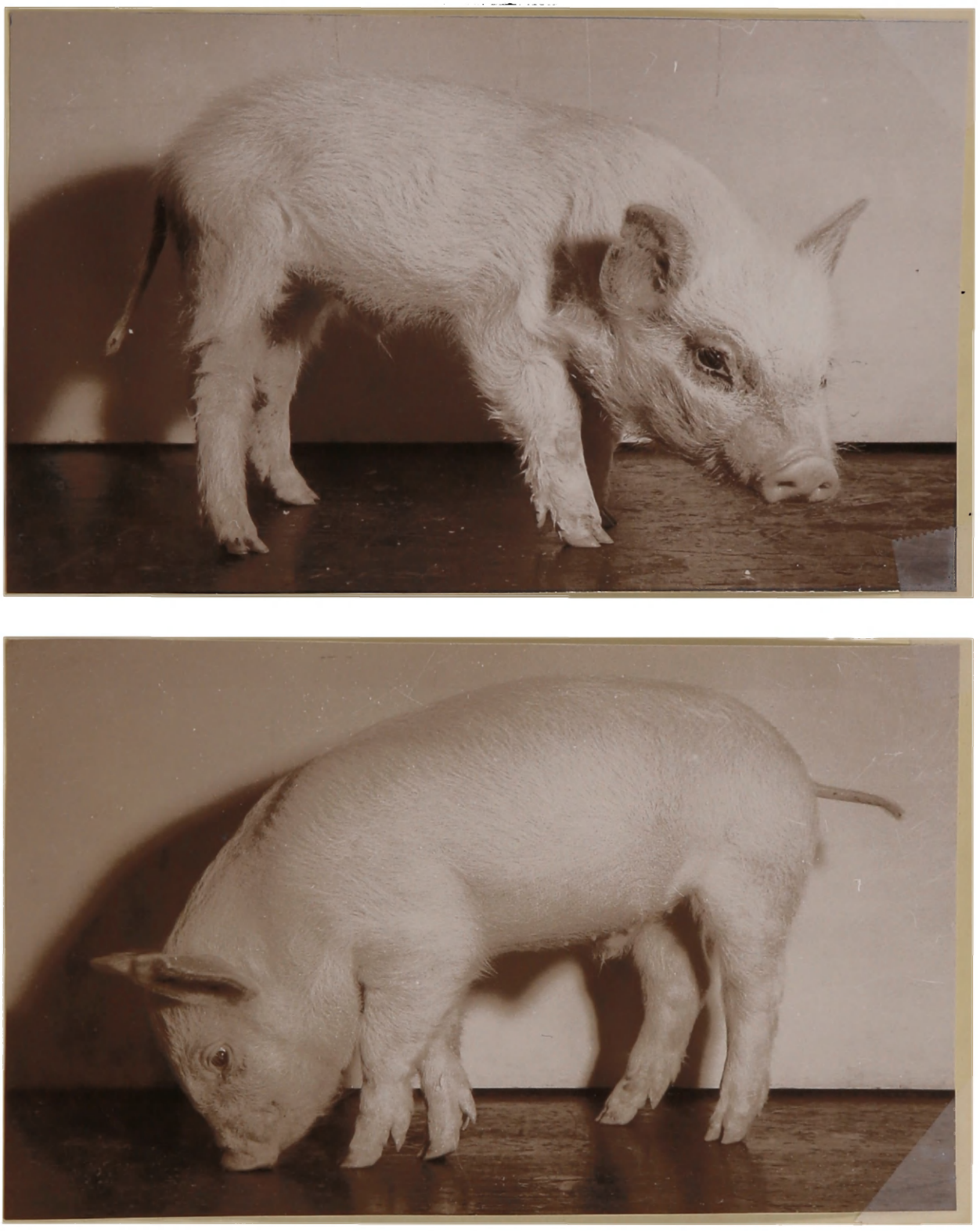


Fig. 3 - The pig on top received no riboflavin in the diet, while the animal below received 3 mg of the vitamin per kilogram of solids.

day, which approaches the normal growth curve presented by Ittner and Hughes (1938). Similar results were obtained by this procedure with two other pigs from lot 2 and two pigs from the basal lot.

One pig from the basal lot and one pig from lot 2 were sacrificed at the end of the 4-week experimental feeding period. In each instance, the liver and kidneys were somewhat mottled and showed evidence of fatty infiltration. In addition, there was excessive fluid in the peritoneal cavity and the pericardial sac. The small intestine, cecum and colon were congested and edema was present in the spiral colon.

The tissues taken at post mortem were placed in formalin, embedded in paraffin and stained with hematoxylin-eosin. Nerve tissue was also stained, using Weil's (1928) method.

Lesions of the eye were confined to the lens, cornea and eyelids. Cataracts were present in the lens at 35 days of age. The cataracts were located posterior to the equator in the zone where the posterior surface of the lens begins to be devoid of epithelium. They were manifested by a swelling and separation of the lens fibers. The epithelial cells, possessing nuclei, were enlarged, elongated and formed the so-called vesicular cells found in cataractous conditions. Ballooning of the columnar cells of the basal layer of the corneal epithelium was observed. There was no evidence of vascularization of the cornea or depigmentation of the iris. There was a ballooning of the tubular glands of the eyelids.

Myelin sheath degeneration was not observed in sections from the cerebrum, cerebellum, thalmus, mid-brain, spinal cord, dorsal root ganglia and sciatic nerves.

The skin showed varying degrees of atrophy and hyperkeratosis of the stratum mucosum. Mucinous degeneration was present in the cecum and colon and evidence of rectal hemorrhage was usually found.

The results of the studies of certain blood constituents are presented in table 5. Blood hemoglobin values of pigs receiving 3 mg and 4 mg of riboflavin per kilogram of solids were significantly higher than for those pigs receiving no riboflavin or 1 mg per kilogram of solids. Although significance was again not obtained, there appears to be a trend in WBC count and neutrophilic concentration similar to that obtained in the second trial. Blood riboflavin level was significantly higher in pigs receiving the three higher levels of dietary riboflavin then in pigs receiving no riboflavin.

Pigs receiving 2.0, 3.0 and 4.0 mg of riboflavin per kilogram of solids in the diet showed no gross symptoms of riboflavin deficiency. However, those pigs receiving 2.0 mg of riboflavin per kilogram of solids gained significantly ($P=0.01$) less rapidly than did the pigs receiving 3.0 and 4.0 mg per kilogram of solids. Furthermore, their efficiency of feed utilization was 20% less than that of pigs in the lots receiving higher levels of riboflavin. There were only slight differences between pigs fed 3.0 mg of riboflavin per

Table 5 - Levels of certain blood constituents of baby pigs receiving different dietary levels of riboflavin[1]

	Level of riboflavin in diet, in mg/kg solids				
	0	1	2	3	4
Hemoglobin[2]	10.1±0.8	10.1±0.8	11.3±0.6	12.8±0.4	12.7±0.6
WBC count[3]	17.7±2.3	15.1±1.6	10.7±2.2	10.2±2.2	11.3±1.6
Neutrophiles[4]	60±15	59±9	49±6	44±6	35±6
Riboflavin[5]	40±4	50±3	55±1	52±3	57±3

[1]Blood samples taken during final week of experiment.
[2]Hemoglobin values expressed in grams percent.
[3]WBC count expressed in thousands per cubic millimeter.
[4]Neutrophiles expressed as a percentage of total leukocyte count.
[5]Blood riboflavin values expressed in micrograms percent.

kilogram of solids and pigs in the positive control group. In all but one instance, these slight differences were in favor of the former group. The one exception was in efficiency of feed utilization. It is apparent that under the conditions of this experiment the riboflavin requirement of the baby pig for normal growth approximates 3.0 mg per kilogram of diet. This is a definitely higher requirement than that of 1.5 to 2.0 mg per kilogram of diet reported by Forbes and Haines (1952).

The higher riboflavin requirement found in this study as compared with that reported by Forbes and Haines (1952) may be due to a number of factors. The environmental temperature in this experiment was 5° to 15°F. lower than that reported by the above authors. Mitchell et al. (1950) have shown that the riboflavin requirement of growing pigs is higher at lower temperatures. Moreover, uncontrolled relative humidity may have been a factor accounting in part for our higher requirement. In addition to the above factors, variation in the genetic make-up of the animals used and the more liberal feeding method employed may have influenced the vitamin requirement found in this study.

Part II--Thiamine Studies

Experiment I

The results of the first trial pertaining to the growth response and feed utilization of baby pigs on different levels of thiamine are presented in table 6. Analysis of the data presented indicates that under the conditions of this experiment the minimum thiamine requirement of the baby pig lies between 1.0 and 1.5 mg per kilogram of solids in the diet.

Pigs receiving no thiamine ate and gained equally as well as the positive control pigs for the first 12 days of experimental feeding. Following this, however, the lot 1 animals developed an increasing degree of anorexia, vomited frequently, gained more slowly, then lost weight, became quite weak and emaciated and finally died after three or four weeks on the thiamine-deficient diet (fig. 14). Similar effects appeared somewhat more belatedly in the lot 2 pigs. Most of the pigs in lot 3 developed anorexia and vomited occasionally very late in the experiment. Two of the pigs from this lot died on the final day of the trial. There was no appearance of abnormal gait, nor were there any outward manifestations of nervous disorders in any of the pigs. All pigs in lots 4 and 5 were free of the aforementioned deficiency symptoms throughout the experimental period.

Table 6 - Response of baby pigs to synthetic milk diets containing different levels of thiamine

	Level of thiamine in diet, in mg/kg solids				
	0.0	0.5	1.0	1.5	2.0
Lot number	1	2	3	4	5
Number of pigs	4	4	4	4	4
Days on test	--[1]	--[2]	32	32	32
Ave. initial wt.(lb.)	4.78±0.18[3]	4.55±0.40	4.75±0.17	4.58±0.12	4.65±0.46
Ave. final wt.(lb.)	--	--	16.24±1.35	21.18±1.00	20.69±1.63
Ave. daily gain (lb.)[4]	--	--	.36±0.04	.52±0.03	.51±0.04
Ave. daily solids consumed (lb.)	--	--	.50±0.03	.63±0.03	.58±0.04
Solids per lb. gain(lb.)[5]	--	--	1.43±0.07	1.21±0.03	1.17±0.04

[1]All pigs from lot 1 had died, had been sacrificed or had received thiamine injections by the 27th day on test.

[2]Three of the pigs in lot 2 had died or had received injections of thiamine by the 27th day on test. The 4th pig died on the day following the close of the experimental feeding.

[3]Standard error of mean.

[4]Lots 4 and 5 gained significantly faster at the 5% level than lot 3.

[5]Lots 4 and 5 were significantly more efficient in food utilization at the 5% level than lot 3.

All deficient pigs which died or were sacrificed showed gross symptoms of thiamine deficiency. In animals dying naturally, there was marked cyanosis noticeable in the skin, nose and mucous membranes. The most common gross finding was a pale yellowish-gray mottled heart. The left ventricle was usually contracted and the right ventricle was quite flabby. The whole heart was less firm than normal and in several instances it was rounded and resembled a myxedematous heart (fig. 15). There was always an excess of pericardial fluid and in many cases the peritoneal and pleural cavities also contained excessive fluid.

In most cases, there was congestion of the liver and the serosa of the small intestine was reddened. The mesenteric vessels were usually injected. In a few of the animals, the liver showed a yellowish mottling suggestive of fat. There was inflammation of the cecum or colon varying from a mild reddening to a severe necrotic enteritis with ulcers and caseous masses adhering to the intestinal wall.

On microscopic examination the hearts of thiamine-deficient pigs showed congestion, focal fragmentation and some necrosis of the muscle fibers. The cells in the inflamed area were primarily macrophages although some lymphocytes and Anitschkow cells were also present. This inflammation was more pronounced in the hearts of pigs from lots 2 and 3 and there was an increase in collagenous fibers as demonstrated by Mallory's aniline blue stain. Sudan IV stains showed

extensive fatty degeneration in the heart tissue of all deficient pigs but the fat droplets were small and tell-tale vacuoles were not readily observed by the hematoxylin-eosin stain. All portions of the myocardium were affected but lesions were more marked near the epicardium. The left ventricle was involved to a greater extent than the right. Heart lesions were not noted in pigs from lots 4 and 5 nor in deficient pigs that had recovered after parenteral treatment with thiamine. These findings are similar to those described by Follis et al. (1943) and Wintrobe et al. (1942) except that fatty changes in the heart were more marked in this series and the neutrophilic exudate described by Follis et al. (1943) was not observed in this experiment.

Congestions of the mucosa of the gastro-intestinal tract and mucoid degeneration of the cecum or colon were observed in pigs from lots 1 and 2. No changes were noted in the central nervous system nor in the sciatic nerves.

Experiment II

The second trial was carried out during the summer months when daytime temperatures were often higher than the controlled temperature of other trials. It is possible that the increased temperature may have had somewhat of a sparing action on the thiamine requirement of the pigs. It is apparent from an analysis of the data presented in table 7 that 1.0 mg of thiamine per kilogram of solids in the diet of

Table 7 - Response of baby pigs to synthetic milk diets containing different levels of thiamine

	Level of thiamine in diet, in mg/kg solids				
	0.0	0.5	1.0	1.5	2.0
Lot number	1	2	3	4	5
Number of pigs	4	4	4	4	4
Days on test	--[1]	32	32	32	32
Ave. initial wt.(lb.)	5.12±0.37[2]	5.09±0.19	5.07±0.22	4.93±0.22	4.90±0.33
Ave. final wt. (lb.)	--	16.51±0.90	20.35±0.87	19.42±0.38	19.48±1.93
Ave. daily gain(lb.)[3]	--	.36±0.02	.48±0.03	.45±0.01	.46±0.05
Ave. daily solids consumed (lb.)	--	.41±0.02	.50±0.02	.46±0.01	.47±0.04
Solids per lb. gain (lb.)[4]	--	1.13±0.04	1.05±0.02	1.04±0.02	1.04±0.03

[1]All pigs from lot 1 had died, had been sacrificed or had received thiamine injections by the end of the trial.
[2]Standard error of mean.
[3]Lots 3, 4 and 5 gained significantly faster at the 10% level than lot 2.
[4]Lots 4 and 5 gained significantly more efficiently at the 10% level than lot 2.

the pig is adequate to promote normal rate and efficiency of gain.

The pigs receiving no thiamine in their diet did not show symptoms of a deficiency as early in this trial as in the former trial. One of these pigs died on the 28th day of experimental feeding. Another pig from this lot received therapeutic thiamine injections beginning on the 30th day and was recovered. The other two pigs from this lot were in a very weak condition at the end of the trial and were sacrificed. Post mortem examination of these pigs showed evidences of thiamine deficiency similar to those previously described. Two of the lot 2 pigs died a few days after the end of the experimental feeding period and exhibited these deficiency symptoms also. One pig from each of the upper three levels was also sacrificed and none of these exhibited any thiamine deficiency symptoms.

