THE EFFECT OF PTEROYLGLUTAMIC ACID, VITAMIN B-12, AND RELATED COMPOUNDS ON *ASCARIDIA GALLI* INFECTIONS IN CHICKS

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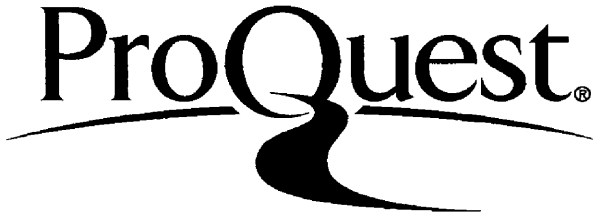

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I. INTRODUCTION

Studies of nutrition in relation to helminthic infections are important in that they provide information on the effect of diet on the epidemiology of parasitic diseases and are a tool for the study of possible mechanisms of resistance to infections. An excellent example in which dietary deficiencies influence the course of infection can be found in the case of hookworm. Hookworm disease is essentially associated with malnutrition (Cruz, 1948). Foster and Cort (1931, 1932, 1935) experimentally demonstrated a definite correlation between undernourishment and susceptibility to Ancylostoma canium, the common dog hookworm. On a very poor diet completely deficient in all vitamins, resistance to this nematode was greatly lowered, the rate of development of the parasite was increased and the egg production of the females also greatly increased. Outbreaks of malaria often accompany or follow impairment of the nutritional level of the population (Hackett, 1937).

Two general methods have been employed to study the nutritional requirements of intestinal helminths; by chemically analyzing the tissues of the parasite for various nutrients and by creating deficiencies in the

diet of the host and ascertaining the effect on the consequent parasitic infection. In studies of the latter type, one must constantly keep in mind that deficiencies in the diet of the host might either influence the resistance of the host to the parasite or might act directly on the parasite.

Ascaridia galli, the common fowl roundworm, is frequently used in nutritional studies. Zimmerman, Vincent and Ackert (1926) first demonstrated that a vitamin deficiency of the host could lower the resistance against A. galli. Since then, Ackert and his co-workers using A. galli made many valuable contributions to this aspect of parasitology.

Physiological and biochemical studies on parasitic helminths are best carried out using in vitro cultivation techniques avoiding possible side effects of the host. Successful in vitro techniques would provide a rational approach to chemotherapy and to knowledge of the metabolism of the parasite which might explain the obligate parasitism of the parasite. The cultivation of any living organism is dependent upon a knowledge of the nutritional requirements of the organism and also a knowledge of the optimal environmental conditions. At present, however, very little is known of the nutrient and optimal environmental

requirements of parasitic helminths and consequently, no true nematode parasite has been successfully cultured *in vitro* through its entire life cycle.

II. LITERATURE

A. Host-Parasite Relations

Ascaridia galli is world-wide in distribution (Ackert, 1931) and in Central United States the incidence can be as high as 49% (Ackert, 1930a). Newer concepts of the morphology and life cycle of A. galli (A. lineata and A. perspicillum as it was then called) were elucidated by Ackert (1931). In natural infections, one cell stage eggs are passed from the female, expelled to the outside in the excreta of the host and, if fertilized, develop to the infective stage in the open without hatching from the shell. The embryonated egg is ingested by the chicken. According to Ackert (1931), larvae 10 to 17 days after hatching in the duodenum of the host, frequently penetrate the duodenal mucosa in young chicks, but after the 17th day, all the larvae are found in the lumen. However, in a more recent report (Ackert and Tugwell, 1948) it was shown that the larvae may penetrate the mucosa as early as the 3rd day of the infection and may remain in it up to the 24th day. The developing larvae undergo three molts and in one month old chicks, maturation of the larvae is completed by the 50th day. Males attain an average length of 63 mm. while the female averages 88 mm.

It would seem that in the period beginning with the 10th day after infection, tissue penetration and clinical symptoms should be the greatest. Beginning with the 10th day, Herrick (1924) reported a loss of appetite, drooping wings, ruffled feathers, general weakness, and loss of weight. The blood sugar level decreases (Ackert and Titus, 1924), and there occurs excessive deposition of urates in the ureters (Ackert, 1930). In the latter report, a marked atrophy of the thymus gland was described, but earlier (Ackert and Morris, 1929) removed the thymus and found that thymectomy had no effect on the resistance of the host to A. galli.

Sadun, et al., (1950) showed that infected chicks developed splenomegaly and hepatomegaly in addition to the characteristic thymic atrophy. Histopathological observations (Sadun, 1950) indicate both intestinal and renal damage occurring 10 days after infection with almost complete regeneration of the intestinal mucosa by the 20th day, whereas the renal condition still persisted. In addition, a severe leucocytosis (eosinophilia) was observed.

Boyd (1947) defined immunity as the degree of resistance to a disease. This resistance can be natural (innate) or acquired. Acquired immunity can be of two types, active acquired immunity in which the host makes its own antibodies,

and passive acquired immunity in which the antibodies are furnished to the host either artificially or congenitally. Since the amount of immunity acquired following infection is generally related to the amount of tissue penetration, and A. galli has a minimum of tissue invasion, it is not surprising to find little acquired immunity. Ackert and Jones (1928) report that resistance is not significantly affected by previous parasitism. Only slight immunity was developed by chicks after vaccination with A. galli antigens (Eisenbrandt and Ackert, 1940). The same authors found that measurable antibodies were seldom produced. Sadun (1949), using the precipitin test, could not demonstrate antibodies against the worm antigens, but was able to show in vitro precipitation of metabolic products in the oral region of the larvae indicative of the presence of an antibody. Sadun reported demonstrating both active and passive acquired immunity (Sadun, 1948, 1949).

Natural immunity, on the other hand, appears to be more important in protecting the chicken against A. galli infections. Genetic constitution of the host, age, and diet all play an important role in natural immunity (Chandler, 1932). Ackert, et al., (1935) showed that heavy breeds or heavier strains within breeds usually

have more resistance than lighter ones. Older chickens are more resistant than young birds indicating age resistance (Ackert, 1935). In 1926, Herrick found that in birds infected at 5 days of age, the larvae increased their length by 5.3 mm. in a 10 day period whereas in birds infected at 103 days of age, the larvae increased only 0.1 mm. in length in a 10 day period. In Herrick's study maximum resistance appeared to occur at the 103rd day, however according to a later report, maximum resistance appeared at the 93rd day (Ackert, et al., 1932).

Many theories have been postulated to explain the phenomena of age resistance for Ascaridia galli. The most plausible one is the Goblet Cell Theory (Ackert, et al., 1939). It was first noted that the older and more resistant fowls had more duodenal goblet cells than younger birds and that the increase in goblet cells, with a concomitant increase in the secretion of mucus, up to 124 days of age closely corresponded to the manifestation of age resistance. It was then found that the duodenal mucus contained a factor inhibitory to A. galli. Later studies (Ackert and Frick, 1940) indicate that the inhibitor is thermostable, and therefore could not be an antigen-antibody relationship since proteins are not thermostable. The inhibitor is water soluble

and only temporary in action appearing to be nutritional in nature (Ackert and Frick, 1947). Chicken serum has also been shown to contain a growth inhibiting substance which increases with age (Carrel and Ebeling, 1921) and which may explain observations that bleeding could lower the resistance of the chickens to the worm (Porter and Ackert, 1933).

In addition to the increase with age in the number of goblet cells and concentration of serum inhibitory factor, it was noted that maximum resistance appeared at about the same time as the development of sexual maturity of the chicken. Accordingly, diethylstilbestrol was injected into young female chickens and found to increase resistance against the nematode (Ackert and Dewhirst, 1950). Sadun (1951), using alpha estradiol for immature females and testosterone propionate for immature males, found that moderate doses of homologous sex hormones also increased resistance, thus confirming Ackert's earlier observation. Sadun suggested that the sex hormones might act by increasing the production or release of specific antibodies.

Nutrition is very important in maintenance of natural or acquired resistance. Of the various dietary factors,

vitamins appear to be involved to the greatest extent, and consequently the next section will be a discussion of the vitamins used in the studies reported in this thesis.

B. Vitamins Used and Their Inter-relationships:

In the years that followed the observation that liver contained an anti-pernicious anemia (APA) factor (Minot and Murphy, 1926), nutritionists, clinicians, and biochemists have studied liver preparations trying to characterize the APA factor. These studies eventually contributed to the isolation and identification of pyridoxine (Elvehjem and Koehn, 1935), pteroylglutamic acid (Stokstad, 1941), and more recently vitamin B-12 (Rickes, et al., 1948).

Pteroylglutamic acid, or PGA, is the most important member of a complex of several chemically different forms comprising the folic acid family. The folic acid complex, found in highest concentrations in green leaves, liver, and yeast, was characterized by adsorption on charcoal and elution by aqueous or alcoholic ammonia, (Robinson, 1951). Members of the complex were biologically active for Streptococcus faecalis and were capable of curing macrocytic anemia, leukopenia and granulocytopenia in chicks and monkeys fed a purified diet (Eddy, 1949). In addition, a deficiency of PGA produced poor feathering and growth (Hogan and Parrott, 1939). Campbell, et al., (1945) reported in PGA deficient chicks a mortality of 50% in a four week period.

At one time there were thought to be at least seven different compounds in the folic acid complex, but recent evidence has shown that there are only three compounds, PGA and two conjugated derivatives of PGA. In the literature, the term folic acid is used synonymously with PGA. Pteroylglutamic acid (liver L. casei factor, vitamin B_c), consists of a pterin group attached through para-amino benzoic acid to glutamic acid (Eddy, 1949). Pteroyltriglutamic acid (yeast fermentation L. casei factor), as the name would imply, contains three glutamic acid residues while the conjugate containing seven glutamic acid residues is termed pteroylheptaglutamic acid (yeast vitamin B_c conjugate). Jukes (1952) included in the folic acid family leucovorin (folinic acid or citrovorum factor) which is a reduced and formylated derivative of PGA and is chemically described by 5 formyl - 5, 6, 7, 8 tetrahydropteroylglutamic acid.

Substitution of the hydroxyl group in the 4 position in PGA with an amino group resulted in a compound possessing growth inhibiting activity (Seeger, et al., 1947). This inhibitor, 4-amino pteroylglutamic acid or aminopterin was antagonistic for PGA in both rats and chicks and the inhibition appeared to be not strictly competitive in nature (Oleson, et al., 1948). Under certain conditions

PGA and leucovorin could prevent the toxic effects of aminopterin with leucovorin appearing to be more effective (Jukes, 1952).

Broquist, et al., (1952) found leucovorin to be 50% as effective as PGA on a weight basis in growth and hematopoietic responses. In the metabolism of PGA in animal tissues, a leucovorin-like compound was produced and aminopterin blocked the formation or liberation of the leucovorin-like compound (Nichol and Welch, 1950a). The results of Skipper, et al., (1950) indicate that aminopterin inhibited the incorporation of C^{14} from formate into nucleic acids and nucleic acid purines.

In addition to the hematopoietic activity of PGA in animals, PGA also maintained hematopoiesis in humans and was related to vitamin B-12. In the middle 1930s Wills observed that megaloblastic anemia of pregnancy was due to a lack of a factor now known as PGA and differentiated it from the APA fraction present in concentrated liver extracts which is now known as vitamin B-12 (Blanck and Wooster, Jr., 1949).

The pathological condition of pernicious anemia is characterized by lesions in the bone marrow and in the central nervous system. Arrested development at the megaloblastic stage results in a decrease in the number

of formed elements in the blood. Both PGA and vitamin B-12 help the blood picture, but only vitamin B-12 ameliorated the degenerative changes in the central nervous system (Jukes, 1952). Castle (1929) explained the etiology of pernicious anemia on the basis of an extrinsic factor and an intrinsic factor both of which were necessary to prevent pernicious anemia. Ternberg and Eakin (1949) reported that the gastric mucosa in the pernicious anemia patient contained in minimal amounts a protein, apoerythein, which normally combined with vitamin B-12 to produce a complex called erythein. The vitamin B-12, tightly bound to apoerythein, is more readily absorbed or is protected from microorganisms and is subsequently absorbed. Thus, apoerythein is the intrinsic factor while vitamin B-12 is the extrinsic factor.

One of the prime functions of vitamin B-12 is the liberation of PGA (Robinson, 1951). Callender and Lajtha (1951) were of the opinion that the hemopoietic factor formed from the interaction of the extrinsic and intrinsic factors (erythein) counteracted the action of an inhibitor which prevented the action of PGA and the citrovorum factor in the production of red blood cells. Thus PGA and vitamin B-12 were not biologically equivalent.

The administration of vitamin B-12 to PGA deficient chicks increased the growth rate but had no effect on feathering (Nichol, et al., 1949). Ascorbic acid enhanced the growth promotion activity of vitamin B-12 and both vitamin C and vitamin B-12 stimulated the synthesis of PGA *in vivo* as shown by higher liver PGA levels (Dietrich, et al., 1949). Likewise, PGA stimulated the *in vivo* synthesis of vitamin B-12. The response of chicks to PGA was influenced by the type of carbohydrate present in the diet. Dextrin or maize meal produced better responses than glucose or starch (Luckey, et al., 1946). A high protein diet resulted in good response to PGA. Although PGA was synthesized by the bacterial flora in the intestine of the chick, the chick cannot utilize this PGA (Robertson, et al., 1946).

In addition to its hemopoietic activity, PGA is concerned in the production of formate from glycine and the subsequent union of formate with glycine to produce serine, the methylation of aminoethanol, homocystine and the pyrimidine ring to produce respectively choline, methionine and thymine, and the introduction of the 2 - and 8 - carbon atoms into the purine ring (Shive, 1951; Broquist, 1952).

Vitamin B-12, in addition to antianemic properties, is also involved in transmethylation reactions. Shaefer, et al., (1949) observed that vitamin B-12 decreased the choline requirement of the chick while Jukes, et al., (1950) found that vitamin B-12 was required by the chick for the methylation of homocystine to methionine and in the presence of methionine and choline, vitamin B-12 was still essential for growth and survival of chicks. Vitamin B-12 deficient chicks on a purified diet did not exhibit a growth response to homocystine but the addition of vitamin B-12, betaine or choline increased the response to homocystine (Jukes and Stokstad, 1952).

PGA did not increase the growth response to homocystine, but the growth response to homocystine plus choline was increased by either PGA or vitamin B-12 (Jukes and Stokstad, 1951). The interrelationship of PGA to methionine was further shown by Dinning, et al., (1951) who found that a PGA deficiency in chicks depressed the formation of methionine from homocystine by liver homogenates plus choline or betaine.

In addition to the above amino acids, vitamin B-12 was reported to be involved in the metabolism of glycine (Menge and Combs, 1950) and Charkey, et al., (1950) found that vitamin B-12 appeared to enhance utilization of

circulating amino acids for building fixed body tissues. In rats on a soybean ration, vitamin B-12 maintained thymus weights when thyroid was included in the ration (Pentz, et al., 1950).

For many years poultry nutritionists had known that protein from animal sources such as liver meal, condensed fish solubles, fish meal, meat scraps and milk by-products contained one or more factors which increased the growth rate of chicks maintained on an all vegetable protein diet (Ewing, 1951). This animal protein factor (APF) appeared to be similar to factor X previously described by Cary, et al., (1946) which was required for growth of rats, and also similar to the unknown growth factor required by chicks occurring in cow manure (Rubin and Bird, 1946). When crystalline vitamin B-12 became available, it was shown that vitamin B-12 had APF activity in the chick (Ott, et al., 1948).

The discovery that vitamin B-12 was formed during the fermentation production of the antibiotic aureomycin led to the use of products from aureomycin fermentation as commercial sources of the animal protein factor (Stokstad, 1950). It was then learned that the APF supplements from crude aureomycin fermentation products produced greater growth increments in chicks than had

vitamin B-12 alone thus indicating the presence of other growth factors (Stokstad, et al., 1949; Whitehill, et al., 1950). Subsequently it was found that pure crystalline aureomycin produced the same growth response as that obtained with the crude aureomycin fermentation products (Stokstad and Jukes, 1950). Thus, the animal protein factor was comprised of at least vitamin B-12 and an antibiotic growth factor with fish meal and meat scraps furnishing only the vitamin B-12 (Stokstad, 1950).

In addition to improving the rate of growth in chicks, aureomycin improved feathering, pigmentation and general appearance of chicks (Stokstad, 1950). It was later found that aureomycin and vitamin B-12 had a mutual sparing action on each other but in the absence of vitamin B-12 aureomycin had no growth stimulation properties (Oleson, et al., 1950). In addition it had been shown that aureomycin had to be present in the diet continuously to be effective (Berg, et al., 1950) which would tend to substantiate the belief that aureomycin functions by influencing the intestinal bacterial flora (Stokstad, 1950).

In this section the intimate relationship between PGA and vitamin B-12 has been discussed together with such related compounds as leucovorin, aminopterin, vitamin C,

aureomycin, etc. The results to be presented in this thesis involve the above nutrients and their relationship to the parasite, *Ascaridia galli*.

C. Nutrition and Parasitism

1. Influence of Dietary Deficiencies of Fat Soluble Vitamins.

Ackert, et al., (1927) first showed the natural resistance of chickens to infections of *A. galli* could be lowered by a deficiency in vitamin A. Previous knowledge led to this discovery since it had been noted that although thrifty one month old chicks showed resistance to *A. galli*, a high incidence of this nematode was found in nature. It was thought that perhaps the diet of the chicks or environment lowered the chick's resistance. In 1923, Emmett and Peacock had found that chicks require vitamin A, and since the food of early spring chicks was likely to be deficient in this vitamin, studies were initiated to ascertain the effect of a deficiency of this vitamin on the resistance of the chick to *A. galli*. Chicks fed a vitamin A deficient diet had more and longer parasites than the control chickens receiving an adequate vitamin A diet. Ackert, et al.,(1931) confirmed and extended this preliminary study. The 1927 investigation had in itself confirmed a previous abstract by Zimmerman, et al.,(1926) that a vitamin deficient diet could lower the resistance of chickens to *A. galli*. Miss Zimmerman (working under

Dr. Ackert) used a thiamine deficient diet to lower the chick's resistance.

A vitamin A deficiency of the host was found to lower the resistance to many other helminths. Hiraishi (1927, 1928) found that he could get higher infestation with Ascaris lumbricoides if the pigs were fed on a vitamin A deficient diet. Wright (1935) obtained a similar lowering of resistance against Toxocara canis and Toxascaris leonina, ascarids in dogs. Spindler (1933) found that vitamin A deficiency in rats lowered their resistance to a superinfection of Nippostrongylus muris, while Lawler (1941) showed that vitamin A deficiency interfered with development of both the natural and acquired resistance of rats to Strongyloides ratti. Complete depletion of the liver stores of vitamin A was necessary before resistance was decreased. Studies using Trichinella spiralis in rats were carried out by McCoy (1934) who found lowered resistance appeared several weeks before the appearance of any other signs of avitaminosis. The lowered resistance of the rats applied to both initial and secondary infections. More recently a conflicting report appeared (Larsh and Gilchrist, 1950) in which it was found that a Vitamin A deficient diet had no effect on the natural resistance of rats to Trichinella spiralis. Krakower, et al., (1940)

found that more schistosomes developed in rats on a vitamin A deficient diet than in rats fed a complete diet and explained their observations on the basis that in the rats on the vitamin A deficient diets fewer juvenile worms were killed in the lungs and liver.

Ackert and Spindler (1929) found A. galli infections were established better in vitamin D deficient chicks than chicks fed an adequate diet. It was reported that vitamin D protected the host against A. galli by inhibiting the development of the worm. Vitamin K, as a dietary supplement, protected chicks against Eimeria tenella infections reducing mortality from 70% to 10% (Baldwin, et al., 1941).

2. Influence of Dietary Deficiencies of Water Soluble Vitamins

In 1926, Zimmerman, Vincent, and Ackert reported in a brief abstract that chickens fed a vitamin B deficient diet for three weeks had more numerous worms which were significantly larger than those found in chickens on an adequate diet. They were apparently the first to show that natural resistance of chickens to helminthic infection could be lowered by nutritional deficiencies. A complete report on these studies appeared in 1931 (Ackert and Nolf) confirming the preliminary studies and showed that the lack of vitamin B caused partial paralysis of the muscles of the digestive tract resulting in a retention of a higher percent of worms.

Watt (1944) reported that a deficiency of thiamine and riboflavin had little effect on a primary infection of Nippostrongylus muris in rats. However, in super-infection the lowering of resistance was much greater which might indicate that the deficiencies interfered primarily with antibody formation. In the same year (Krakower, et al., 1944) found that Schistosoma mansoni grew normally in vitamin C deficient guinea pigs, but noticed that abnormal worm egg shells were produced.

More recently, Sadun and his co-workers (1949) have shown that a deficiency of pteroylglutamic acid (PGA) lowered the resistance of chicks to A. galli. The deficiency of PGA adversely affected the chicks, resulting in growth retardation, thymic atrophy, hepatomegaly, anemia and leucopenia. It was suggested that there was an interference with the formation of antibodies caused by the thymic atrophy and leucopenia which resulted in lowered resistance. This experiment showed that A. galli was not adversely affected by the absence of PGA from the host diet since this nematode was not inhibited by the lack of this vitamin in the diet of the host. But this work did not demonstrate whether A. galli required this vitamin in its nutrition.

The highly purified diet used by Sadun, containing 200 micrograms of PGA per 100 grams diet and minimal amounts of vitamin B-12, did not support normal development of A. galli in chicks fed this diet which was claimed to be adequate for good growth of the chickens. A commercial starter mash (which presumably contained vitamin B-12) allowed worms to be more numerous and longer. When liver was added to the purified diet, the worms were longer and more numerous than the worms in chickens fed only the purified diet but smaller and less numerous than the worms

found in those chickens fed the commercial starter mash. It would appear that one or more factors found in natural food stuffs and present in liver is required by the parasite or that these factors interfere with the resistance of the chicken.