Experiment III

A third trial was conducted to aid in determining a resolution of the somewhat differing results obtained in the two previous trials. A summary of the growth and feed consumption data is presented in table 8. There were no significant differences in mean values of rate or efficiency of gain between any two of the three lots receiving 1.0 mg or more of thiamine per kilogram of solids in the diet. However, vomiting occurred occasionally in pigs on the 1.0 mg

Table 8 - Response of baby pigs to different levels of dietary thiamine

	Level of thiamine in diet, in mg/kg diet				
	0.0	0.5	1.0	1.5	2.0
Lot number	1	2	3	4	5
Number of pigs	3	3	3	3	3
Days on test	--[1]	--[2]	32	32	32
Ave. initial wt.(lb.)	4.30±0.26[3]	4.30±0.32	4.25±0.15	4.27±0.03	4.42±0.48
Ave. final wt. (lb.)	--	--	18.14±0.30	19.19±0.17	21.07±2.30
Ave. daily gain (lb.)	--	--	.43±0.02	.47±0.004	.52±0.08
Ave. daily solids consumed (lb.)	--	--	.48±0.02	.49±0.02	.54±0.05
Solids per lb. gain(lb.)	--	--	1.10±0.02	1.06±0.04	1.05±0.03

[1]All pigs in lot 1 had died before the end of the experimental feeding period.

[2]One of the pigs from lot 2 died before the end of the experimental feeding period and another pig died shortly thereafter.

[3]Standard error of mean.

level and two of these pigs showed a somewhat reduced appetite late in the experiment. One of these pigs was sacrificed and a fine yellow speckling of the left ventricle of the heart was observed.

Thus it would seem that while 1.0 mg of thiamine per kilogram of solids may be adequate to produce normal rate of gain under the conditions of this experiment, it may be insufficiend to prevent the onset of subclinical pathological conditions in the animal. Post mortem studies on all of the pigs in lots 1 and 2 revealed varying degrees of the thiamine deficiency syndrome previously described.

A summary of the pig growth and feed consumption data for all three trials is presented in table 9. Statistical treatment of this data was given, using the method of Snedecor (1946) for analyzing multiple classification variance permitting the removal of replication error. An analysis of these composited data indicates that under the conditions of these experiments the minimum thiamine requirement of the baby pig lies between 1.0 and 1.5 mg per kilogram of solids in the diet and further suggests that the 1.5 mg level should be considered a practical minimum thiamine concentration in the baby pig's diet. Since the daily dietary solids intake of each baby pig constituted approximately 5% of its body weight throughout the experiments, this requirement value is equivalent to that of 75 mcg per kilogram body weight daily reported by Moustgaard (1953) for older pigs.

Table 9 - Response of baby pigs to synthetic milk diets containing different levels of thiamine

	Level of thiamine in diet, in mg/kg solids				
	0.0	0.5	1.0	1.5	2.0
Lot number	1	2	3	4	5
Number of pigs	11	11	11	11	11
Days on test	--[2]	--[2]	32	32	32
Ave. initial wt.(lb.)	4.77±0.21[3]	4.68±0.23	4.73±0.18	4.62±0.15	4.68±0.25
Ave. final wt.(lb.)	--	--	18.25±0.83	19.96±0.50	20.35±1.12
Ave. daily gain (lb.)[4]	--	--	.42±0.02	.48±0.01	.49±0.02
Ave. daily solids consumed (lb.)	--	--	.50±0.02	.53±0.03	.53±0.03
Solids per lb.gain(lb.)[5]	--	--	1.20±0.06	1.11±0.03	1.09±0.03

[1]Pigs were taken from the sow when three or four days old and placed on a depletion diet containing no thiamine for four days. Pigs were then assigned to lots and started on the feeding trial.

[2]Data on growth and feed consumption for lots 1 and 2 are not included since most of these pigs had died, been sacrificed in extremis or received thiamine injections before the end of the experimental feeding period.

[3]Standard error of mean.

[4]Lots 4 and 5 gained significantly faster at the 10% level than lot 3.

[5]Lots 4 and 5 were significantly more efficient in food utilization at the 5% level than lot 3. The pigs in the second and third replications of all three lots were significantly more efficient in food utilization at the 1% level than the pigs in the first replication.

Studies of the blood hemoglobin values and the white cell counts revealed no significant trend which could be related to the level of thiamine in the diet. A summary of the initial and final levels of these blood components are shown in table 10 in which the blood thiamine levels of the pigs late in the experiment are also presented. These latter values exhibit a positive relation between dietary and blood thiamine levels.

Table 10 - Initial and final levels of certain blood constituents of baby pigs receiving different dietary levels of thiamine[1]

	Level of thiamine in diet, in mg/kg solids				
	0.0	.5	1.0	1.5	2.0
Hemoglobin[2]					
Initial	12.3±0.4	11.8±0.5	11.5±0.5	11.7±0.3	11.7±0.3
Final	10.7±1.2	10.8±0.8	11.0±0.4	12.1±0.3	12.0±0.4
WBC count[3]					
Initial	10.1±0.9	13.0±1.0	10.1±0.7	11.4±1.0	13.2±1.5
Final	15.6±2.8	12.9±2.9	10.8±0.9	13.5±1.1	11.5±1.1
Thiamine[4,5]					
Final	4.2±1.0	4.3±1.2	7.3±1.9	9.0±0.9	11.8±1.8

[1]Blood samples taken during first and fifth week of trial.
[2]Hemoglobin values expressed in grams percent.
[3]WBC count expressed in thousands per cubic millimeter.
[4]Blood thiamine values expressed in micrograms percent.
[5]Blood thiamine levels in pigs from lots receiving 1.5 and 2.0 mg of thiamine per kilogram of solids were significantly higher (P=.05) than in pigs receiving no thiamine or .5 mg per kilogram solids.

The percentage of the total body weight which either the weight of the heart or the adrenals comprised was considerably greater in the deficient animals. This appeared to be due not so much to hypertrophy of these organs as to the re-

duction in body weight of the deficient pigs. Thyroid weight as a percentage of the body weight did not appear to be appreciably affected. A summary of the relation of these organ weights to the total body weight of the pigs autopsied in this study are present in table 11.

Table 11 - Relation of certain organ weights to total body weight of baby pigs receiving different dietary levels of thiamine[1]

	Level of thiamine in diet, in mg/kg solids			
	0.0	0.5	1.0	1.5 & 2.0
Number of pigs	8	6	5	4
Heart	.78±.02[2]	.92±.12	.77±.06	.53±.04
Adrenals	.031±.004	.023±.006	.011±.000	.010±.003
Thyroid	.012±.002	.014±.002	.010±.003	.009±.002

[1]Values are expressed as a percent of the total body weight.
[2]Standard error of mean.

Three deficient pigs received thiamine injections in an attempt to overcome the deficient condition. One of the pigs failed to respond and died shortly thereafter exhibiting most of the symptoms heretofore mentioned. The other two pigs, however, showed an almost immediate response to the injection. Their heart rates returned from bradycardia to normal within a few minutes. Their appetites improved rapidly and within 24 hours they were gaining in body weight. Thereafter intraperitoneal injections of 100 to 500 mg of thiamine were given at weekly intervals and these pigs when placed on the positive control diet continued to gain normally over a three-week recovery period. These pigs were

then killed and post mortem examination showed no gross evidence of a deficiency except a few light colored areas on one pig's heart which was no longer flabby.

Electrocardiograms were taken weekly during the second and third trials on all pigs to determine if this were a more sensitive measure of thiamine adequacy than any of the other criterial measures used. Other purposes for taking ECGs were to establish knowledge of electrocardiographic patterns of normal pigs and to determine what deviations from the normal pattern were manifested by thiamine deficient pigs.

Electrocardiograms from the positive control animals usually showed positive P, R and T waves in the standard leads although T was sometimes inverted in all of these leads and very often inverted or small in lead I and often barely perceptible in lead III. P was often barely discernible and sometimes inverted in lead III. The S wave was usually not perceptible in lead I. There were no consistent differences between the positive and the negative control groups in the direction of any of the waves in the standard leads.

The duration of the heart cycle in positive control pigs was usually uniform although a slight degree of sinus arrhythmia appeared not to be abnormal. A marked sinus arrhythmia was observed in most of the deficient pigs.

The P, R and T waves were usually all inverted in the

AVR unipolar lead. In the AVL lead T was usually inverted and both P and T were often small or not perceptible. T was occasionally inverted in the AVF lead. T was usually more pronounced on the chest unipolar lead than on any other lead. Again, no consistent differences either in wave magnitude or direction between positive and negative control groups were apparent.

The heart rates (taken from the electrocardiograms) of all pigs were above 200 beats per minute during the first week. The average was 266 beats per minute with a range of 202 to 294 bpm. This rate had dropped sharply by the second week (average 189 bpm) and then only gradually thereafter as the age advanced. This is shown in table 12 and figure 4.

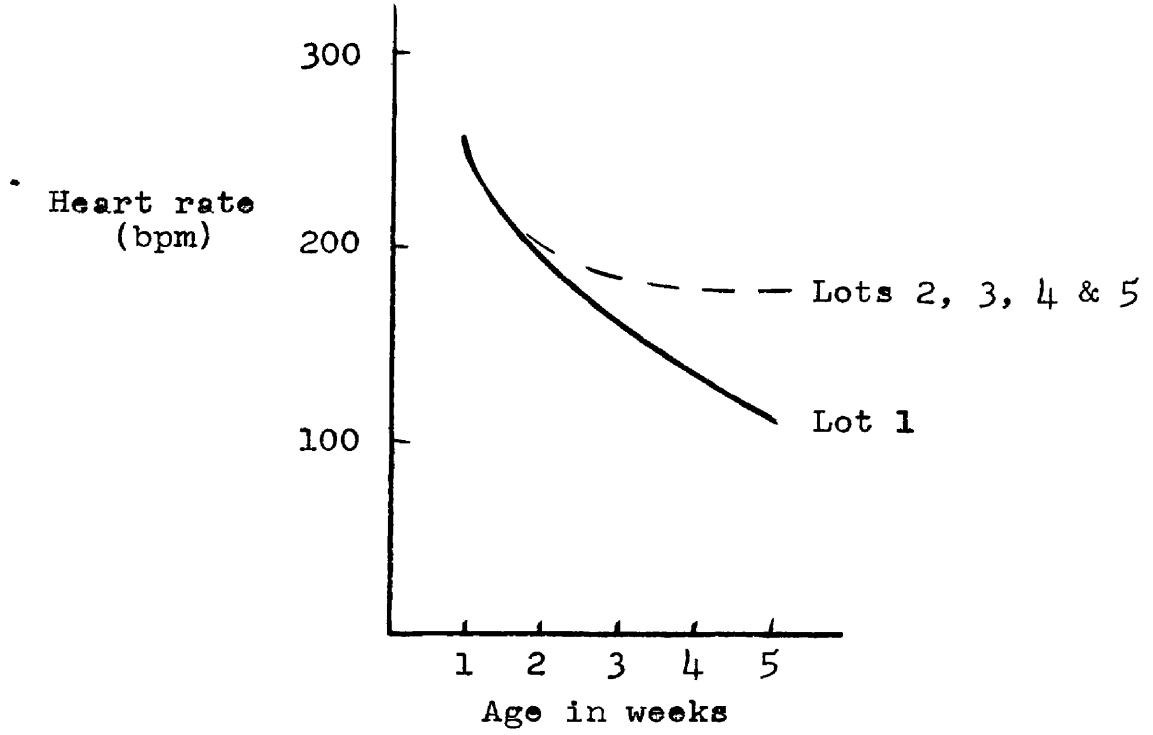


Fig. 4 - Heart rate as affected by age and dietary level of thiamine.

Table 12 - Heart rate[1] response of baby pigs to different levels of dietary thiamine

Week of age	Level of thiamine in diet, in mg/kg solids									
	0.0		0.5		1.0		1.5		2.0	
1	(2)[2]	272±12[3]	(2)	270±0	(2)	283±3	(2)	248±46	(2)	255±15
2	(7)	176±10	(7)	189±4	(7)	195±10	(7)	195±8	(7)	186±10
3	(7)	177±7	(7)	183±9	(7)	197±12	(7)	192±10	(7)	189±10
4	(6)	151±15	(6)	166±10	(7)	182±9	(7)	162±10	(7)	162±11
5	(4)	124±11	(6)	174±4	(7)	177±7	(7)	179±9	(7)	188±7

[1]Heart rates are recorded in beats per minute.

[2]Numbers in parenthesis are the number of pigs considered in determining the mean heart rate for the adjacent entry. These parenthetical numbers apply also to corresponding entries in tables 13 to 17 inclusive.

[3]Standard error of the mean.

The pigs receiving no thiamine did not indicate a pronounced bradycardia until after the characteristic symptoms of anorexia, vomiting and reduced rate of gain appeared. This usually occurred when the pigs had been on no thiamine for two weeks and were hence about three weeks old. In each instance the heart rate was reduced further as the degree of thiamine deficiency progressed. The lowest recorded heart rate was 100 for one of the deficient pigs several hours before sudden death occurred. Three negative control pigs had died before ECGs could be taken in the final week.

The PR interval in the heart cycle is a measure of the time relation between auricular and ventricular contractions. A lengthening of the PR interval of more then 50 percent above normal denotes a delay in conduction from the auricles to the ventricles. This is first degree auriculoventricular block. The absence of the ventricular phase in a cycle is termed second degree auriculoventricular block. A summary of the PR interval data is presented in table 13 and figure 5. The PR interval was lengthened in most of the deficient pigs. The longest PR interval recorded was .14 seconds in two of the deficient pigs, and indicates first degree auriculoventricular block. The average PR interval of the negative control pigs during the final week was 25 percent longer than the average PR interval of each of the other lots.

Table 13 - PR interval[1] response in baby pigs to different dietary levels of thiamine

Week of age	Level of thiamine in diet, in mg/kg solids				
	0.0	0.5	1.0	1.5	2.0
1	.06±.005	.06±.000	.06±.000	.06±.000	.06±.000
2	.07±.003	.07±.003	.07±.003	.07±.003	.07±.004
3	.07±.003	.07±.000	.07±.004	.07±.002	.07±.004
4	.09±.007	.08±.003	.08±.001	.08±.002	.08±.002
5	.10±.010	.08±.001	.08±.001	.08±.002	.08±.001

[1]Duration of PR interval is expressed in seconds.