Protozoan parasites especially malarial organisms have been studied more extensively than helminths with respect to water soluble vitamin deficiencies in the diet of the host. The following table summarizes the influence of dietary deficiencies of vitamins and protein upon protozoan infections:

A. Deficiency Inhibiting Growth of Parasite (Or Increasing Resistance to the Parasite)

Growth Factor	Parasite	Host	Reference
Thiamine	Eimeria nieschulzi	Rat	Becker & Dilworth, 1941
Riboflavin	E.nieschulzi	Rat	Becker, 1942
Ascorbic Acid	Plasmodium knowlesi	Monkey	McKee & Geiman, 1946
Riboflavin	P.lophurae	Chick	Seeler & Ott, 1944
Pantothenic Acid	P.gallinaceum	Chick	Brackett, et al., 1946
Vitamin B Complex	Trypanosoma equiperdum	Rat	Reiner & Paton, 1932

B. Deficiency Decreasing Resistance to Parasite

Growth Factor	Parasite	Host	Reference
Vitamin B Complex	T.brucei	Pigeon	Saiazzo, 1929
Pantothenic Acid	T.lewisi	Rat	Becker, et al., 1947
Pyridoxine	E.nieschulzi	Rat	Becker & Dilworth, 1941
Folic Acid	P.lophurae	Chick	Seeler & Ott, 1945a
Biotin	T.lewisi	Rat	Caldwell & Györgyi, 1947
Biotin	P.lophurae	Chick	Trager, 1943
Biotin	P.lophurae	Duck	Trager, 1943
Biotin	P.cathemerium	Duck	Trager, 1943
Biotin	P.gallinaceum	Chick	Seeler, et al., 1944
Protein	P.lophurae	Chick	Seeler & Ott, 1945b

Tables A and B, although not complete, do demonstrate the variable effects of different vitamin deficiencies against various parasites in different hosts. It is interesting to note that a deficiency of riboflavin or the deficiency of folic acid or biotin directly or indirectly is of benefit to the parasite. For a more complete discussion of this topic the reader is referred to the reviews by Schneider (1946) and Von Brand (1952).

3. Influence of Other Nutritional Factors

Ackert and Beach (1933) showed that animal protein deficiencies influenced resistance of chicks to parasitism. A control cereal ration having adequate plant protein, vitamins, and minerals supplemented by animal proteins such as meat meal and skim milk produced the most resistant fowls. When the skim milk was deleted from the diet, resistance decreased, and when peanut meal (plant protein) replaced the meat meal, the chickens were least resistant to *A. galli*. This would indicate that skim milk and other animal proteins increased resistance to the parasite. Peanut meal was thought to be a less satisfactory source of amino acids than meat meal which resulted in decreased resistance. Beach and Davis (1925) had previously shown that skim milk provided resistance against *Eimeria tenella* (*E. avium*).

Branson (1944) found that a 14.3% soybean oil meal supplement to an otherwise adequate ration was as effective as meat scrap and powdered skim milk in maintaining resistance against *A. galli* infections. In a more recent study, Ackert and Riedel (1946) found that the addition of skim milk to an adequate diet decreased the number of worms found to 40% the number present in controls fed only an adequate diet. It was stated that a milk supplement

could be used in the control of A. galli. The beneficial action of skim milk could not be explained but Riedel and Ackert (1950) thought it might be due to the ease of digestibility of milk casein or to the high concentrations of lysine and tryptophane present in skim milk. Riedel (1950) however could not demonstrate that lysine increased resistance of chickens to A. galli. No reports concerning tryptophane have appeared but Todd (1951a) using the amino acid, methionine, was unable to demonstrate that methionine had any effect on the resistance against A. galli.

Chickens maintained on a glucose solution administered parenterally were found to harbor fewer and smaller worms than those fed a normal diet (Ackert and Whitlock, 1935). Ackert and his co-workers (1940) continued these studies confirming that worms thrive better in normally fed chickens than those nourished only by glucose injections. It was noted that little or no growth of the worms resulted upon substitution of glucose injections for the normal diet. The fewer and shorter worms were explained by the partial starvation of the host decreasing the amount of food available to the parasite thereby slowing and stopping the growth of the nematodes.

A carbohydrate rich diet generally benefits intestinal parasites whereas a high protein diet is often harmful to

the parasites (Von Brand, 1952). These changes in the host's diet might influence the pH of the intestinal contents and change the bacterial flora (Hegner, 1937). Read (1950) pointed out that a high lactose content in the diet in addition to influencing the pH might influence the oxidation-reduction potential of the intestinal contents, the intestinal emptying time and possible vitamin synthesis by the intestinal bacterial flora.

Donaldson and Otto (1946) demonstrated an interference with development of acquired immunity on a protein deficient diet in rats infected with Nippostrongylus muris. When a diet of whole milk was fed to rats, Porter (1935) showed that more adults of N. muris developed than in rats fed on regular stock ration. Since an anemia was also present in rats fed only whole milk, it was concluded that a deficiency of iron lowered the resistance. Ackert and Gaafar (1949) found that a deficiency of phosphorus limited A. galli infections in chicks. The following year, Gaafar and Ackert (1950) found that a deficiency of calcium also limited A. galli infections whereas a manganese deficiency had no effect on the infection. In infections of Heterakis gallinae in chicks, a deficiency of calcium and phosphorus was found to favor the development of H. gallinae (Clapham, 1934). Harwood and Luttermoser (1938) found that a manganese

deficiency lowered the resistance of chicks to Railletina cesticillus infections.

At the present time, most commercial chick rations include one or more of the common antibiotics as a growth stimulant. Todd (1951b) studied the effects of penicillin, streptomycin and neomycin upon A. galli infections and found that penicillin had anthelminthic properties. However, Todd did not use pure penicillin but a base containing both penicillin and vitamin B-12 and it is possible that the anthelminthic effect observed was due to the presence of vitamin B-12. Further studies using pure antibiotics must be conducted before the action of penicillin on the parasite could be definitely ascertained.

III. PROBLEMS

The recent studies by Sadun and his co-workers (Sadun, et al., 1950) on the effect of PGA on infections of *Ascaridia galli* in chicks on a purified synthetic diet was the starting point of the studies reported herein. Sadun showed that a deficiency of PGA decreased resistance of chicks resulting in increased numbers of worms which were longer than those in birds receiving PGA in the diet. At the time the above studies were carried out, vitamin B-12 was unavailable and therefore it would seem that Sadun was actually working with a double deficiency of both PGA and vitamin B-12 in his PGA deficient birds. From this, one might assume that his control birds which were given adequate PGA were still deficient in vitamin B-12.

Since then, adequate supplies of crystalline vitamin B-12 have become available and it was deemed highly desirable to initiate studies to elucidate the relationship that may exist between PGA and vitamin B-12 with respect to resistance and pathology of chicks infected with *A. galli*.

The following criteria were employed to study the effect of the vitamins:

a. number and length of worms recovered at necropsy 3 weeks after infection of the birds.

b. mortality rates.

c. weight gain during the 3 week period after infection to the time of necropsy.

d. organ weights relative to body weight including liver, spleen, and thymus.

In view of the fact that newly hatched birds possess body reserves of vitamin B-12 which had been carried over in the egg the complete depletion of vitamin B-12 of chicks on a synthetic diet could not definitely be ascertained. Previously in this thesis it was mentioned that vitamin B-12 was related to protein metabolism and thus, when protein metabolism was accelerated, body reserves of vitamin B-12 would be rapidly expended. Use of thyroid active materials such as iodinated casein (Robinson, 1951) or administration of high levels of protein (Hartman, et al., 1949) would effectively accelerate protein metabolism. Accordingly use was made of a 70% soybean oil meal diet to further study the effect of a deficiency of vitamin B-12.

Closely related to the biological activity of vitamin B-12 and folic acid is leucovorin and ascorbic acid (vitamin C). It is currently believed by some investigators that vitamin B-12 functions in the transformation of PGA to a leucovorin-like compound (Welch and Nichol, 1952). If this is true then the

biological activity of leucovorin should be greater than PGA and equal to the combined activity of PGA and vitamin B-12. Vitamin C on the other hand appeared to be synergistic in nature. Dietrich, et al., (1949) reported that vitamin C enhanced vitamin B-12 activity and both vitamin C and vitamin B-12 stimulated *in vivo* synthesis of PGA. Studies were subsequently initiated as part of this thesis problem to ascertain the relationship of leucovorin and vitamin C to PGA and vitamin B-12 deficiencies in chicks infected with *A. galli*.

The results of many previous investigations indicate a general lowering of resistance to *A. galli* infections when the chicks were placed on deficient diets. The diverse nature of the ingredients depleted from the diet would seem to indicate that the effect of any vitamin on worm infections is not specific to that vitamin but is a generalized response. If this is true then omitting other vitamins as yet untested should also lower the resistance of the chick. Pyridoxine was chosen and the effect of a pyridoxine deficiency was studied using the same criteria as previously employed.

Sadun, et al., (1950) showed that a deficiency of PGA caused rapid atrophy of the thymus gland. In the present work the dramatic effect of PGA on the thymus gland was

used as a basis of comparison to ascertain further the interaction of PGA, vitamin B-12, vitamin C, and leucovorin. In addition, the effect of the parasite on the thymus gland was also studied.

The highly purified synthetic diet used, containing adequate PGA and vitamin B-12, did not support normal development of A. galli in chicks whereas a commercial mash did contain a factor which stimulated the development of the worms. Sadun appreciated the absence of the A. galli stimulation factor but felt that vitamin B-12 might possess A. galli stimulation properties. The results reported in this thesis indicate that vitamin B-12 at the level of 5 micrograms per 100 grams diet does not stimulate the growth of A. galli but actually depressed the growth of the worm. The studies reported herein terminated with several studies designed to ascertain the identity of the A. galli factor present in a natural product ration.

IV. MATERIALS AND METHODS

Day-old, straight run, single comb, white leghorn chicks, used in the studies reported herein, were obtained at seven week intervals from the same approved commercial hatchery to insure uniformity of chicks. Upon arrival in the laboratory, the chicks were banded, weighed, and segregated into experimental groups of uniform weight. The chicks were then housed in an electrically controlled brooder and immediately placed on the various experimental diets with feed and water given ad libitum.

For experimental infection of the chicks, eggs were teased from the uteri of living female *Ascaridia galli* and allowed to develop *in vitro* to the infective stage, incubated at room temperature or at 30°C. Fertilized eggs, incubated in Petri dishes at 30°C containing 30 ml. of 0.5% formalin to prevent mold and fungus growth in the egg cultures, became infective in about sixteen days whereas at room temperature it took approximately twenty-one days. The cultures were aerated daily by removing the cover of the Petri dish and rotating the dish. An alternate method suggested by Riedel (1951) was also used with success. This method consisted of keeping the entire mature female worm in 0.5% formalin until ova were required

at which time the ascarids were ground up in a Waring blendor. The resulting suspension was filtered through several thicknesses of cheesecloth and concentrated by centrifugation.

After embryonation, the eggs were washed with distilled water and then placed for several hours in 0.05N naOH to remove mucus, resulting in a more homogeneous suspension. The eggs were washed again and counts made using a Stoll pipette.

The experiments were designed according to the four pen technique of Roe and Collins (1943). This technique insured adequate experimental controls and utilized the following groups:

I. Complete Control Diet - Uninfected

II. Complete Control Diet - Infected

III. Experimental Diet - Uninfected

IV. Experimental Diet - Infected.

Groups I and II provide information on the severity of the infection and the physiological condition of the chicks. Group III demonstrates the effect of the experimental diet on the normalcy of the chicks and Groups II and IV provide the basic data of the effect of the diet on the infection.

After a preliminary period of two weeks, during which time all body reserves of the vitamin in question were depleted, the chicks were infected with approximately five hundred embryonated eggs administered orally with a pipette. After a period of time (21 days) all the animals were sacrificed and the worms removed using a modified hydraulic flushing system (Ackert and Nolf, 1929). This method consisted of fasting the birds 12-18 hours prior to sacrificing by ether, thereby decreasing the amount of intestinal contents. The small intestine was freed of all mesenteries and cut into one foot strips. The strips of intestine were flushed with hot water under pressure into one quart mason jars. Ten ml. of 10% formalin was added to each jar and the jars stored in the refrigerator until such time as they could be examined. Separation of the worms from intestinal debris was accomplished using Jenner's Stain, the white worms not staining while the intestinal debris stains blue.

Number of worms per animal and the average worm length were used as criteria for ascertaining the effect of various experimental diets on the parasitism. Worm length was measured by a photographic apparatus (Ackert, et al.,1940); the shadow of the worm was magnified 8.5 times and projected on the ground glass of a photographic

bellows. The length of the shadow was traced on onion skin paper and worm length ascertained using a milled tracing wheel (map measure). When the length of very small larvae had to be ascertained, use was made of a camera lucida. In addition to the above criteria, chick body weights and the weights of the liver, spleen, and thymus were recorded. A Hanson dietic scale was used to weigh the chicks; spleen and thymus weights were obtained using a Roller-Smith precision balance while liver weights were obtained by use of a triple beam balance with a sensitivity of .01 grams.

Three types of diets were employed:

a. Purified synthetic diet (Sadun, et al., 1950) to which various factors were added or removed.

b. Natural basal diet low in vitamin B-12 (Miller and Groschke, 1950).

c. Commercial starter mash as a positive control.

The purified synthetic diet (Table I, Appendix) consisted essentially of purified ingredients such as vitamin-free casin, gelatin, L-cystine, cellulose, salts, fish liver oil, lard, all the vitamins known to be required by chicks, and a source of carbohydrate. In the early experiments, corn starch was employed as the carbohydrate source, but the resulting impacted beak condition, which interfered with feed consumption, necessitated use of

Cerelose (trade name for glucose). This change in the nature of the carbohydrate employed had no effect on the nature of the A. galli infections studied (Table IV, Appendix).

The salt mixture (Jones and Foster, 1942) used in the purified synthetic diet was designed for rats and some modification was necessary to adapt it for use with chicks. Analysis of the original salt mixture indicated 0.7581% Ca and 0.4428% P with a Ca/P ratio of 1.712. According to the recommended nutrient allowances (RNA) for 0-8 week-old chicks (Ewing, 1951) there should be 1% Ca, 0.6% P and a Ca/P ratio of 1.67. Consequently, sufficient $Ca_3(PO_4)2$ and K_2HPO_4 were added to the salt mixture to provide the following: 0.99% Ca, 0.59% P and a Ca/P ratio of 1.67. Table II (Appendix) gives the composition of the adjusted salt mixture.

The addition of the two above mentioned salts increased the salt concentration from 5.00% to 5.77%. The concentration of fish liver oil was decreased from 1.3% to 0.53%. Crystalline mineral stable vitamin D_3 and crystalline vitamin A acetate were added to the vitamin mixture to meet the RNA requirements. The vitamin D_3 mixture, spray-dried with carbohydrate, is highly stable by inhibiting contact between the minerals that normally destroy

vitamin D in a few weeks (Bowman Feed Products, Inc. Technical Bulletin MS-D). The preparation of crystalline vitamin A acetate was stablized with gelatin and a sugar.

The concentration of calcium pantothenate, niacin, vitamin K, and biotin was increased whereas the vitamin E concentration was decreased from 24 mgm.% mixed tocopherols to 4.0 mgm.% alpha - tocopherol acetate in accordance with RNA standards. The only other modification consisted in the omission of the lactone of 2 methyl-3 hydroxy-4 carboxy-5 hydroxy methyl pyridine (beta pyracin) from the vitamin mix. This compound, unavailable commercially, was originally thought to possess anti-anemic activity for chicks and accelerated the growth of the first week (Scott, et al., 1944). However, later studies by the same laboratory (Scott, et al., 1946), showed that if PGA were present in the diet at the level of 100 micrograms per hundred grams of diet, pyracin did not have any activity.

The synthetic diet, made up in 50 pound lots, was prepared fresh at approximately ten day intervals. Use was made of a special table with the top covered with tin plating to hand-mix the ingredients of the synthetic diet. The presence of lard in the diet necessitated a special technique to insure adequate mixing of all ingredients. The lard was allowed to soften, and then

worked into most of the casein. To the remainder of the casein, fortified feeding oil and alpha tocopherol were added to form a premix which later was added to the casein-lard mixture. Cellulose and salts were then mixed into the above mixture. Vitamins, manganese sulfate, L-cystine, and choline chloride were added to the gelatin, and after thorough mixing, the gelatin mixture was worked into the bulk of the ration. The addition of Cerelose completed the preparation of the purified synthetic diet. The completed diet generally was divided into five ten-pound lots and various supplements added depending upon the requirements of the experiment. The diet was stored in the refrigerator prior to use, to prevent the development of rancidity which might destroy certain vitamins in the diet.

The natural basal diet consisted of soybean oil meal, ground yellow corn, dehydrated alfalfa meal, oyster shell flour, B-Y feed, fish oil, iodized salt, manganese sulfate and crystalline niacin and choline chloride. The diet, prepared in 100 pound lots, was mixed in a Homart concrete mixer (Sears Roebuck & Co.). All supplements or modifications to the basal diet were made at the expense of corn. This soybean-corn diet was designed to deplete rapidly all body reserves of vitamin B-12 and thus serve for the

assay of vitamin B-12 and products possessing Animal Protein Factor (APF) activity.

Miller and Groschke (1950), using a 50% soybean diet, obtained good depletion of vitamin B-12 in Rhode Island Red Chicks, but in the studies reported herein, 50% soybean did not deplete the vitamin B-12 reserves in white leghorn chicks. A 70% soybean diet (Table III, Appendix) was used and found to be successful without excessive mortality.

Two commercial chick rations, Zinn's Michigan State Chick Starter Mash and Kasco Chick Starter Mash, were employed as positive controls. The exact composition of neither ration was available but both contained natural products such as soybean oil meal, dehydrated alfalfa meal, ground yellow corn, oats, wheat bran and middlings. Salts and vitamins were present in amounts conforming to RNA standards. In addition, both rations contained vitamin B-12 and antibiotic supplements. The Zinn ration contained 350 micrograms vitamin B-12, 200 milligrams penicillin and 900 milligrams aureomycin per 100 pounds feed. The Kasco ration contained 600 micrograms vitamin B-12 and 500 milligrams of an unidentified antibiotic per 100 pounds feed.

V. RESULTS

A. The Effect of PGA, Vitamin B-12 and Related Compounds on Infections of Ascaridia Galli

Chicks on a purified synthetic diet, deficient in both PGA and vitamin B-12, harbored more worms which were longer than those found in birds fed a complete diet. These differences were highly significant when analyzed using the "t" test method (Snedecor, 1946). In the analysis of scientific data, biologists and statisticians, as a matter of convention, designate a "t" value as a significant value when it exceeds the value corresponding to a probability of .05 (Snyder, 1940). A highly significant value is one that exceeds the .01 value (1%) indicating that there is less than 1 chance in a 100 that the difference between the two groups is due to chance or that there is highly significant evidence against the Null hypothesis which postulates that the difference between two means is due to variations within the same general population. The higher the "t" value, the greater the possibility that the Null hypothesis does not hold and that the differences are not due to chance. For all data, with the exception of the rates of mortality, the standard deviation and standard error were calculated for use in determination of the "t" value. The Chi Square

value was used to ascertain significance of mortality differences between two groups (Kendall, 1947).

As was earlier mentioned in this section, a simultaneous deficiency of both PGA and vitamin B-12 resulted in increased worm numbers and increased worm length. A PGA deficiency alone, in all cases, allowed an increase in the number of worms found but had no consistent effect on worm length indicating that PGA influenced the ability of the chick to hold down worm numbers but had no effect on the growth of the worm once the worm had become established in the intestine of the chick. A deficiency of vitamin B-12 alone, on the other hand, had no effect on worm numbers but in all cases permitted an increase in worm length. The above results are summarized in Tables I and II.

The presence of worms in birds previously fed 500 embryonated A. galli eggs had no effect on the rate of mortality, weight gain in a three week period, or on the relative weight* of liver, spleen, and thymus. Likewise the presence of the parasite had no effect on the above criteria in birds on a vitamin B-12 deficient diet.

* Organ weight per 100 grams body weight.

Since no differences were observed between infected and normal birds, the results of all experiments pertain only to infected birds. The only observed difference between normal and infected birds was noted in the thymus of chicks on a 70% soybean oil meal diet. The presence of the parasite decreased the relative weight of the thymus gland. In a later section of results, the effect of various nutrients on the thymus gland will be discussed.

A deficiency of PGA, in all cases, increased the mortality rate, decreased the weight gain, and resulted in hepatic hypertrophy, a decrease in the relative weight of the spleen, and thymic atrophy (Table III). A very extensive atrophy of the thymus gland is one of the prime characteristics of a PGA deficiency. A deficiency of vitamin B-12 had no effect on the mortality rate or on the relative weight of the liver or spleen, but decreased the weight gain and the relative weight of the thymus gland. The Appendix contains several photographs showing 5 week old chicks on various experimental diets.

The addition of vitamin B-12 to a PGA deficiency in chicks had no effect on the mortality rate, weight gain, relative organ weight, and mean worm number (Table IV). A definite highly significant reduction of worm length was noted upon the addition of vitamin B-12 to PGA

deficient chicks which further substantiated the action of vitamin B-12 on worm length.