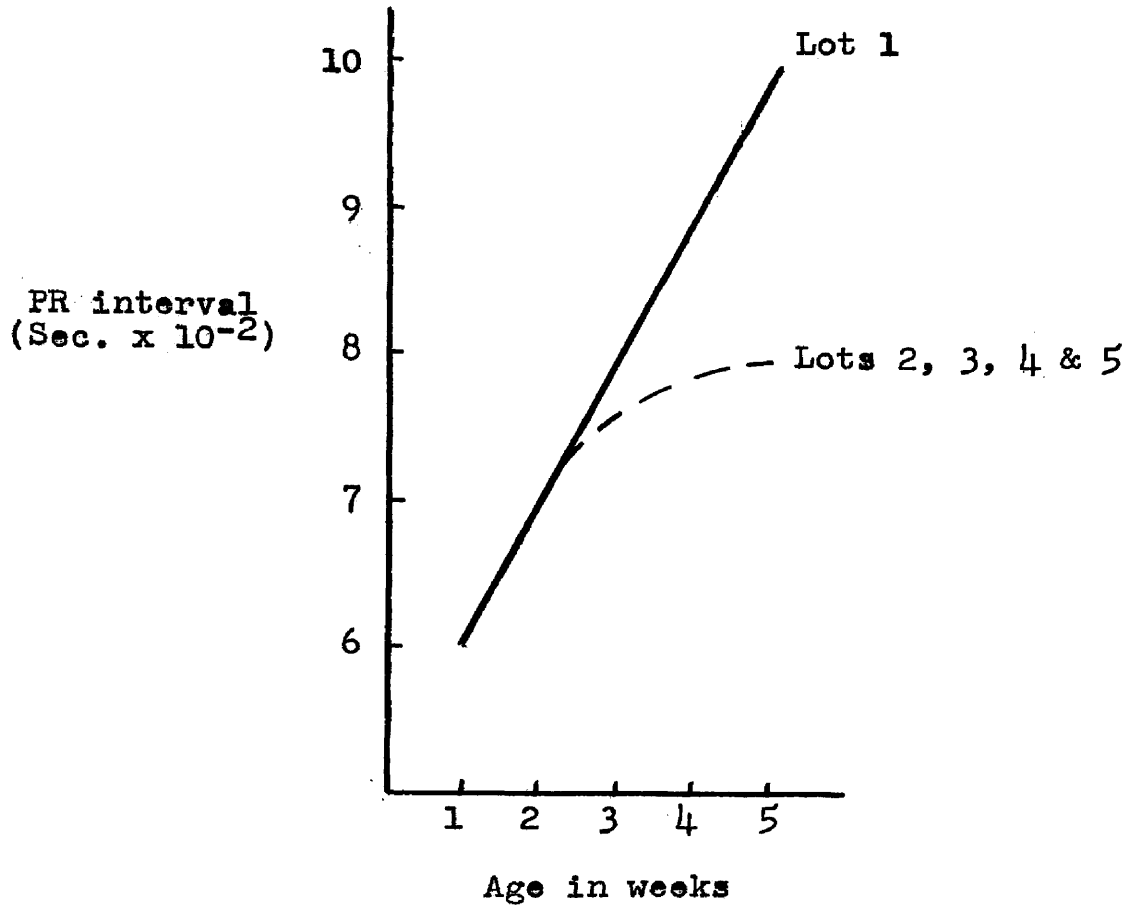


Fig. 5 - PR interval as affected by age and dietary level of thiamine.

The interval between the initial and final ventricular complexes is known as the ST segment. This segment should be isoelectric and a great deviation from this indicates anoxia in the myocardium. A great elevation of this segment was found in the ECG of only one deficient animal. This is apparent in the ECG of pig 15-4 in figure 13. The ST interval represents the period during which depolarization persists during ventricular systole. A lengthening of the ST interval will be noted in deficient pigs in table 14 and figure 6.

Table 14 - ST interval[1] response in baby pigs to different dietary levels of thiamine

	Level of thiamine in diet, in mg/kg solids				
Week of age	0.0	0.5	1.0	1.5	2.0
1	.06±.005	.06±.000	.06±.000	.06±.010	.06±.005
2	.08±.004	.08±.004	.08±.004	.08±.003	.08±.003
3	.09±.002	.09±.004	.08±.004	.08±.005	.08±.007
4	.12±.010	.12±.008	.10±.005	.10±.002	.10±.005
5	.16±.017	.12±.009	.10±.007	.10±.007	.10±.005

[1]Duration of ST interval is expressed in seconds.

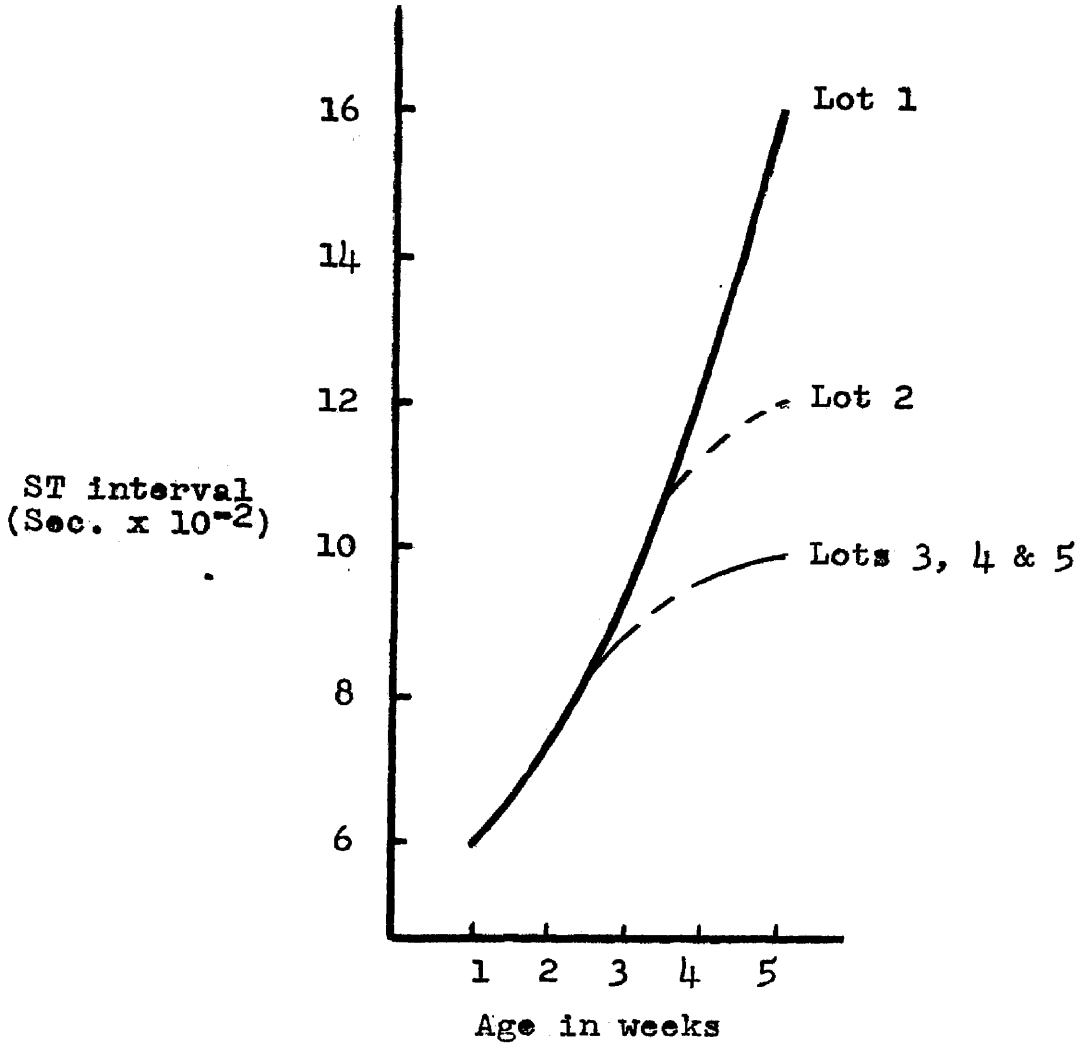


Fig. - 6 - ST interval as affected by age and dietary level of thiamine.

The QRS complex occurs at the start of ventricular systole. The length of this interval is prolonged in cases in which excitation spreads by abnormal routes. In these experiments there seemed to be no lengthening of this interval due to thiamine deficiency and it was consistently .03 or .04 seconds in duration.

The QRST interval is known as the electrical systole. The duration of this complex is approximately the same as

that of mechanical ventricular systole. A lengthening of this period is due to a delaying of the bundle-branch spread of excitation and to a lessening in the repolarizing ability of the myocardium. That this interval is lengthened in animals which are thiamine deficient is shown in table 15 and figure 7.

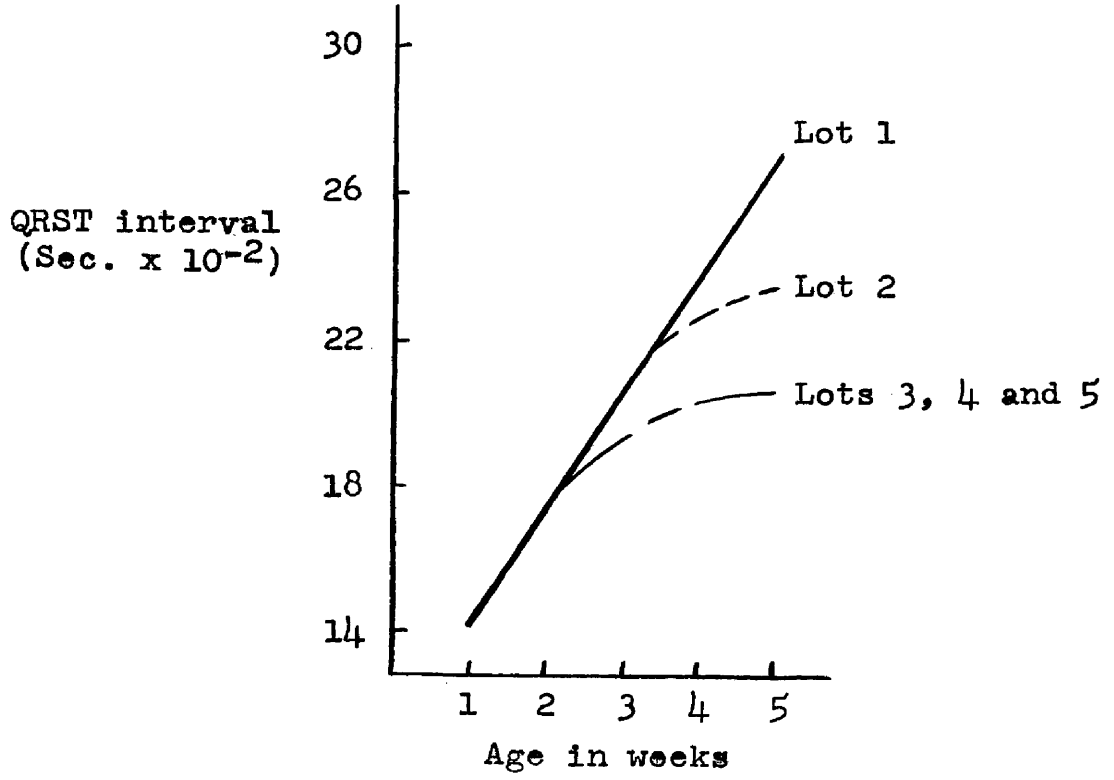


Fig. 7 - QRST interval as affected by age and dietary level of thiamine.

The T wave results from a repolarization of the ventricular septum and apical portions of the heart. The end of the T wave coincides with the end of ventricular systole. Thiamine deficiency in the pigs in this experiment resulted in a moderate lengthening of the duration of the T wave

Table 15 - QRST interval[1] response in baby pigs to different dietary levels of thiamine

Week of age	Level of thiamine in diet, in mg/kg diet				
	0.0	0.5	1.0	1.5	2.0
1	.14±.010	.14±.005	.14±.005	.14±.020	.14±.010
2	.19±.004	.18±.004	.19±.007	.18±.005	.19±.005
3	.19±.003	.19±.005	.18±.008	.19±.006	.18±.007
4	.24±.017	.23±.010	.21±.008	.21±.003	.21±.007
5	.27±.016	.23±.007	.21±.008	.20±.007	.20±.004

[1]Duration of QRST interval is expressed in seconds.

suggestive of a decreased ability of the thiamine deficient pig to bring about a restitution of the polarized state of the myocardium. This effect of thiamine deficiency upon T wave duration is shown in table 16.

Table 16 - T wave duration[1] response in baby pigs to different dietary levels of thiamine

	Level of thiamine in diet, in mg/kg diet				
Week of age	0.0	0.5	1.0	1.5	2.0
1	.06±.005	.06±.005	.06±.000	.06±.005	.06±.005
2	.07±.002	.06±.002	.07±.002	.06±.002	.07±.003
3	.07±.004	.07±.003	.06±.004	.06±.003	.06±.002
4	.07±.004	.07±.004	.07±.002	.07±.002	.07±.002
5	.08±.009	.07±.006	.06±.002	.06±.004	.06±.003

[1]Duration of T wave is expressed in seconds.

The systolic portion of the cycle increases as thiamine deficiency occurs. This is shown in table 17 and figure 8.

Table 17 - Bazett's constant ($K = \frac{QT}{\sqrt{RR}}$) response in baby pigs to different dietary levels of thiamine

	Level of thiamine in diet, in mg/kg solids				
Week of age	0.0	0.5	1.0	1.5	2.0
1	.30±.015	.31±.010	.31±.005	.29±.015	.29±.010
2	.33±.012	.32±.008	.33±.009	.32±.010	.32±.007
3	.33±.005	.33±.007	.33±.012	.34±.009	.33±.010
4	.38±.025	.38±.015	.37±.012	.35±.005	.35±.009
5	.39±.020	.39±.011	.36±.013	.35±.009	.35±.008

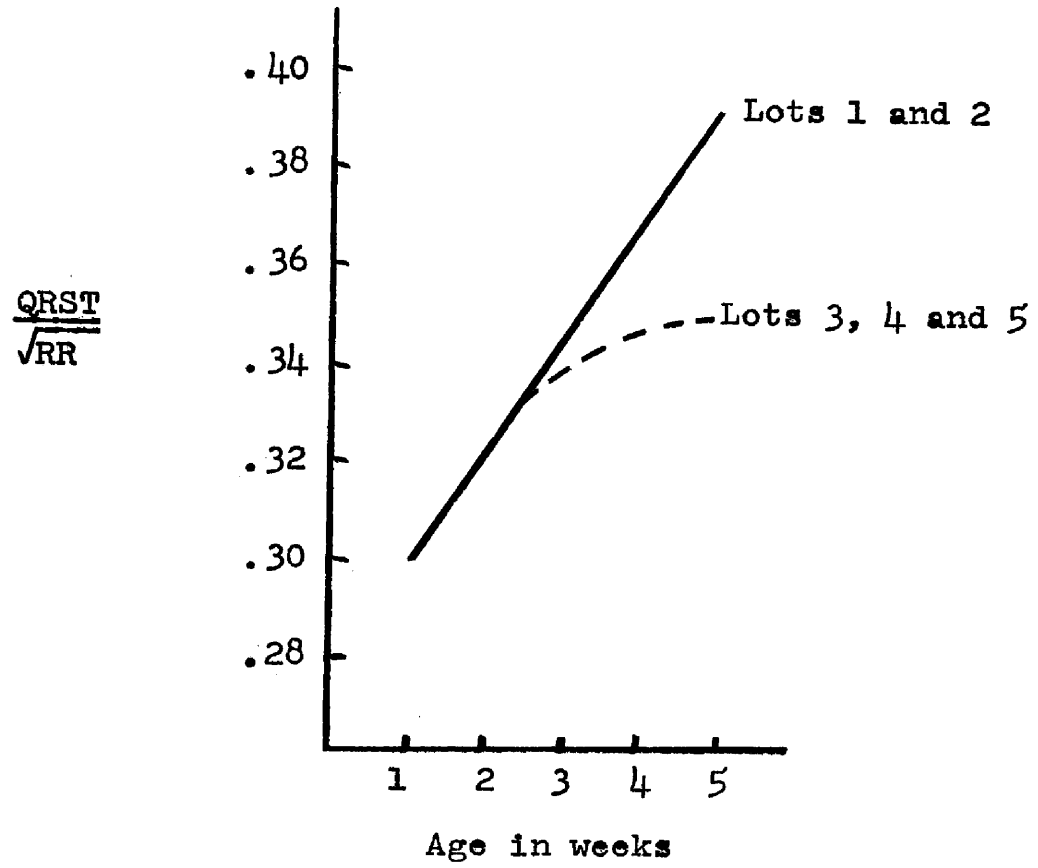


Fig. 8 - Bazett's constant as affected by age and dietary level of thiamine.