In order to study further the effect of a vitamin B-12 deficiency, use was made of a 70% soybean oil meal natural product ration. When 500 embryonated eggs were administered to the chicks, the absence of vitamin B-12 in the diet had no effect on worm number, but resulted in increased worm length and decreased weight gain. These results are not unlike those obtained using a purified synthetic diet. When a higher infective dose (2500 embryonated eggs) was administered to the chicks, vitamin B-12 was found to have a definite beneficial action in the reduction of worm numbers as seen in Part A, Table V. The chicks on the basal diet, deficient in vitamin B-12, had a mean worm number of 358 whereas in the presence of vitamin B-12, a mean worm number of 5.7 was obtained.

In non-infected birds a deficiency of vitamin B-12 had no effect on the relative weight of the liver. Likewise, the parasite in the presence of vitamin B-12 had no effect on the liver. However, a vitamin B-12 deficiency combined with an infection of Ascaridia galli produced definite hepatic hypertrophy. On the other hand, thymic atrophy occurred in the absence of vitamin B-12, and also in the presence of the parasite. Greatest thymic atrophy

was observed when vitamin B-12 deficient birds were infected with A. galli. The following relative thymic weights taken from Part B of Table V indicate the great extent of thymic atrophy:

Infected - no vitamin B-12 _____.186

Infected - presence of vitamin B-12 _____.310

Not infected - no vitamin B-12 _____.326

Not infected - presence of vitamin B-12 _____.530

The results of deficiencies of vitamin B-12 and PGA are summarized in Tables I to V.

TABLE I. THE EFFECT OF PGA AND VITAMIN B-12 DEFICIENCIES ON THE AVERAGE NUMBER OF ASCARIDIA GALLI HARBORED BY CHICKS FED A SYNTHETIC DIET

DIET / Exp. No.	1			2			3			5			10		
	Mean Worm No.*	No. Chicks Used Start	End	Mean Worm No.	No. Chicks Used Start	End	Mean Worm No.	No. Chicks Used Start	End	Mean Worm No.	No. Chicks Used Start	End	Mean Worm No.	No. Chicks Used Start	End
Deficient in PGA and vitamin B-12	55	9	1	29.0	14	1	-	-	-	-	-	-	49.7	20	7
Complete Diet	4.6	14	10	1.4	13	13							0.6	14	14
Level of Significance Between Means	**			**									Exceeds 1%		
PGA Deficient Diet	32.3	19	4	19.5	13	2	-	-	-	90.6	27	7	8.2	21	11
Complete Diet	4.6	14	10	1.4	13	13				3.0	16	14	0.6	14	14
Level of Significance Between Means	**			**						Exceeds 1%			Exceeds 1%		
vitamin B-12 Deficient Diet	25.5	15	11	3.6	14	14	2.1	11	11	-	-	-	1.6	18	18
Complete Diet	4.6	14	10	1.4	13	13	1.9	13	13				0.6	14	14
Level of Significance Between Means	Exceeds 5%			None			None						None		

* Average number of worms harbored per surviving chick.

** Insufficient number of surviving birds on deficient diet do not permit statistical analysis.

TABLE II. THE EFFECT OF PGA AND VITAMIN B-12 DEFICIENCIES ON THE AVERAGE LENGTH OF ASCARIDIA GALLI HARBORED BY CHICKS FED A SYNTHETIC DIET

DIET / Exp. No.	1	2	3	5	10
Deficient in PGA and vitamin B-12	16.32*	10.00	-	-	4.47
Complete diet	10.98	4.38			2.97
Level of Significance Between Means	Exceeds 1%	Exceeds 1%			Exceeds 1%
PGA Deficient Diet	8.99	6.45	-	4.33	4.58
Complete Diet	10.98	4.38		3.23	2.97
Level of Significance Between Means	None	Exceeds 5%		None	Exceeds 1%
vitamin B-12 Deficient Diet	14.49	7.96	4.92	-	3.62
Complete Diet	10.98	4.38	3.67		2.97
Level of Significance Between Means	Exceeds 1%	Exceeds 1%	Exceeds 1%		Exceeds 1%

* Length in mm.

TABLE III. EFFECT OF DEFICIENCIES OF PGA AND OF VITAMIN B-12 UPON CHICKS INFECTED WITH ASCARIDIA GALLI*

PGA Deficiency

Criteria	Diet		Level of Significance Between Means
	Deficient	Complete	
% Mortality	66.2	7.4	Exceeds 1%
Weight Gain (2-5 Weeks)	37	151	Exceeds 1%
Weight of Liver**	3.58	3.21	None
Weight of Spleen	.107	.182	None
Weight of Thymus	.122	.480	Exceeds 1%

Vitamin B-12 Deficiency

Criteria	Diet		Level of Significance Between Means
	Deficient	Complete	
% Mortality	6.9	7.4	None
Weight Gain (2-5 Weeks)	127	151	Exceeds 1%
Weight of Liver	3.22	3.21	None
Weight of Spleen	.182	.182	None
Weight of Thymus	.406	.480	Exceeds 5%

* These results represent a composite of several experiments involving 58 vitamin B-12 deficient chicks and 80 PGA deficient chicks.

** Organ weight per 100 grams body weight.

TABLE IV. EFFECT OF VITAMIN B-12 ON A PGA DEFICIENCY IN CHICKS INFECTED WITH ASCARIDIA GALLI*

Criteria	Diet		Level of Significance Between Means
	Basal (No vitamin B-12)	Basal + vitamin B-12**	
% Mortality	63.2	66.2	None
Weight gain (2-5 weeks)	33	37	None
Weight of Liver***	4.13	3.58	None
Weight of Spleen	.072	.107	None
Weight of Thymus	.077	.122	None
Mean Worm Number	44.6	37.7	None
Mean Worm Length in mm.	10.26	6.09	Exceeds 1%

* These results represent a composite of several experiments involving 43 PGA deficient infected birds and 80 PGA deficient infected birds which in addition received vitamin B-12.

** 5 micrograms vitamin B-12 per 100 grams diet.

*** Organ weight per 100 grams body weight.

TABLE V. STUDIES ON A VITAMIN B-12 DEFICIENCY PRODUCED BY A 70% SOYBEAN OIL MEAL RATION

PART A - EFFECT ON ASCARIDIA GALLI INFECTIONS

No. Ova Given per Chick	Diet	Criteria		
		Mean Worm No.*	Mean Worm Length in mm.	Weight Gain (2-5 weeks)
500	BASAL**	4.4	8.38	138
500	BASAL + B-12***	1.8	3.53	207
	Level of Significance Between Means	None	Exceeds 1%	Exceeds 5%
. . . . .				
2500	BASAL	358.0	7.95	122
2500	BASAL + B-12	5.7	3.52	192
	Level of Significance Between Means	Exceeds 1%	Exceeds 1%	Exceeds 5%

* Using 12-15 birds per experimental group.

** 70% soybean oil meal basal diet.

*** 5 micrograms vitamin B-12 per 100 grams diet.

TABLE V. PART B - THE EFFECT OF A VITAMIN B-12 DEFICIENCY AND THE PARASITE ON ORGAN WEIGHTS

Criteria	No. Ova Given per Chick	Diet		Level of Significance Between Means**
		Basal	Basal + B-12	
Liver*	500	4.82	3.95	Exceeds 1%
	0	4.23	4.07	None
	Level of Significance Between Means***	None	None	
Spleen	500	.129	.123	None
	0	.101	.159	None
	Level of Significance Between Means	None	None	
Thymus	500	.186	.310	Exceeds 5%
	0	.326	.530	Exceeds 1%
	Level of Significance Between Means	Exceeds 5%	Exceeds 1%	

* Organ weights per 100 grams body weight.

** To ascertain effect of vitamin B-12.

*** To ascertain effect of the parasite.

In view of the synergistic action exhibited by vitamin C to PGA and vitamin B-12, studies were initiated to ascertain the effect of vitamin C on infections of A. galli. As seen in Table VI, the addition of 100 mgm. percent vitamin C to a PGA deficient diet had no effect on the mortality rate, mean worm number, and mean worm length. It was however noted (Table VII) that whereas in the presence of vitamin B-12, vitamin C had no activity, in the absence of vitamin B-12, vitamin C significantly decreased the mortality rate of the chicks, the mean worm number and worm length and in addition increased the chick weight gain. The addition of PGA and vitamin B-12 to vitamin C (Table VIII) did not further reduce the mortality rate or the worm length, but did significantly decrease the mean worm number.

Previously in this section, evidence was presented which indicated that PGA controlled worm numbers whereas vitamin B-12 controlled worm length. The addition of vitamin C to a double deficiency of PGA and vitamin B-12 significantly decreased both worm numbers and worm lengths. The effect of vitamin C was most pronounced with respect to worm length since the addition of vitamin B-12 and PGA did not further decrease worm length. However the addition of PGA and vitamin B-12 did further decrease worm numbers,

a property apparently controlled by PGA. Thus these results seem to indicate that vitamin C can in part replace vitamin B-12 activity on the parasite.

A similar experiment was designed using leucovorin in place of vitamin C to ascertain whether leucovorin is biologically equivalent to PGA and vitamin B-12 as a means of furnishing additional data to support the concept that leucovorin is the active form of PGA the transformation of which is aided by vitamin B-12 (Physician's Bulletin, 1953). It was found that leucovorin could replace PGA with respect to reduction of worm numbers with vitamin B-12 having no effect on worm numbers. The addition of vitamin B-12 to a basal diet containing PGA significantly reduced worm length, but the addition of vitamin B-12 to a basal diet containing leucovorin in place of PGA did not significantly reduce worm numbers indicating that leucovorin is biologically equivalent to PGA plus vitamin B-12. Additional confirmation will be discussed in a later section of results using the thymus gland as a testing criteria.

The effects of vitamin C and leucovorin are tabulated in Tables VI to IX.

TABLE VI. EFFECT OF VITAMIN C ON A PGA DEFICIENCY IN CHICKS ON A SYNTHETIC DIET INFECTED WITH *ASCARIDIA GALLI*

Exp. Group	Diet	No. Birds Used	% Mortality	Weight Gain (2-5 Weeks)	Mean Worm Number	Mean Worm Length in mm.
5A	BASAL (No PGA)	27	74	25	90.6	4.33
5B	5A + 100 mgm. % vitamin C	19	58	66	30.1	4.45
	Level of Significance Between Means		None	None	None	None
. . . . .						
7B	BASAL (No PGA)	24	67	42	67.5	2.89
7D	7B + 100 mgm. % vitamin C	21	67	81	29.3	2.56
	Level of Significance Between Means		None	None	None	None

TABLE VII. THE EFFECT OF VITAMIN C ON A PGA DEFICIENCY IN THE PRESENCE AND ABSENCE OF VITAMIN B-12

Criteria	Diet: Basal (No PGA)	Diet: Basal + 100 mgm.% vitamin C	Level of Significance
% Mortality			
No B-12	40	8	Exceeds 1%
+ B-12	67	67	None
Weight Gain (2-5 Weeks)			
No B-12	47	99	Exceeds 1%
+ B-12	42	81	None
Mean Worm Number			
No B-12	19.5	3.9	Exceeds 1%
+ B-12	67.5	29.3	None
Mean Worm Length in mm.			
No B-12	4.77	2.56	Exceeds 1%
+ B-12	2.89	2.44	None

TABLE VIII. THE EFFECT OF VITAMIN C IN THE PRESENCE AND ABSENCE OF BOTH PGA AND VITAMIN B-12 IN CHICKS INFECTED WITH *ASCARIDIA GALLI*

Exp. Group	Diet	No. Birds Used	% Mortality	Weight Gain (2-5 Weeks)	Mean Worm Number	Mean Worm Length in mm.
7A	BASAL (No PGA, No B-12)	25	40	47	19.5	4.77
7C	7A + 100 mgm. % vitamin C	24	8	99	3.9	2.56
7E	7C + (200 microgm. PGA and 5 microgm. B-12 per 100 grams diet)	24	0	264	0.4	2.81
	Level of Significance Between 7C and 7E		None	Exceeds 1%	Exceeds 5%	None

TABLE IX. THE EFFECT OF LEUCOVORIN ON A PGA DEFICIENCY IN THE ABSENCE AND PRESENCE OF VITAMIN B-12 IN CHICKS INFECTED WITH ASCARIDIA GALLI

Exp. Group		No. Birds Used	% Mortality	Final Weight	Mean Worm Number	Mean Worm Length in mm.
10A	BASAL (No PGA-No B-12)	20	65	106	49.7	4.47
10F	BASAL + PGA + B-12	14	0	387	.6	2.97
	Level of Significance Between Means		Exceeds 1%	Exceeds 1%	Exceeds 1%	Exceeds 1%
10A	BASAL	20	65	106	49.7	4.47
10C	BASAL + Leucovorin	18	0	349	1.8	3.44
	Level of Significance Between Means		Exceeds 1%	Exceeds 1%	Exceeds 1%	Exceeds 1%
10B	BASAL + PGA	18	0	343	1.6	3.62
10F	BASAL + PGA + B-12	14	0	387	.6	2.97
	Level of Significance Between Means		None	None	None	Exceeds 5%
10C	BASAL + Leucovorin	18	0	349	1.8	3.44
10F	BASAL + PGA + B-12	14	0	387	.6	2.97
	Level of Significance Between Means		None	None	None	None

A pyridoxine deficiency in chicks was used to test a concept that a deficiency of any vitamin could affect the resistance of chicks against infections of A. galli resulting in increased worm numbers and increased worm length. A complete deficiency of pyridoxine resulted in slightly less than a 100% mortality in birds at the end of four weeks and subsequently sub-optimal levels of pyridoxine (50 micrograms per 100 grams diet) were used. Very little mortality was observed at this level of pyridoxine as compared to the RNA level of 400 micrograms per 100 grams diet. However birds on this low pyridoxine diet did not gain weight as rapidly as those birds on the normal diet and also a highly significant increase in worm numbers and worm length was observed in birds fed the low pyridoxine diet (Table X).

TABLE X. EFFECT OF A PYRIDOXINE DEFICIENCY ON INFECTIONS OF ASCARIDIA GALLI IN CHICKS

Exp. Group	Diet	No. Birds Used	% Mortality	Weight Gain (2-5 Weeks)	Mean Worm Number	Mean Worm Length in mm.
3C	BASAL (No Pyridoxine)	25	96	37	(Not infected)	
3D	3C + 400 microgm. Pyridoxine per 100 grams diet	6	0	245	(Not infected)	
5C	BASAL (50 microgm. Pyridoxine per 100 grams diet)	19	5	57	15.6	6.06
5D	5C + 400 microgm. Pyridoxine per 100 grams diet (complete diet)	14	0	194	3.0	3.23
	Level of Significance Between Means of 5C & 5D		None	Exceeds 1%	Exceeds 1%	Exceeds 1%

B. The Effect of Various Nutrients on the Thymus Gland

The dramatic atrophy of the thymus gland in the absence of PGA is an excellent tool for elucidating the interactions of PGA with other compounds. Weekly weights of the thymus gland from 0 to 5 weeks of age were taken and these results are tabulated in Table XI and shown graphically in Graph I (Appendix). A deficiency of vitamin B-12 also significantly decreased thymic weight although to a less dramatic degree than caused by a PGA deficiency. In Section A of results it was mentioned that in addition to a vitamin B-12 induced thymic atrophy, the parasite also induced a thymic atrophy, with the greatest degree of atrophy occurring in infected vitamin B-12 deficient birds. Additional confirmation of these results are tabulated in Table XII and shown graphically in Graph II (Appendix).

The addition of vitamin C to PGA deficient chicks significantly increased the relative weight of the thymus gland but did not approach the level produced by the addition of PGA to the diet (Table XIII). On the other hand, the relative weight of the thymus gland in the presence of leucovorin is almost equal to the weight level of the thymus gland in the presence of both PGA and vitamin B-12 (Table XIV). The weight of the thymus gland in the presence of leucovorin is significantly

greater than the weight produced by PGA alone or by vitamin B-12. This is additional evidence that leucovorin is biologically equivalent to PGA plus vitamin B-12. Graphs III and IV (Appendix) indicate clearly the interactions of PGA, vitamin B-12, vitamin C, and leucovorin with respect to the relative weight of the thymus gland.

TABLE XI. THE EFFECT OF A DIETARY PGA DEFICIENCY UPON THE THYMUS WEIGHT* IN BIRDS RECORDED AT ONE WEEK INTERVALS

	Thymus Weight per 100 grams Body Weight	
Time in Weeks	Absence of PGA	Presence of PGA
0	.156**	.156
1	.107	.228
2	.126	.402
3	.076	.400
4	.079	.494
5	***	.508

* Thymus weight per 100 grams body weight.

** Each value represents the mean of five birds used.

*** No survivors at end of experiment.

TABLE XII. THE EFFECT OF A VITAMIN B-12 DEFICIENCY UPON THE THYMUS GLAND IN NORMAL AND INFECTED BIRDS FED A 70% SOYBEAN DIET

	Thymus Weight per 100 Grams Body Weight			
	Absence of Vitamin B-12		Presence of Vitamin B-12	
Time in Weeks	Normal	Infected	Normal	Infected
0	.186*	-	.186	-
1	.253	-	.286	-
2	.147	-	.254	-
3	.252	.200	.391	.374
4	.312	.170	.412	.286
5	.374	.186	.530	.310

* Each value represents the mean of five birds used with the exception of the values at five weeks which represents the mean of fifteen birds used.

TABLE XIII. EFFECT OF VITAMIN C ON A PGA DEFICIENCY INDUCED THYMIC ATROPHY IN CHICKS INFECTED WITH ASCARIDIA GALLI

Exp. Group	Diet	Thymus Weight*
7B	BASAL (No PGA)	.089
	7B + 100 mgm. % vitamin C	.197
	Level of Significance (Between 7B & 7D)	Exceeds 5%
7D	7B + 100 mgm. % vitamin C	.197
7E	7D + 200 microgm. PGA/100 grams diet	.579
	Level of Significance (Between 7D & 7E)	Exceeds 1%

* Thymus weight per 100 grams body weight.

TABLE XIV. EFFECT OF LEUCOVORIN ON A PGA DEFICIENCY INDUCED THYMIC ATROPHY IN THE PRESENCE AND ABSENCE OF VITAMIN B-12 IN CHICKS INFECTED WITH ASCARIDIA GALLI

Diet	Absence of B-12	Presence of B-12	Level of Significance**
BASAL (No PGA-No B-12)	.069*	.132	Exceeds 1%
BASAL + Leucovorin (400 micrograms per 100 grams diet)	.531	.419	None
Level of Significance Between Means	Exceeds 1%	Exceeds 1%	
BASAL + PGA (200 micrograms per 100 grams diet)	.380	.543	Exceeds 1%
BASAL + Leucovorin	.531	.419	None
Level of Significance Between Means	Exceeds 5%	Exceeds 5%	

. . . . . .

Exp. Group	Diet	Thymus Weight*
10A	BASAL (No PGA - No B-12)	.069
10C	10A + Leucovorin	.531
10F	10A + PGA + B-12	.543
	Level of Significance Between 10C and 10F	None

* Thymus weight per 100 grams body weight.

** To ascertain effect of vitamin B-12.

C. Studies on the Ascaridia Galli Stimulation Factor Present in Natural Product Rations

The existence of a factor present in a commercial starter mash which stimulated the growth of Ascaridia galli is shown in Table XV. Sadun and his co-workers (1950) were of the opinion that vitamin B-12 or the animal protein factor might possess A. galli stimulation properties. The results of this thesis indicate that vitamin B-12 at the level of 5 micrograms per 100 grams diet does not stimulate worm growth but on the contrary depressed worm length. Since the animal protein factor was found to be composed of both vitamin B-12 and aureomycin, the effect of addition of .01% aureomycin to a complete synthetic diet was studied. The level of aureomycin used had no effect on the mean worm number or on the worm length. Negative results were obtained when 100 mgm.% vitamin C and 0.4% Wilson Liver powder 1:20 were added to the diet. Likewise negative results were obtained upon addition of 10% soybean oil meal (experimental groups 6A and 6D) and also upon addition of 5% dried alfalfa (Table XVI).

These preliminary studies indicate that .01% aureomycin, 100 mgm.% vitamin C and 0.4% Wilson Liver powder 1:20, 10% soybean oil meal and 5% dried alfalfa added separately to an otherwise adequate and pure synthetic diet did not possess any A. galli stimulation properties.

TABLE XV. COMPARISON BETWEEN A COMPLETE SYNTHETIC RATION AND A COMMERCIAL RATION

Exp. Group		Weight Gain (2-5 Weeks)	Mean Worm Number per Chick	Average Worm Length in mm.
1E	Starch Synthetic*	160	4.6	10.98
1H	Comm. (Zinn)**	237	5.8	22.90
	Level of Significance Between Means		None	Exceeds 1%
. . . . .				
5D	Cerelose Synthetic*	194	3.0	3.23
5F	Comm. (Zinn)**	120	3.8	9.94
	Level of Significance Between Means		None	Exceeds 1%

* Contains PGA and B-12.

** Contains PGA, B-12, APF and antibiotics.

TABLE XVI. EFFECT OF VARIOUS SUPPLEMENTS TO COMPLETE SYNTHETIC RATIONS UPON WORM NUMBERS AND WORM LENGTH IN CHICKS INFECTED WITH ASCARIDIA GALLI

Exp. Group	Supplements to Diet	No. Birds	Mean Worm Number per Bird	Mean Worm Length in mm.
3D	None	13	1.9	3.67
3E	.01% Aureomycin	11	2.0	3.04
	Level of Significance Between Means		None	None
6A	None	14	3.6	2.11
6B	100 mgm.% vitamin C & .4% Wilson Liver Powder 1:20	15	1.3	2.72
	Level of Significance Between Means		None	None
6A	None	14	3.6	2.11
6D	10% Soybean Oil Meal	15	2.6	2.25
	Level of Significance Between Means		None	None
11A	None	14	3.6	3.25
11B	5% Dried Alfalfa	14	0.5	2.94
	Level of Significance Between Means		None	None

VI. DISCUSSION

A. Interrelationships between PGA, Vitamin B-12, Leuvocorin, and Vitamin C

The results of these investigations provide additional data to substantiate a generalized concept that a deficiency of any vitamin would lower the resistance of chicks against infections of *Ascaridia galli*. A deficiency of vitamin A (Ackert, et al., 1931), vitamin D (Ackert and Spindler, 1929), "the vitamin B complex" (Zimmerman, et al., 1926), and more recently "PGA" (Sadun, et al., 1950) all produced increased worm numbers and increased worm length. The results reported herein add pyridoxine to the above list of vitamins.