It appears tenable from the data presented that electrocardiographic recordings are not the most sensitive test for measuring dietary thiamine adequacy for the baby pig. Both rate of growth and efficiency of feed utilization seem to be more sensitive. However, the ECG does supply information which makes possible a more complete knowledge of the thiamine deficiency syndrome in the baby pig. Electrocardiographic recordings from several of the positive and negative control pigs are shown in figures 9, 10, 11, 12 and 13.

Fig. 9 - The seven lead recordings made on all electrocardiograms. Leads I, II and III are the standard leads. Lead V is the unipolar chest lead and the AVR, AVL and AVF leads are unipolar leads from the right front, left front and the left hind legs respectively. This ECG was taken from positive control pig 15-1 late in the experiment. Heart cycle length is uniform and waves P, R and T are of positive potential in all leads except the AVR lead in which all these potentials are in a negative direction.

Heart rate is 190 bpm, PR time is .08 seconds and QRST duration is .20 seconds. P and T are barely perceptible in lead III.

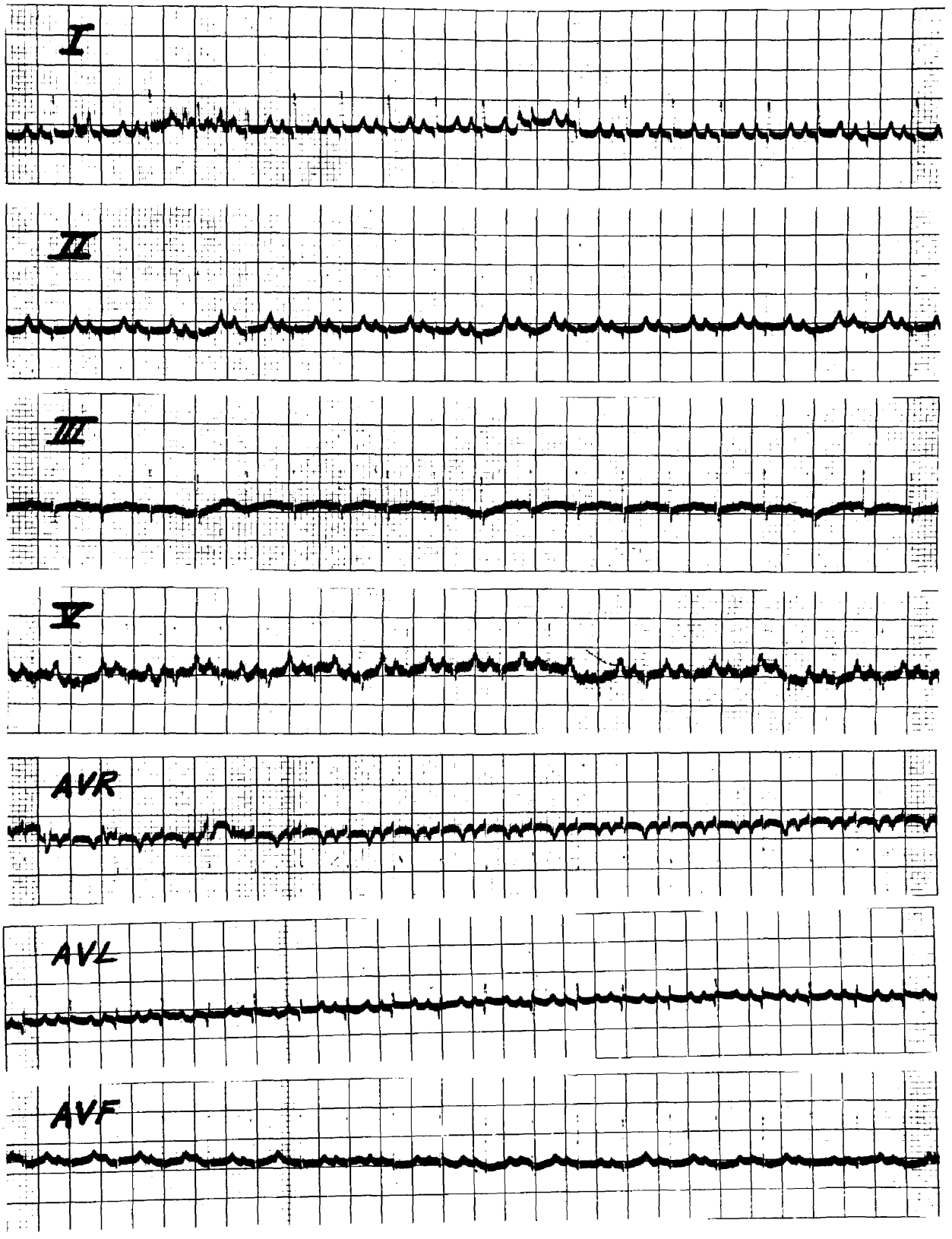


Fig. 9 - Electrocardiogram from positive control pig 15-1.

Fig. 10 - The seven lead tracings on the ECG of negative control pig 15-11 taken late in the experiment after pronounced external thiamine deficiency symptoms were observed. Second degree A-V block is the most pronounced manifestation observed. A moderate sinus arrhythmia and bradycardia are apparent. PR time is .12 seconds, QRST time is .24 seconds.

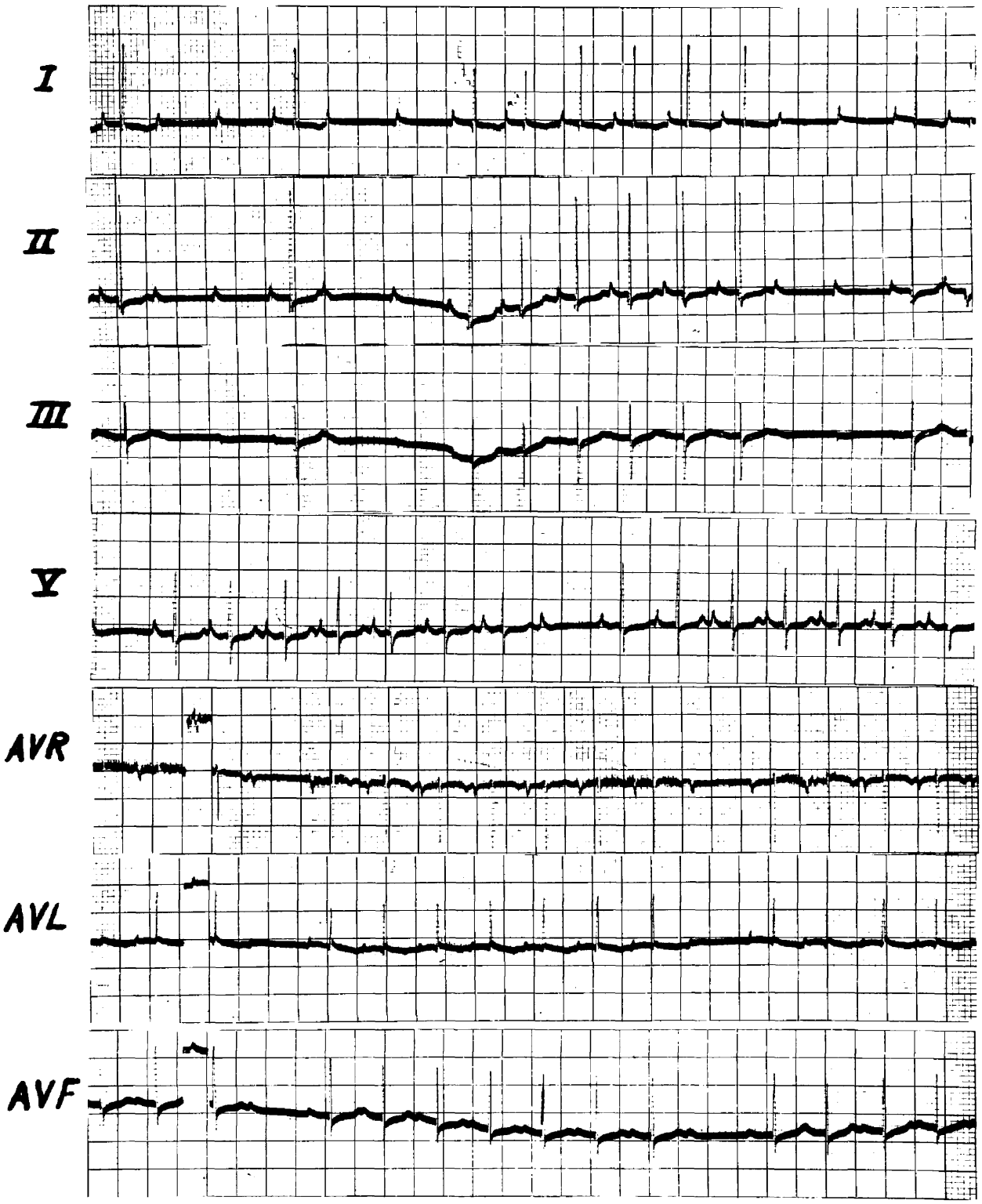
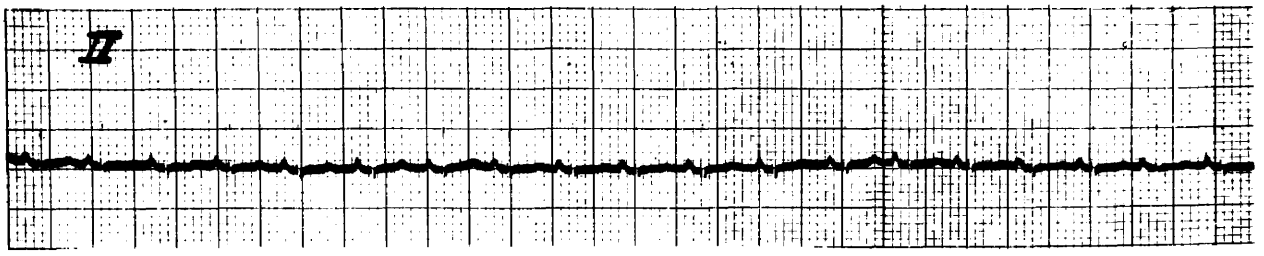
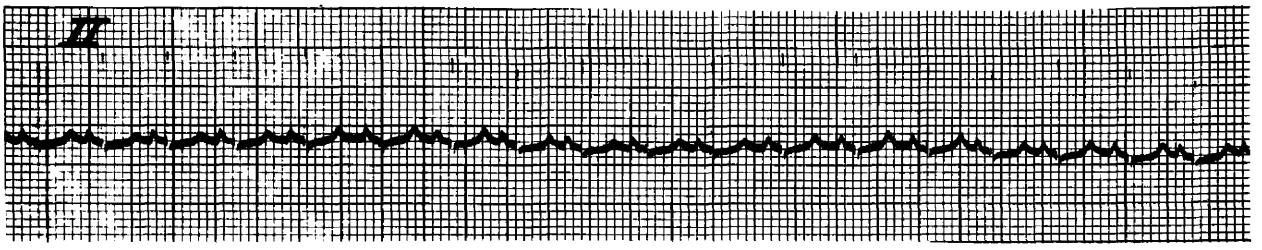


Fig. 10 - Electrocardiogram from pig 15-11 while in a state of acute thiamine deficiency.

Fig. 11 - The heart rates of the positive control pigs remained relatively constant throughout the experiment. In pig 12-1 the heart rate was 180 to 190 bpm in all recordings. The heart cycles are uniform in length. The PR interval is .08 seconds, the QRST interval is .18 to .20 seconds and the T wave potential is less than .2 millivolts in lead II.