Ackert and Beach in 1933 first noted that infected chicks on an all vegetable diet also had less resistance to *A. galli* than birds fed a vegetable diet supplemented with either skim milk or meat meal. Ackert and his students postulated many theories to explain the nature of the factor present in skim milk or in meat meal which stimulated resistance. With the elucidation of the identity of the animal protein factor, it is reasonable to assume that Ackert and Beach were actually working with a deficiency of vitamin B-12.

As had been mentioned earlier in this thesis, Sadun and his co-workers (1950) probably worked with a double deficiency of PGA and vitamin B-12 and found that in accordance with previous investigations, this deficient diet resulted in larger and more numerous worms. The results of this thesis confirm Sadun's work in that a double deficiency of PGA and vitamin B-12 had the same effect on the infection as had been reported by Sadun. However a deficiency of PGA alone had an effect only on the number of worms present whereas a deficiency of vitamin B-12 had an effect only on worm length. At first glance, these results seem out of line with the concept of a vitamin deficiency influencing both worm number and worm length. However, a consideration of the intimate inter-relations between PGA and vitamin B-12, leads to the conclusion that both vitamins were acting conjointly.

The belief that leucovorin is the active form of PGA (Welch and Heinle, 1951), and that vitamin B-12 participates in the formation of leucovorin from PGA in chicks (Dietrich, et al., 1951) seems to explain the observed effects of PGA and vitamin B-12 on the infections of Ascaridia galli. If one considers PGA and vitamin B-12 to be biologically equivalent to leucovorin, it follows that a deficiency of leucovorin would result in increased worm numbers and

increased worm length, an observation demonstrated in this thesis. The results of Nichol and Welch (1950b), who showed that vitamin C augmented an enzyme system present in liver and kidney which converted PGA to leucovorin, tend to explain the observation reported in this thesis that vitamin C appeared to act synergistically with PGA in the absence of vitamin B-12, and in the presence of PGA and vitamin C, the increased formation of leucovorin affected the parasite. The action of vitamin C is not specific inasmuch as it can be replaced by glucoascorbic acid (Nichol and Welch, 1950b).

Other investigations indicate a possibility that vitamin C exerts an important effect on the bacterial flora of rats (Daft, 1951). The results of Waisman, et al., (1951), using aureomycin, seemed to indicate that the bacterial flora might be involved in the formation of leucovorin. Welch and Nichol (1952), offered the following explanation as to the mechanism of aureomycin activity "It would appear that aureomycin, by inhibiting the growth of certain species of microorganisms in the intestine, either limits the destruction of CF (leucovorin), formed microbially in the intestine, or promotes its synthesis, perhaps by permitting appropriate strains of microorganisms to flourish."

Thymus weight studies confirmed the interrelationships existing between PGA, vitamin B-12, vitamin C, and leucovorin in chicks infected with Ascaridia galli. Use of the thymus gland as a criterion provided additional evidence to support the concept that leucovorin is biologically equivalent to PGA and vitamin B-12. Studies on the thymus gland offer the biochemist an excellent tool to ascertain the relationships existing between dietary factors and nucleoprotein synthesis. It is common knowledge that the thymus gland is one of the richest sources of nucleoprotein in the body. Nucleoproteins are composed of proteins linked with nucleic acids. PGA, vitamin B-12, and leucovorin are all involved in nucleic acid synthesis predominantly in the formation of the nucleoside, thymidine which when linked with H_3PO_4 forms a nucleotide, multiples of which form nucleic acids (Physician's Bulletin, 1953). Leucovorin, formed from PGA with the assistance of vitamin B-12 and vitamin C aids in the transformation of uracil to thymine. Vitamin B-12 appears to play its chief role in nucleoprotein metabolism by promoting the formation of thymidine from the carbohydrate desoxyribose and thymine previously formed from uracil (Physician's Bulletin, 1953).

B. Nutritional Requirements of Helminths

At this time the fact should be reemphasized that a vitamin deficiency of the host might either influence the resistance of the host to the parasite or might act directly on the parasite. Although no definite conclusions can as yet be drawn, the lowering of host resistance against the parasite appears to be more logical.

Very little has been actually learned of the vitamin requirements of the parasite itself. It is apparent that absence of the vitamin had no adverse effect on the parasite. Ackert (1930b) stated that the larvae of A. galli did not require vitamins A, B-complex or D during the first third of its growth period. Read (1950) in reference to Ackert's work, concluded that A. galli during the first third of its growth period is independent of the diet of the host as a vitamin source.

The existence of an Ascaridia galli stimulation factor present in a natural product diet appeared to be the first indication that A. galli had a nutritional requirement for a factor present in the diet of the host. Sadun, et al., (1950) reported that a preparation of injectable liver concentrate (Lederle) contained some A. galli growth stimulation properties. Preliminary studies reported in this thesis attempting to elucidate the sources of

this factor in natural products were unsuccessful. Aureomycin, vitamin C, Wilson liver powder 1:20, soybean oil meal, and dried alfalfa all lacked any worm growth stimulation properties. Additional studies should be initiated to test the remaining components of the natural products diet for A. galli growth stimulation properties.

Chandler and his colleagues at the Rice Institute, working with the tapeworm, Hymenolepis diminuta, have contributed much to the knowledge of the nutrient requirements of H. diminuta. Hymenolepis diminuta has been found to require some factor in the vitamin B complex for normal egg production (Hager, 1941). Chandler (1943) and Addis and Chandler (1944, 1946) showed that this species is independent of vitamins A, D, and E for growth but required them for normal establishment. H. diminuta was found to be independent of protein and thiamin in the diet of the host, but was very dependent upon carbohydrates and some other factor for normal establishment and growth present in brewer's yeast, which was not any of the eight members of the vitamin B complex known at that time. This unknown yeast factor was originally thought to be required only by female rats, but Beck (1950) showed that over longer periods of depletion both males and females require it.

Recently, Chandler and his co-workers (1950) using radio-active thiamin demonstrated that H. diminuta obtained its vitamins from the host and not by bacterial synthesis in the intestine of the rat or by its own synthesis. The thiamin content of H. diminuta was found to be constantly independent of the diet of the host and also independent of parenteral injections of thiamin into the host. Following parenteral injection of radio-active thiamin, the specific activity of thiamin in the cestode and in the intestine of the rat was the same showing that the host supplied thiamin to the parasite.

Raillietina cesticillus, a poultry tapeworm, is dependent upon an adequate carbohydrate supply in the diet of the host (Reid, 1942). Following starvation for 24 hours, 94% of the glycogen store of the parasite was utilized and the strobila was lost. It was also found that the glycogen content of the worm was correlated with the normal feeding habits of the chicken. Reid (1945a) found likewise using A. galli that starvation resulted in expulsion of worms and that glycogen stores of the parasite were utilized. Comparisons made between in vivo and in vitro glycogen utilization (Reid 1945b) showed that under both conditions the same amount of glycogen was utilized. These data would validate use

of *in vitro* techniques to study the glycogen metabolism of *R. cesticillus*. At present, very little is known of carbohydrate metabolism in parasitic helminths except that in the case of *A. galli*, glycogen could be synthesized from the Cori ester (Rogers and Lazarus, 1949), and, that likewise, using *A. galli* there is some evidence that the Krebs Cycle is present (Massey and Rogers, 1949).

In this thesis a concept has been maintained that a vitamin deficiency of the host decreased resistance to *A. galli* infections. A vitamin deficiency usually results in decreased weight gains and thus one could postulate that the better the nutrition of the host, the greater the weight of the bird, and consequently the higher the degree of resistance to this parasite. Todd and Hansen (1951) do not believe that host resistance should be defined in terms of action on the parasite, but maintained that resistance of domestic animals be defined in terms of ability of the host to gain weight properly. They showed statistically that the heaviest birds at necropsy harbored the greatest number of worms and the longest worms. These authors were of the opinion that the greater the resistance of the chick against the parasite, the lower the weight gain since the energy employed by the animal to decrease worm numbers and worm length prevented efficient weight gains.

It might be possible to reconcile both viewpoints by a consideration of the type of diet employed. Todd and Hansen used a natural product diet which presumably contained the A. galli stimulation factor. Thus the resistance mechanisms of the chick had to overcome the action of the A. galli stimulation factor in addition to the initial attempts of the parasite to maintain itself in the gut and the parasite's inherent tendency to grow. On the other hand, the purified synthetic diet lacked the A. galli stimulation factor and therefore less energy was expended to prevent maturation of the worms. Thus the concept that the better the nutrition of the bird, the higher the body weight, and consequently the greater the resistance to A. galli, would apply specifically to chicks on the purified synthetic diet.

VII. SUMMARY AND CONCLUSIONS

The results of the studies reported herein on the effect of nutrition on infections of *Ascaridia galli* in chicks substantiate a generalized concept that a vitamin deficiency of the host decreases resistance against the parasite which is manifested by increased worm numbers and worm length. The results also indicate the following conclusions:

I. Effect of PGA, vitamin B-12, and related compounds on infections of Ascaridia galli

a. A simultaneous deficiency of vitamin B-12 and PGA using a highly purified synthetic diet resulted in increased worm numbers and worm length in chicks infected with 500 embryonated eggs of *A. galli*. A single deficiency of PGA only increased worm numbers whereas a vitamin B-12 deficiency alone increased only worm length.

b. The addition of vitamin B-12 to PGA deficient chicks infected with *A. galli* reduced worm length significantly.

c. A deficiency of vitamin B-12 in chicks fed a 70% soybean oil meal diet infected with 500 embryonated *A. galli* eggs produced the same results as had been observed using the purified synthetic diet. When

2500 embryonated eggs were administered vitamin B-12 significantly reduced worm numbers in addition to a reduction of worm length.

d. A simultaneous deficiency of vitamin B-12 and PGA in birds on a synthetic diet increased chick mortality rates, decreased chick weight gains, and resulted in hepatic hypertrophy, a decrease in the relative weight of the spleen, and thymic atrophy. A single deficiency of PGA had the same effect on the chicks as had been reported above for the simultaneous deficiency of vitamin B-12 and PGA. A deficiency of vitamin B-12 alone only decreased the weight gain and the relative weight of the thymus gland.

e. Infected vitamin B-12 deficient chicks suffered from hepatic hypertrophy whereas the parasite in the presence of vitamin B-12 had no effect on the liver, and a deficiency of vitamin B-12 in non-infected birds had no effect on the relative weight of the liver.

f. The addition of 100 mgm.% vitamin C decreased chick mortality rates, mean worm numbers and worm length in chicks deficient in both vitamin B-12 and PGA, but had no effect in the presence of vitamin B-12.

g. Evidence is presented to support the concept that leucovorin is biologically equivalent to vitamin B-12 and PGA and thus a simultaneous deficiency of vitamin B-12 and PGA is in reality a deficiency of leucovorin.

h. A complete pyridoxine deficiency resulted in almost a 100 percent mortality at the end of a four week period. Chicks fed a low pyridoxine diet harbored more numerous worms which were longer than birds fed a complete synthetic diet.