First week of experiment



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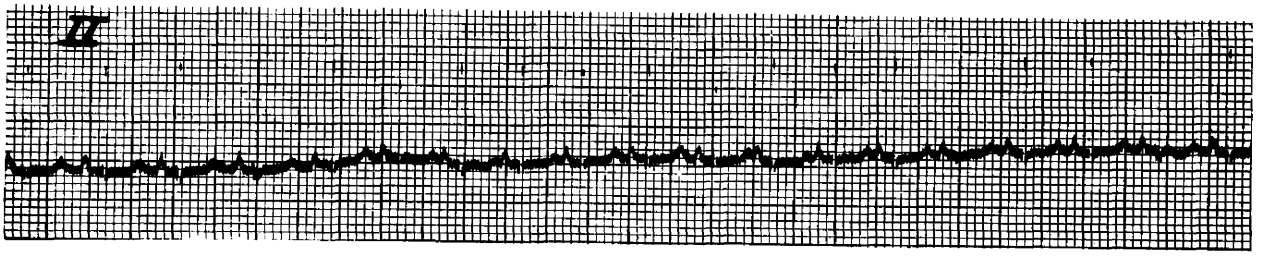
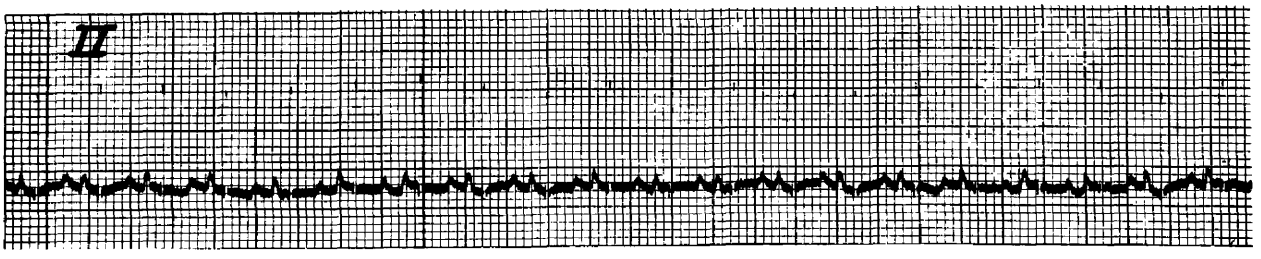


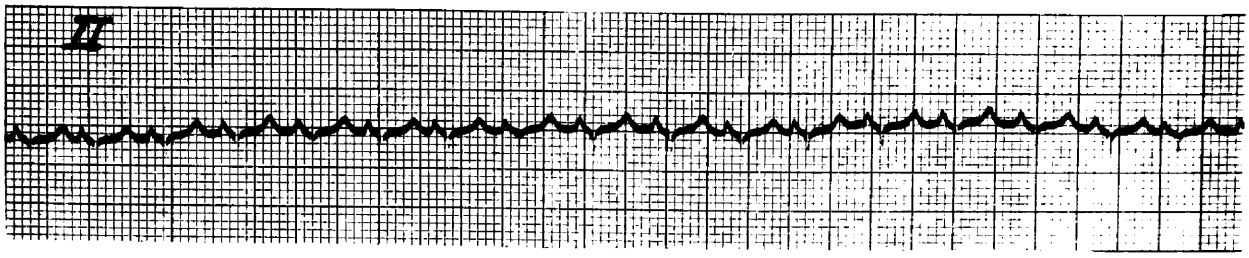
Third week of experiment



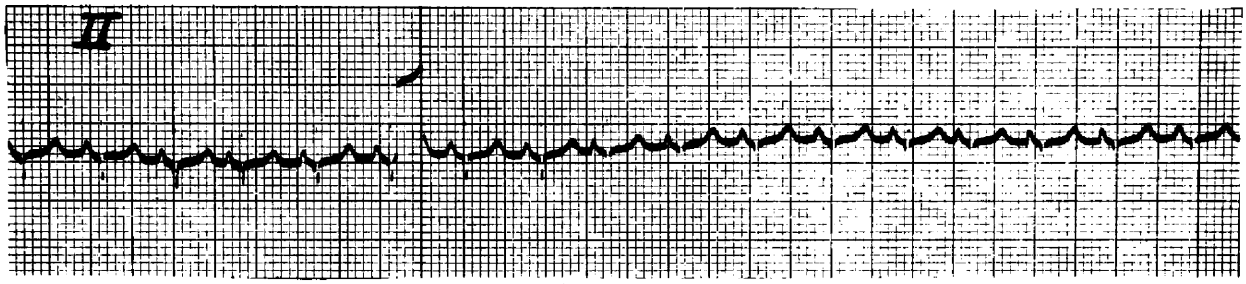
Fourth week of experiment

Fig. 11 – Electrocardiograms of positive control pig 12-1 taken weekly throughout the experiment.

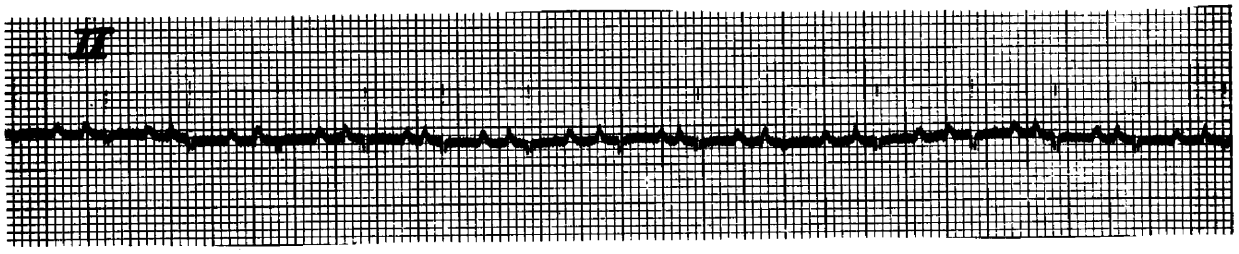
Fig. 12 - Most of the negative control pigs began to show a slight to marked bradycardia during the third week of the experiment. By the fourth week of the experiment the bradycardia was quite pronounced. The heart rate of pig 12-6 was about 180 bpm during both the first and second weeks of the experiment. By the third week of the experiment the heart rate was down to 140 bpm and in the fourth week was 100 bpm. On the final recording the PR time was .14 seconds (first degree A-V block), the QRST interval duration was .34 seconds and the T wave potential was .3 to .5 millivolts in lead II.



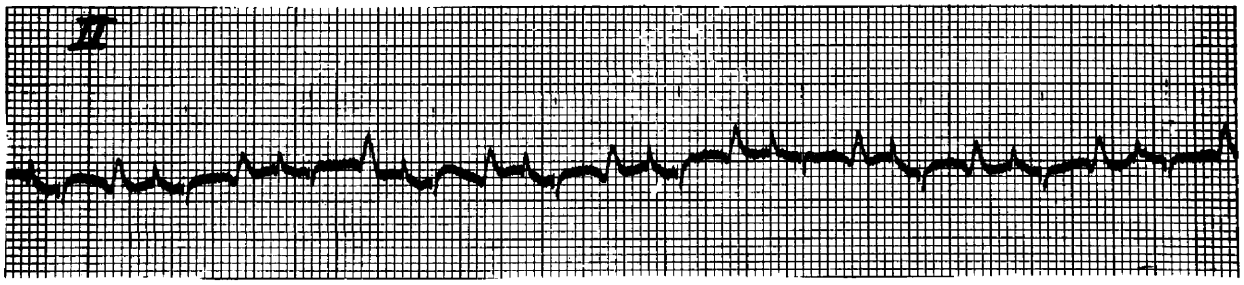
First week of experiment



Second week of experiment



Third week of experiment



Fourth week of experiment

Fig. 12 - Electrocardiograms of negative control pig 12-6 taken weekly throughout the experiment.

Fig. 13 - ECG findings in these thiamine deficient pigs reveal varying degrees of bradycardia in all pigs. A marked sinus arrythmia is exhibited by pigs 16-4, 15-11, and 15-4. First degree auriculoventricular block is apparent in pig 12-6 and second degree AV block is exhibited by pig 15-11. The duration of the QRST interval is excessive in all pigs except 15-4. The duration of the PR interval is greatly lengthened in pigs 12-6 and 15-11. The ST segment is greatly elevated in pig 15-4. T wave potential was abnormally high in pigs 12-6, 15-9 and 15-4.

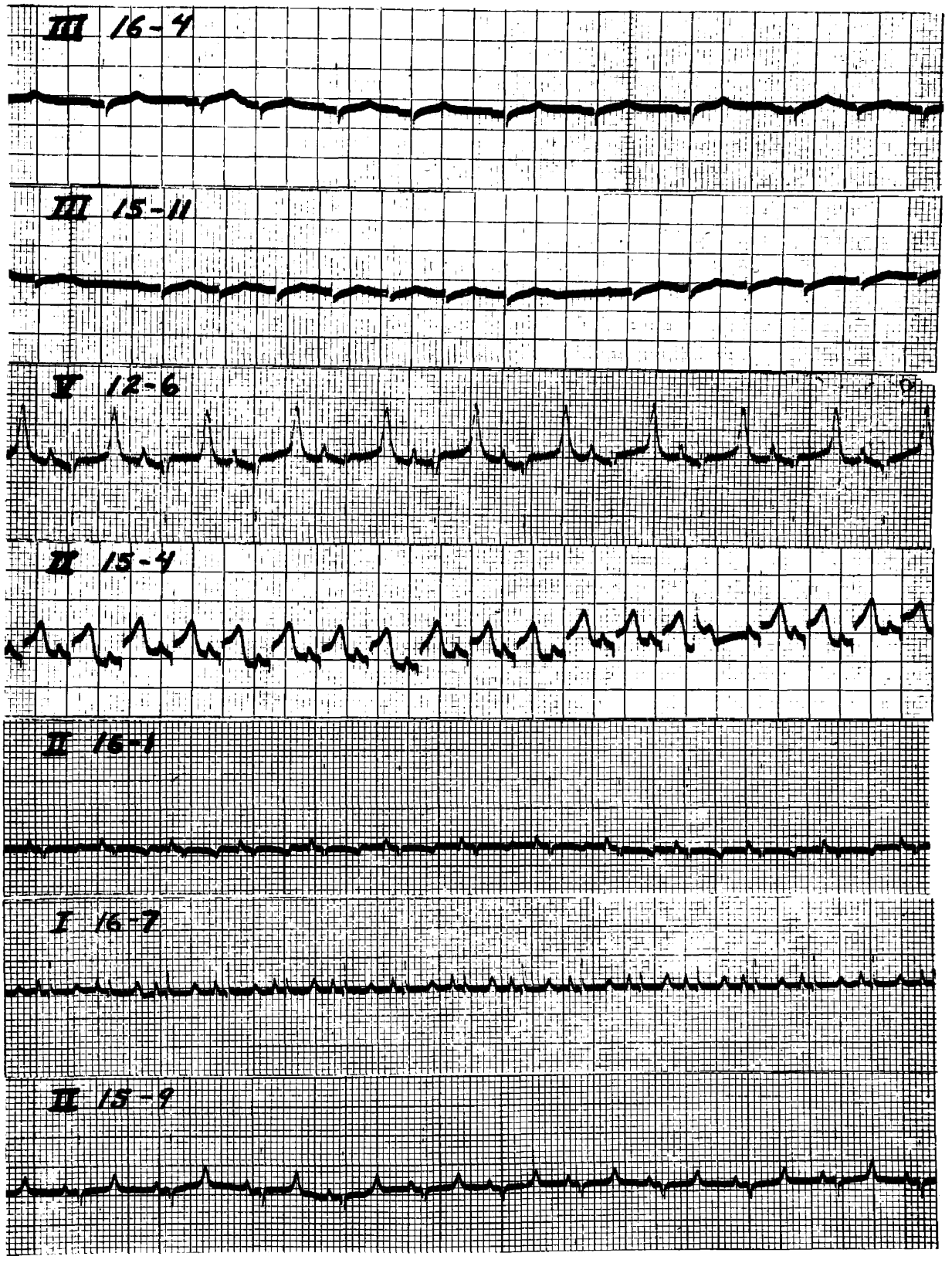


Fig. 13 - Electrocardiograms taken from thiamine-deficient pigs.

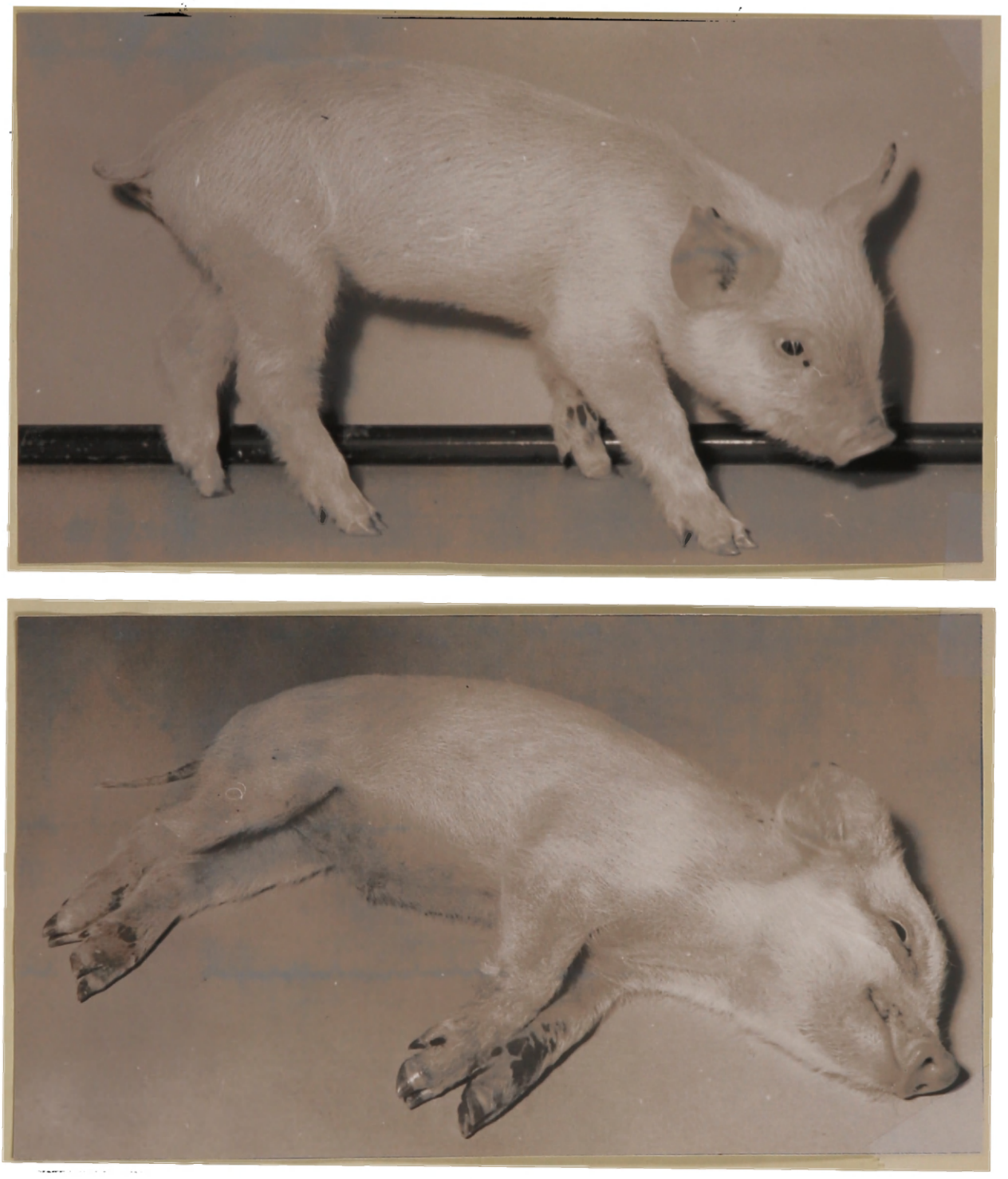


Fig. 14 - Two photographs of thiamine-deficient pig 15-11. The lower photograph was taken a few minutes after the upper photograph. Sudden death occurred ad interim. This pig had been on the negative control diet for 23 days and weighed 2.68 kilograms. This pig had manifested second degree A-V block on the ECG taken 4 days previously.

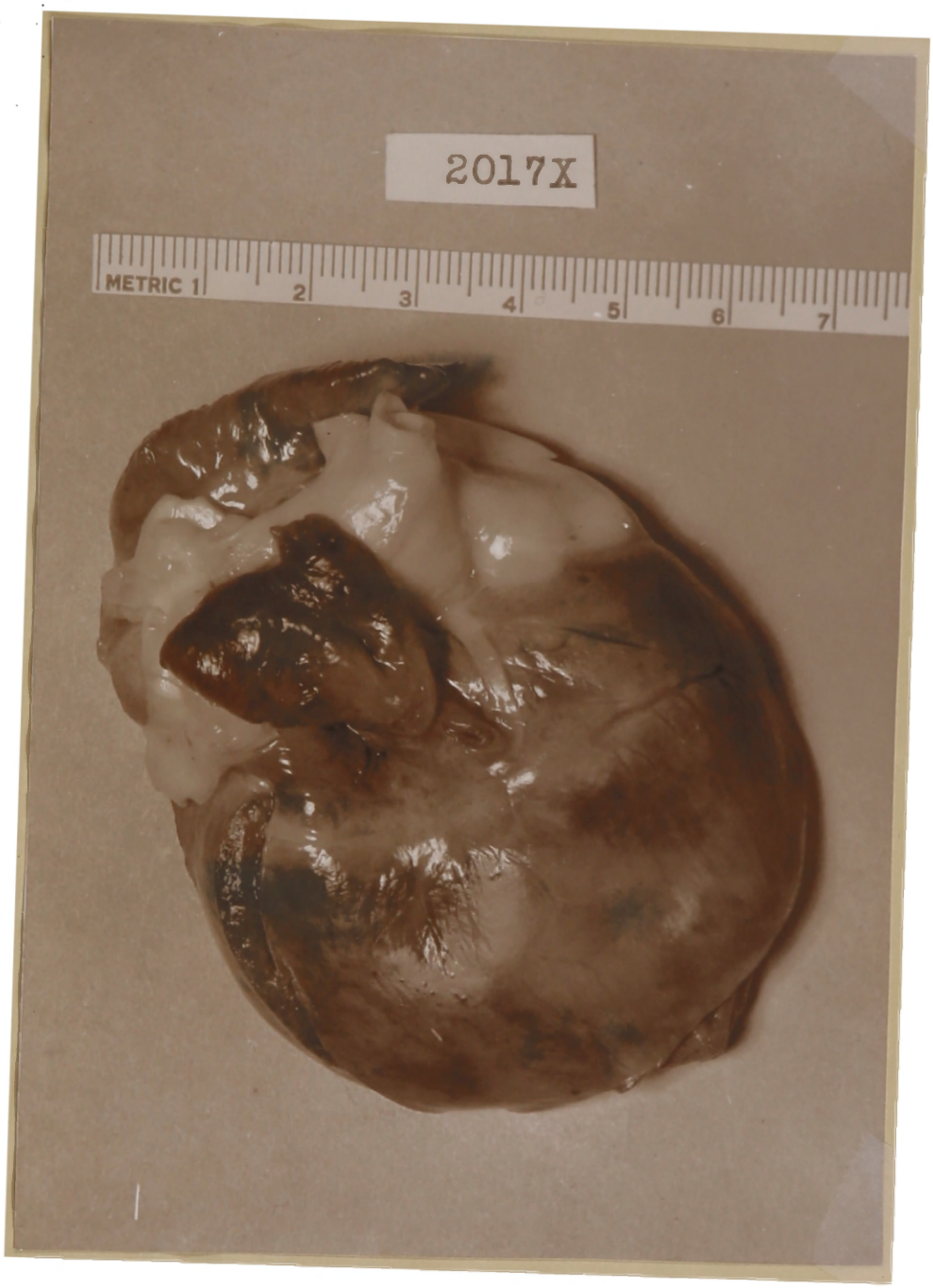


Fig. 15 - Heart of pig 15-4. This pig died after 5 weeks on a regime of .5 mg thiamine per kilogram of solids. A pale mottling is present, the whole heart is flabby and resembles a myxedematous heart. Heart weight represents 1.38% of the total body weight.

Part III--Pyridoxine Studies

Experiment I

A summary of the results of the first pyridoxine trial are presented in table 18. On the basis of these experimental results and using the rate of daily gain, daily feed intake and efficiency of gain as criteria for determining pyridoxine requirement, it seems apparent that the minimum B_6 requirement of the baby pig does not exceed 1 mg per kilogram of solids in the diet. The growth curves presented in figure 16 support this conclusion.

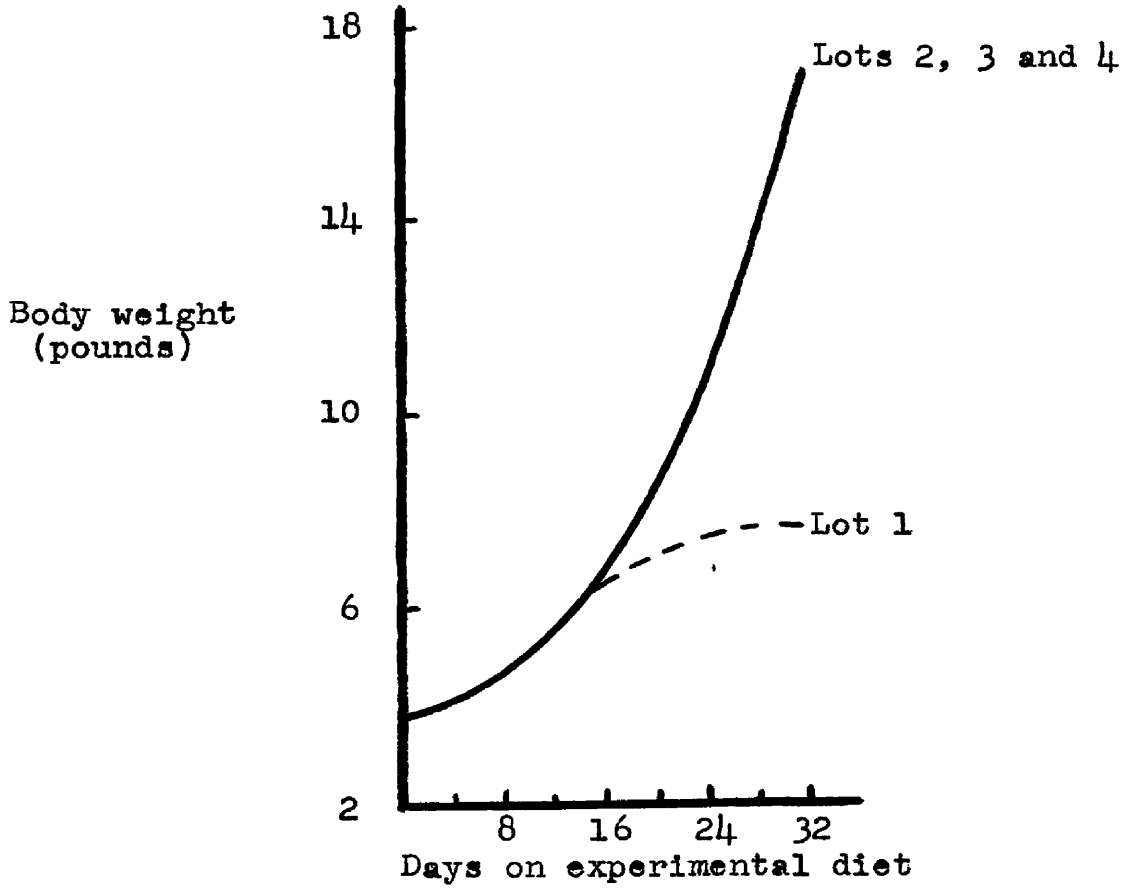


Fig. 16 - Growth response of baby pigs to different dietary levels of B_6.

Table 18 - Response of baby pigs to synthetic milk diets containing different levels of pyridoxine

	Level of pyridoxine in diet, in mg/kg solids			
	0	1	2	4
Lot number	1	2	3	4
Number of pigs	4	4	4	4
Days on test	32	32	32	32
Ave. initial wt.(lb.)	3.72±.18	3.78±.22	3.78±.20	3.72±.37
Ave. final wt.(lb.)	7.63±.55	16.04±.55	15.78±.77	16.50±.71
Ave. daily gain(lb.)[1]	.12±.01	.39±.01	.38±.02	.40±.01
Ave. daily solids consumed(lb.)[2]	.24±.01	.40±.01	.40±.02	.40±.01
Solids per lb. gain (lb.)[3]	1.98±.14	1.04±.02	1.06±.03	1.00±.01
Blood hemoglobin[4] level(gm/100ml)	7.8±.70	11.7±.50	12.9±.40	13.0±.60
Red blood cell count(million/mm^3)	6.11±.76	7.06±.09	7.24±.70	7.37±.61

[1]All lots receiving pyridoxine gained significantly faster than the lot receiving no pyridoxine. (P=.01)

[2]All lots receiving pyridoxine consumed significantly more feed than the lot receiving no pyridoxine. (P=.01)

[3]All lots receiving pyridoxine were significantly more efficient in food utilization than the lot receiving no pyridoxine. (P=.01)

[4]Blood hemoglobin and RBC values taken during 4th week of experiment. Blood hemoglobin values of all lots receiving pyridoxine were significantly higher (P=.05) than the lot receiving no pyridoxine.

An analysis of the weekly hemoglobin and RBC count values lends further support to the conclusion that 1 mg per kilogram of solids is adequate. All blood samples taken from the negative control pigs from the third week of experimental feeding until their death (fifth week) showed pale microcytic erythrocytes with anisocytosis. That hypochromia develops rapidly in pyridoxine deficiency is apparent from figure 17.

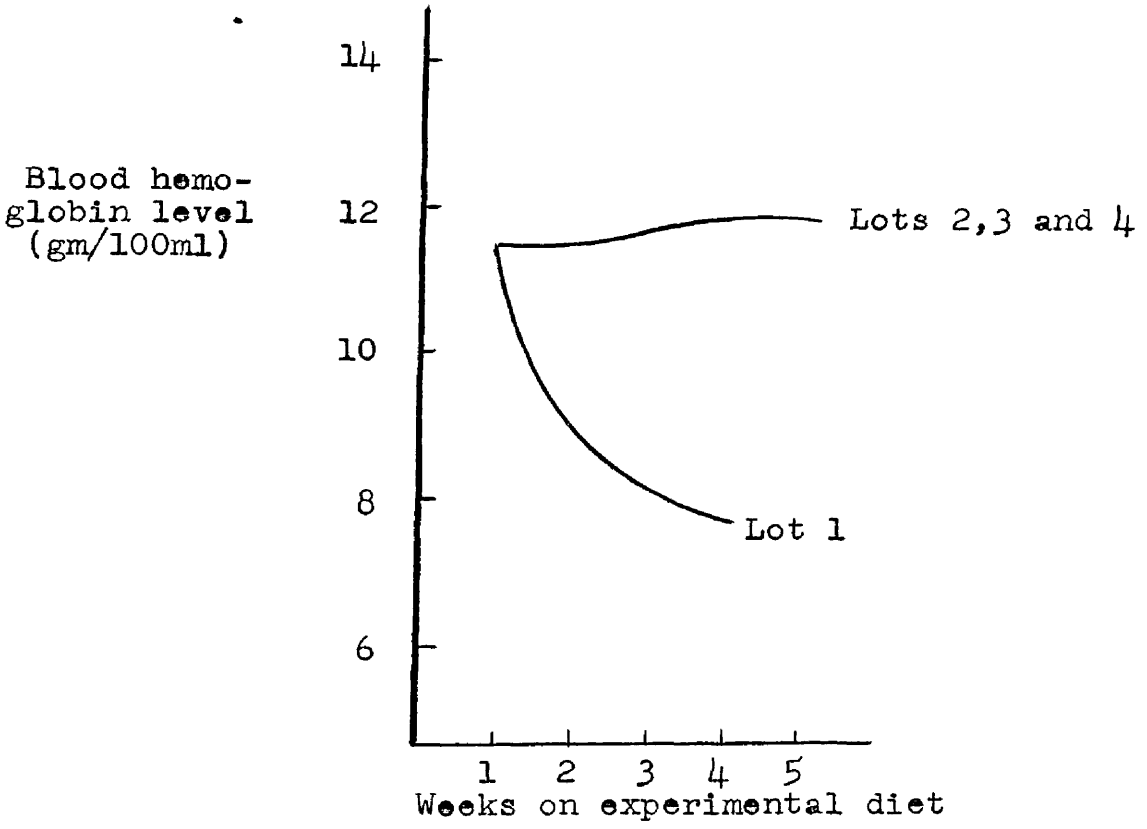


Fig. 17 - Blood hemoglobin response of baby pigs to different dietary levels of pyridoxine.

A concurrent experiment, in which two pigs received the positive control level of pyridoxine supplementation and were pair fed with two of the negative control pigs, indicated that the hypochromic microcytic anemia and its subse-

quent effects was not due to the reduced dietary intake of the negative control pigs but rather was due specifically to the absence of B_6 in the diet as shown in table 19.

Table 19 - Comparison of rate of gain, feed efficiency, blood hemoglobin level and red blood cell count of positive control pigs pair fed with negative control pigs

	Level of pyridoxine in diet, in mg/kg solids	
	0	4
Lot number	5	6
Number of pigs	2	2
Days on test	32	32
Ave. initial wt.(lb.)	3.45±0.20	3.01±0.11
Ave. final wt.(lb.)	6.75±0.50	9.24±0.18
Ave. daily gain (lb.)	.10±0.01	.20±0.01
Ave. daily solids consumed (lb.)	.23±0.01	.23±0.01
Solids per lb.gain(lb.)	2.21±0.10	1.18±0.02
Blood hemoglobin level (gm/100 ml)[1]	8.3±0.50	14.4±0.40
Red blood cell counts (million/mm3)[1]	5.94±1.20	9.11±0.30

[1]Blood hemoglobin and RBC count values taken during 4th week of experiment.

At no time during this trial were epileptiform seizures observed in any of the pigs. The pigs in lot 1 began to manifest anorexia during the second week of the trial and reduced growth rate was a proximal sequel. These pigs became weak and emaciated late in the trial and three of them died on the second day following the close of the experimental feeding period. The fourth pig from this lot died two days later. Post mortem studies of these pigs consistently revealed the presence of generalized anasarca.

Experiment II

Many of the baby pigs started on the second trial were small and weak. None of the pigs did well on the synthetic milk diet at first and scouring appeared in every pig. This necessitated a drastic reduction in feed offered for consumption in an effort to stop the scouring. Oral doses of terramycin in the milk and drinking water seemed to help stop the scouring. As a result of the diarrhea and the low caloric intake the pigs were in a weak condition. This weak condition persisted and feed consumption remained low or was arbitrarily reduced because of the susceptibility of the pigs to a recurrence of the morbid diarrhetic state. During the third week of experimental feeding all pigs were given intraperitoneally two 10 ml doses of a multiple B-vitamin solution. The B-vitamins were in a sterile saline solution and consisted of 5 mg niacinamide, 5 mg calcium pantothenate, 2 mg riboflavin, 2 mg thiamine, 250 mcg folic acid and 5 mcg B_{12} per ml solution. No noticeable improvement followed and feed consumption remained low throughout the trial. Consequently, the growth and feed consumption data obtained in this experiment were considered invalid.

In this trial epileptiform seizures were observed in the negative control pigs on 29 recorded occasions. These seizures occurred on every observed occasion either immediately prior to, during or just after feeding. Although the seizure appeared spontaneously, its onset seemed to be

stimulated by the excitement or anxiety of the feeding experience. The seizures were of varying degrees of severity and duration. On each observed occasion the seizure came suddenly, the pig began to shake and tremble convulsively and occasionally threw himself on his back pawing wildly. The seizure would close with the pig stretching himself prone, apparently dead, with foam appearing at the mouth. Shortly thereafter the pig would relax and still later rise weakly and walk over to the feed trough and consume slowly the milk. On no occasion was the duression sustained for more than two or three minutes. Seizures were observed only in the negative control group.

Experiment III

A presentation of the general results of the third trial is made in table 20. Statistical analysis of the data pertaining to rate of gain, feed consumption and feed efficiency indicate that the minimum pyridoxine requirement of the baby pig is not in excess of .5 mg B_6 per kilogram of solids in the diet. However, the subclinical data obtained, i.e. blood hemoglobin level and urine xanthurenic acid concentration, both indicate that the .5 mg level and possibly the .75 mg level of pyridoxine administration may be too low for the total well-being of the pig.

Data obtained from the blood and urine studies indicate that both blood hemoglobin level and urine xanthurenic acid

Table 20 - Response of baby pigs to synthetic milk diets containing different levels of pyridoxine[1]

	Level of pyridoxine in diet, in mg/kg solids				
	0.0	0.5	0.75	1.0	2.0
Lot number	1	2	3	4	5
Number of pigs	4	3	3	4	4
Days on test	32	32	32	32	32
Ave. initial wt.(lb.)	4.28±0.51[2]	4.20±0.50	4.68±0.40	4.16±0.12	4.06±0.22
Ave. final wt.(lb.)	8.49±0.77	16.76±1.54	18.96±0.75	17.05±0.60	17.69±0.53
Ave. daily gain(lb.)[3]	.13±0.01	.39±0.04	.45±0.02	.41±0.02	.43±0.02
Ave. daily solids consumed (lb.)[4]	.21±0.02	.39±0.01	.42±0.02	.39±0.02	.39±0.01
Solids per lb.gain(lb.)[5]	1.57±0.10	1.00±0.06	.94±0.02	.96±0.03	.92±0.01
Blood hemoglobin level (mg/100 ml)[6]	6.2±0.1	7.9±1.3	9.9±0.9	10.3±0.7	11.2±0.9
Red blood cell count (million/mm^3)[6]	5.04±0.10	5.92±0.37	5.96±0.25	6.44±0.25	5.81±0.15
Urine XA acid concentration (mcg/ml)[6]	64±26	23±9	16±2	0	0

[1]Pigs were taken from the sow when 4 days old and placed on a depletion diet containing no pyridoxine for 4 days. Pigs were then assigned to lots and started on the feeding trial.

[2]Standard error of mean.

[3]All lots gained significantly faster than lot receiving no pyridoxine. (P=.01)

[4]All lots consumed significantly more solids than lot receiving no pyridoxine.(P=.01)

[5]All lots significantly more efficient in food utilization than lot receiving no pyridoxine. (P=.01)

[6]Blood hemoglobin, RBC and XA excretion values taken during last week of experiment.

concentration are more sensitive tests for adequacy of pyridoxine supplementation than is pig growth. Graphs showing a summary of the progressive changes exhibited in growth, blood hemoglobin level and xanthurenic acid concentration are presented in figures 18, 19 and 20 respectively.

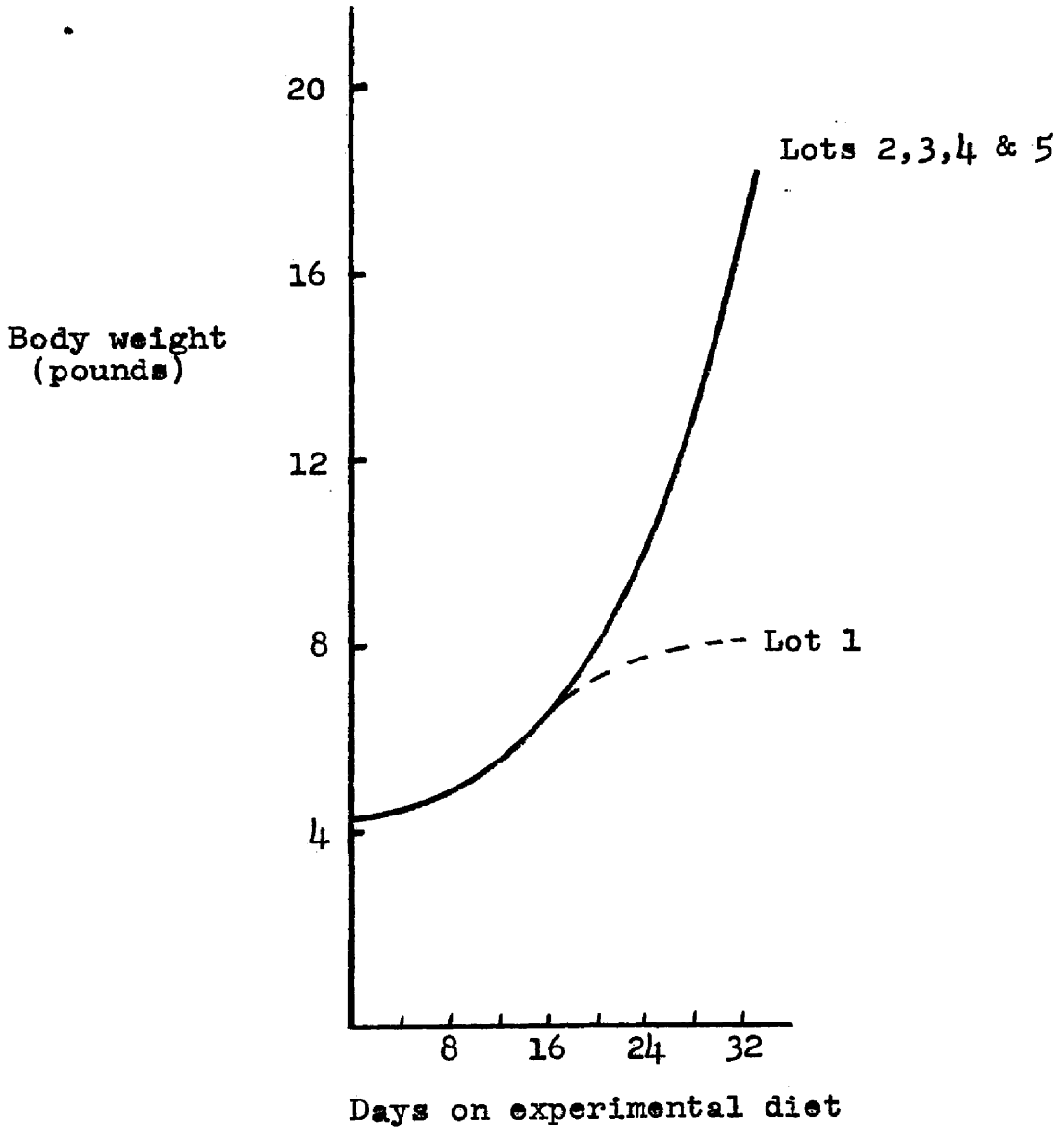


Fig. 18 - Growth response of baby pigs on different levels of pyridoxine intake.

It is apparent from figure 18 that differences in body weight between the lots receiving pyridoxine and the lot receiving no pyridoxine were not obtained until after the pigs had been on the experimental diets for three weeks or more. No significant differences between any two of the lots receiving various levels of pyridoxine developed during the trial.

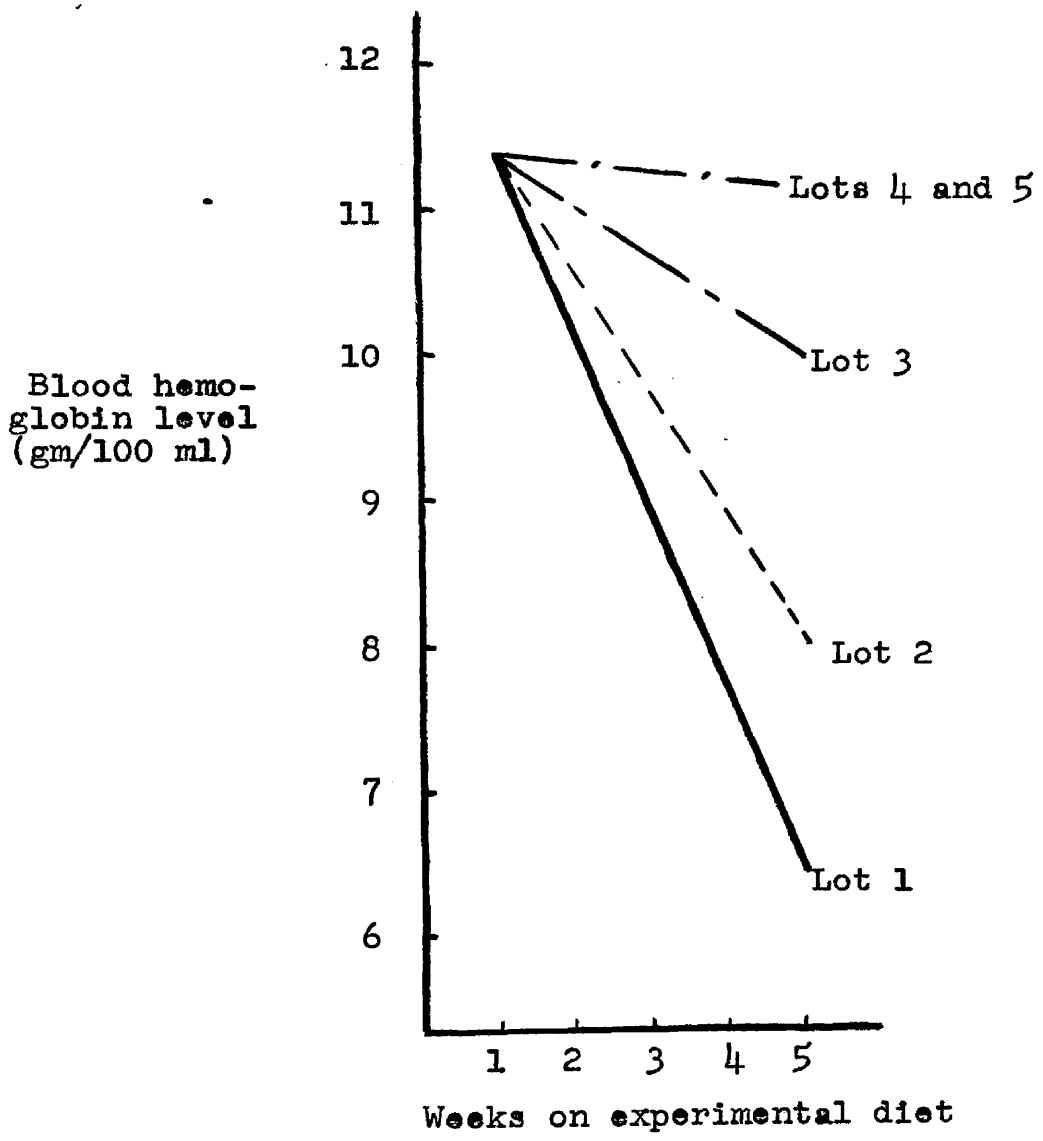


Fig. 19 - Blood hemoglobin response of baby pigs to different dietary levels of pyridoxine.

Differences in blood hemoglobin level and urine xanthurenic acid concentration between the lot receiving no pyridoxine and lots receiving pyridoxine were present before two weeks of the trial had elapsed. As the trial progressed, differences developed in both blood hemoglobin level and urine xanthurenic acid concentration between lots receiving the various levels of dietary pyridoxine.

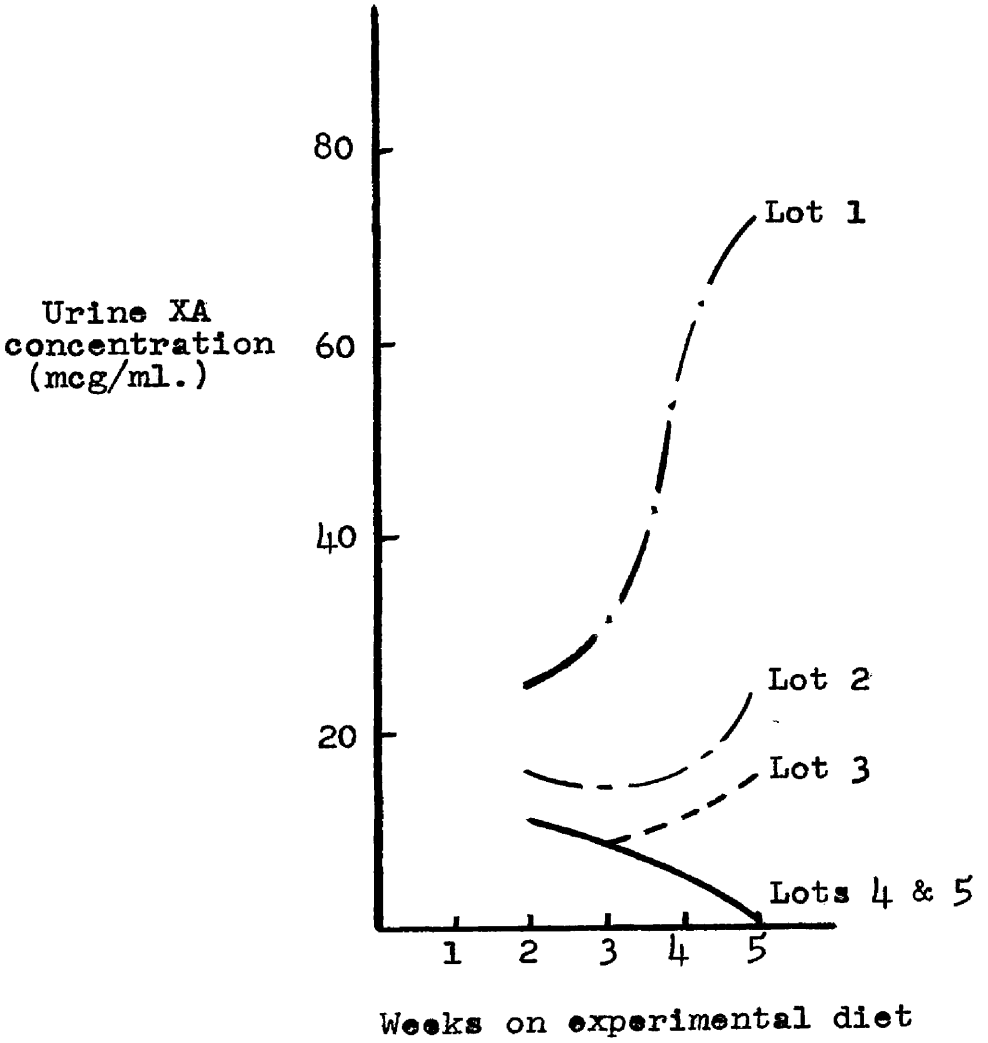


Fig. 20 - Urine xanthurenic acid concentration of baby pigs on different dietary levels of pyridoxine.

Only pigs in lot 1 exhibited epileptiform convulsions during the course of the experiment. Vomiting with expulsion of copious amounts of a thick yellowish-green fluid was an occasional occurrence in the lot 1 pigs as the deficiency developed. One of the pigs from this lot died on the final day of the experimental feeding period and all other pigs receiving no pyridoxine were weak and emaciated at this time. One of the three remaining pigs was autopsied in this weak condition. Generalized anasarca was evidenced by both these pigs when post mortem examinations were made. There was an excessive amount of peritoneal fluid present and both the liver and heart were somewhat fatty.

The remaining two pigs from lot 1 were placed on the positive control diet and one of them received a single intraperitoneal injection of 50 mg of pyridoxine. Within a few days the anorexia subsided and normal gains followed in both pigs. By the end of the 24-day recovery period both pigs were making excellent gains. The blood hemoglobin value and RBC count rose to normal within one week in the case of the pig which received the B_6 injection and within two weeks in the case of the pig receiving only the dietary source of pyridoxine. Neither of the pigs had a recurrence of the epileptiform seizures after treatment commenced and the urine xanthurenic acid excretion dropped sharply. The recovery of the pig receiving only the dietary source of

pyridoxine is shown in figures 21 and 22. The pig which received the injection was sacrificed at the end of the 24-day recovery period. Post mortem examination revealed no abnormalities.

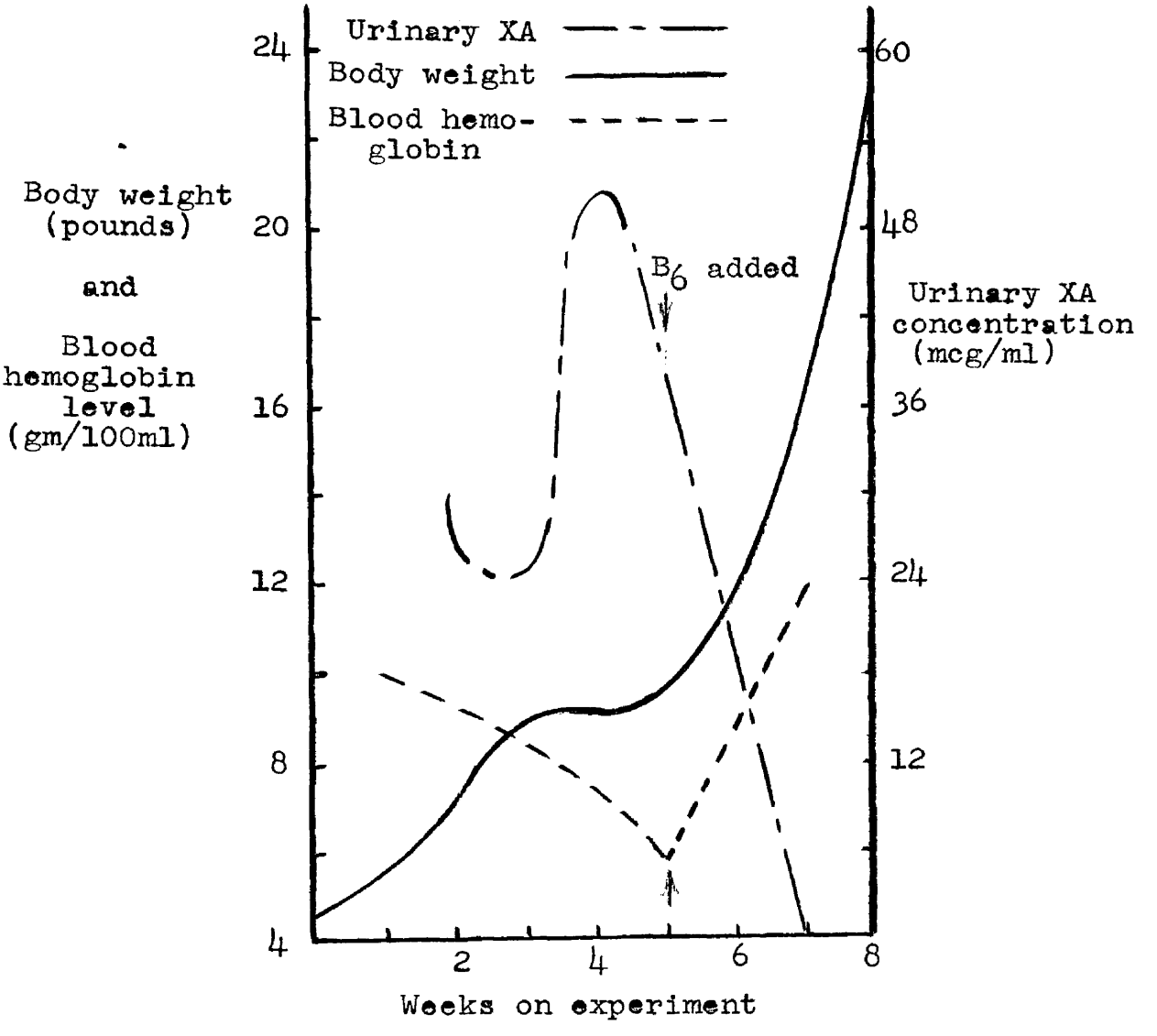


Fig. 21 - Response of negative control pig 44-3 to dietary supplementation of pyridoxine.

Data are presented in table 21 giving a general comparison between pair fed positive and negative dietary pyri-

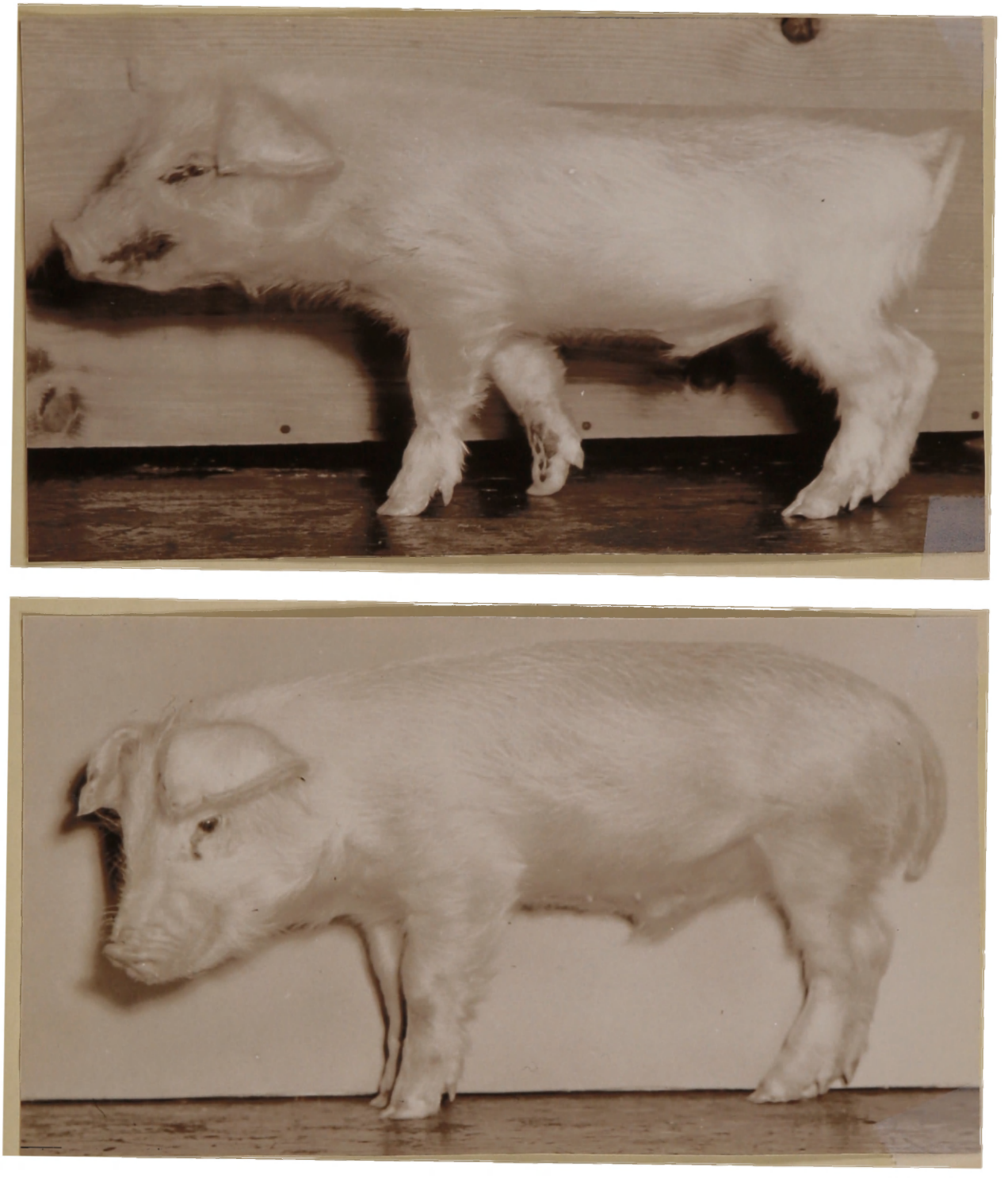


Fig. 22 - Two photographs of negative control pig 44-3. The upper photo was taken at the end of the 32 day experimental feeding period when the pig weighed 9 pounds. The lower photo was taken after 3 weeks of dietary pyridoxine treatment at which time the pig weighed 22 pounds.

doxine control pigs. A statistical analysis of this data was not made because of the small number of animals. However, the data do show that the limited fed pigs receiving pyridoxine gained 50 percent faster and hence 50 percent more efficiently than those pigs receiving no pyridoxine. Furthermore, the pigs receiving pyridoxine showed normal blood hemoglobin level and RBC count indicating again that the oligocythemic microcytic hypochromic anemia which developed in the negative control pigs was a specific effect of pyridoxine deficiency and did not result from the ensuing anorexia.

Table 21 - Comparison of rate of gain, feed efficiency, blood and urine data of positive control pigs pair fed with negative control pigs

	Level of B_6 in diet, in mg/kg solids	
	0	2
Lot number	7	6
Number of pigs	2	2
Days on test	32	32
Ave. initial wt. (lb.)	3.35±.09	3.37±.00
Ave. final wt. (lb.)	7.09±.48	9.13±.64
Ave. daily gain (lb.)	.12±.01	.18±.02
Ave. daily solids consumed (lb.)	.19±.02	.19±.02
Solids per lb. gain (lb.)	1.58±.05	1.03±.02
Blood hemoglobin level (gm/100 ml)[1]	6.4±.1	12.2±.1
Red blood cell counts (million/mm^3)[1]	5.22±.01	6.86±.20
Urine xanthurenic acid concentration (mcg/ml)[2]	37±13	5±5

[1]Blood hemoglobin and RBC count values taken during 5th week of experiment.

[2]XA excretion values taken during 4th week of experiment.

Lymphocyte production appeared to be impaired in the acutely deficient pigs in the pyridoxine trials. This impairment of lymphocyte production was not pronounced until the 4th week of the experiment and occurred only in the negative control group. This is shown in table 22.

Table 22 - Lymphocyte[1] response in baby pigs receiving different dietary levels of pyridoxine[2]

	Level of B_6 in diet, in mg/kg solids						Pair fed[3]
	0.0	0.5	0.75	1.0	2.0	4.0	
No. of pigs	8	3	3	8	8	4	4
1st week	59±3	53±6	59±2	59±3	63±5	58±5	55±1
2nd week	54±4	62±9	57±10	58±3	52±5	47±1	57±3
3rd week	52±4	56±8	54±5	56±5	56±5	60±4	57±8
4th week	41±5	57±4	60±2	52±2	55±3	62±5	62±5
5th week	33±6	59±4	63±3	60±5	60±5	64±4	55±4

[1]Lymphocytes expressed as percent of total WBC count.
[2]Trials one and three combined.
[3]Positive control pigs pair fed with negative controls.

Conclusions

From an examination of the results of this study one may reasonably conclude that under the conditions of these trials:

1. The minimum riboflavin requirement of the baby pig approximates 3.0 mg per kilogram of dietary solids intake.

2. The riboflavin deficiency syndrome in the baby pig consists of a rough haircoat, a heavy sebaceous exudate on the skin, decreased appetite, decreased growth rate, lens cataracts, cecal and colon mucinous degeneration and rectal hemorrhage.

3. Riboflavin deficiency symptoms in the baby pig may be rapidly alleviated by riboflavin supplementation.

4. The minimum thiamine requirement of the baby pig approximates 1.5 mg per kilogram of dietary solids intake.

5. The thiamine deficiency syndrome in the baby pig consists of severe anorexia, vomiting, emaciation, loss of weight, cyanosis of nose and skin and sudden death. Common gross finding are a pale yellowish-gray mottled heart with flabby ventricles and a myxedematous appearance, excess of pericardial fluid and inflammation of the cecum and colon. Microscopic examination reveals necrosis of the cardiac muscle fibers, fatty degeneration in the heart tissue and

mucoid degeneration of the cecum and colon. Electrocardiographic studies reveal bradycardia, sinus arrhythmia, auriculoventricular block and lengthening of the PR and the QRST intervals. Blood thiamine levels are low.

6. Good gaining ability and general well-being may be rapidly restored to thiamine deficient pigs which receive thiamine therapy.

7. The minimum pyridoxine requirement of the baby pig approximates 1.0 mg per kilogram of dietary solids intake.

8. The pyridoxine deficiency syndrome in the baby pig consists of anorexia, reduced growth rate, vomiting and epileptiform seizures. The most common gross autopsy finding is a generalized anasarca. Blood studies reveal a reduced hemoglobin level, microcytosis, anisocytosis, oligocythemia and decreased lymphocyte production. Urine analysis reveals a greatly increased level of xanthurenic acid excretion.

9. The decreased production of blood hemoglobin, red blood cells and lymphocytes and the impaired utilization of tryptophan are specific effects of pyridoxine deficiency and not due to inanition.

10. Pyridoxine deficiency symptoms are rapidly alleviated by oral administration of pyridoxine.

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