II. The Effect of Various Nutrients on the Thymus Gland

a. A deficiency of PGA resulted in marked thymic atrophy. The addition of vitamin B-12 to PGA deficient chicks had no effect on the thymus gland. However in the presence of PGA, vitamin B-12 significantly increased the relative weight of the thymus gland.

b. In addition to a thymic atrophy caused by a deficiency of vitamin B-12, the parasite also induced a thymic atrophy with the greatest degree of atrophy occurring in infected vitamin B-12 deficient birds.

c. The addition of vitamin C to PGA deficient chicks significantly increased but did not approach the relative weight of the thymus gland, produced by the addition of PGA to the diet.

d. In the presence of leucovorin, the relative weight of the thymus gland was almost equal to the thymus weight in the presence of both PGA and vitamin B-12.

III. Studies on the Ascaridia Galli Stimulation Factor

a. Evidence is presented to confirm the presence of a factor in natural product diets which stimulated the growth of A. galli.

b. Preliminary studies indicate that .01% aureomycin, 100 mgm.% vitamin C and 0.4% Wilson liver powder 1:20, 10% soybean oil meal and 5% dried alfalfa added separately to an otherwise adequate and pure synthetic diet did not possess any A. galli stimulation properties.

APPENDIX

TABLE I - COMPOSITION OF COMPLETE SYNTHETIC DIET

	%
"Vitamin-Free" Casein.	25.00
Gelatin.	10.00
Corn Starch or Cerelose.	51.00
Cellulose (Ruffex)	3.00
L-Cystine.	0.30
Lard	4.00
Salts (See Table II)	5.77
Fortified Feeding Oil (3860A/300D)	0.53
$MnSO_4 \cdot 4H_2O$	0.10
Choline Chloride	0.20
	Mgm. per 100 Grams
Thiamine Chloride.	0.40
Riboflavin	0.80
Pyridoxine HCl	0.60
Calcium Pantothenate	2.00
Niacin	3.00
i-inositol	50.00
PABA	15.00
Menadione (Vitamin K).	0.05
Biotin	0.02
Folic Acid	0.20
Vitamin B-12	0.005
Vitamin D_3-Mineral Stable (200,000 I.C.U/gram)	0.44
Vitamin A Acetate - Stabilized (500,000 USP/gram)	1.76
Alpha Tocopherol Acetate	4.00

TABLE II - SYNTHETIC DIET SALT MIXTURE

	Grams per 100 Grams Diet
$NaCl$	0.696
KH_2PO_4	1.944
K_2HPO_4	0.170
$CaCO_3$	1.907
$Ca_3(PO_4)_2$	0.600
$MgSO_4$	0.286
$FeSO_4 \cdot 7H_2O$	0.135
KI	0.004
$MnSO_4 \cdot 2H_2O$	0.022
$ZnCl_2$	0.0013
$CuSO_4 \cdot 5H_2O$	0.0024
$CoCl_2 \cdot 6H_2O$	0.00012

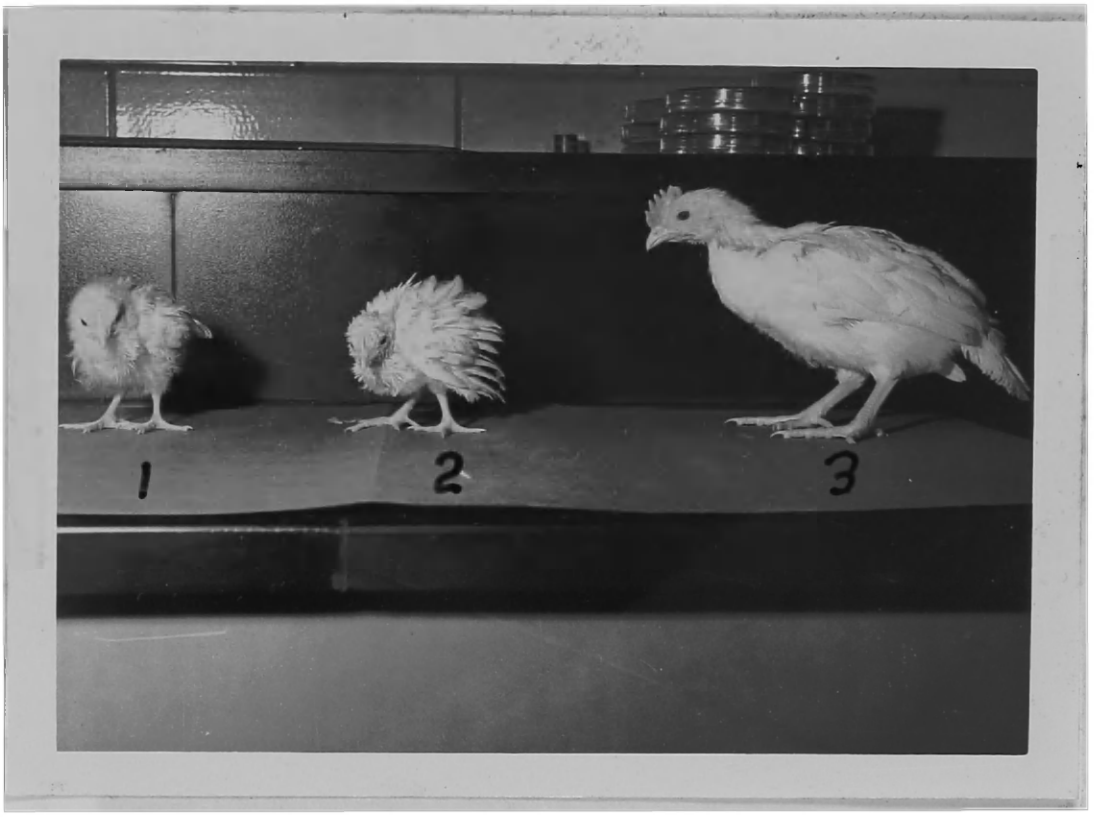
TABLE III - COMPOSITION OF NATURAL BASAL CHICK DIET

	%
Soybean Oil Meal (44% Protein)	70.00
Ground Yellow Corn	19.40
Dehydrated Alfalfa Meal	5.00
Oyster Shell Flour	1.50
B-Y Feed (100 micrograms Riboflavin per gram)	0.30
Fish Oil (3860A/300D)	0.20
Salt (Iodized)	0.50
Choline Chloride	0.10
Niacin	0.005
Manganese Sulfate	0.022

TABLE IV. A COMPARISON OF THE EFFECT OF STARCH AND CERELOSE AS THE SOLE CARBOHYDRATE SOURCE IN THE SYNTHETIC PURIFIED RATION ON CHICKS INFECTED WITH ASCARIDIA GALLI

	Carbohydrate Used		Level of Significance Between Means
	Starch	Cerelose	
No. birds used	19	14	
Criteria			
% Mortality	0	0	None
Weight gain 2-5 weeks	175	194	None
Mean worm number	7.3	3.0	None
Mean worm length in mm.	3.04	3.23	None

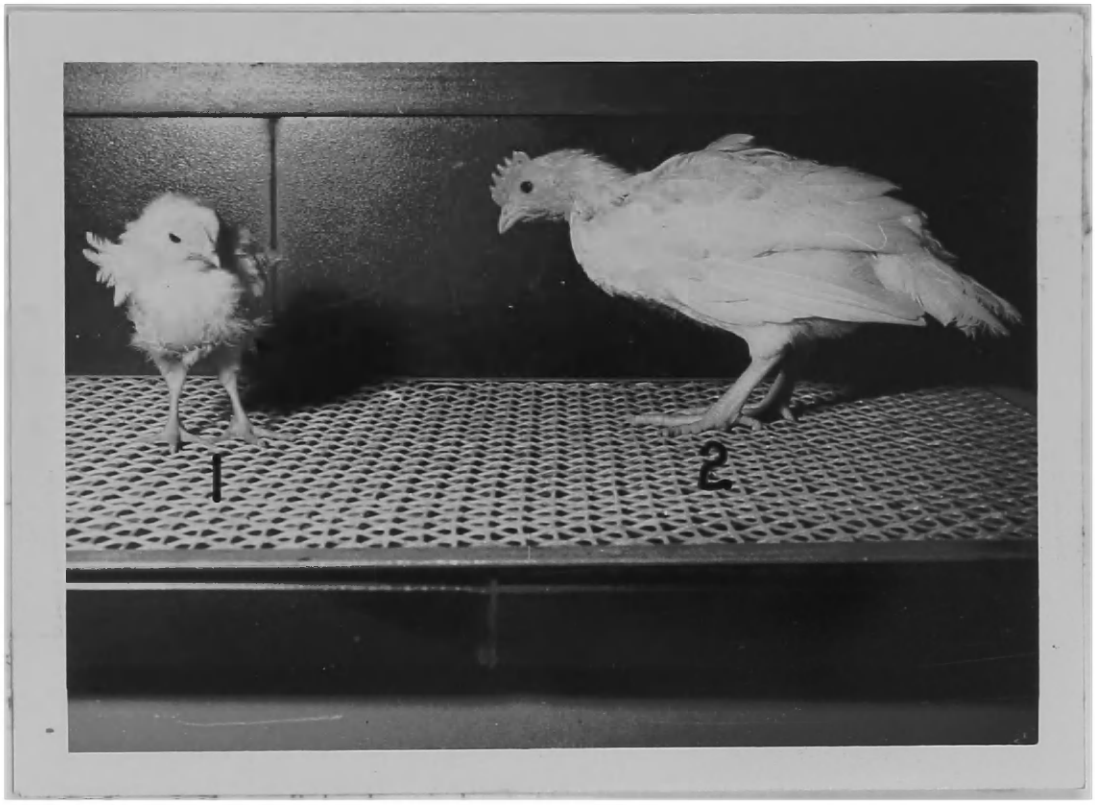
FIGURE I. The Effect of Deficiencies of PGA and Vitamin B-12 on Chicks Infected with Ascaridia Galli.



Bird 1*	Bird 2	Bird 3
Basal Diet (No PGA, no vitamin B-12)	Basal Diet + 5 micrograms vit. B-12 per 100 grams diet	Basal Diet + 5 micrograms vit. B-12 + 200 micrograms PGA

* All birds are five weeks of age.

FIGURE II. The Effect of a PGA Deficiency on Chicks Infected with Ascaridia Galli.



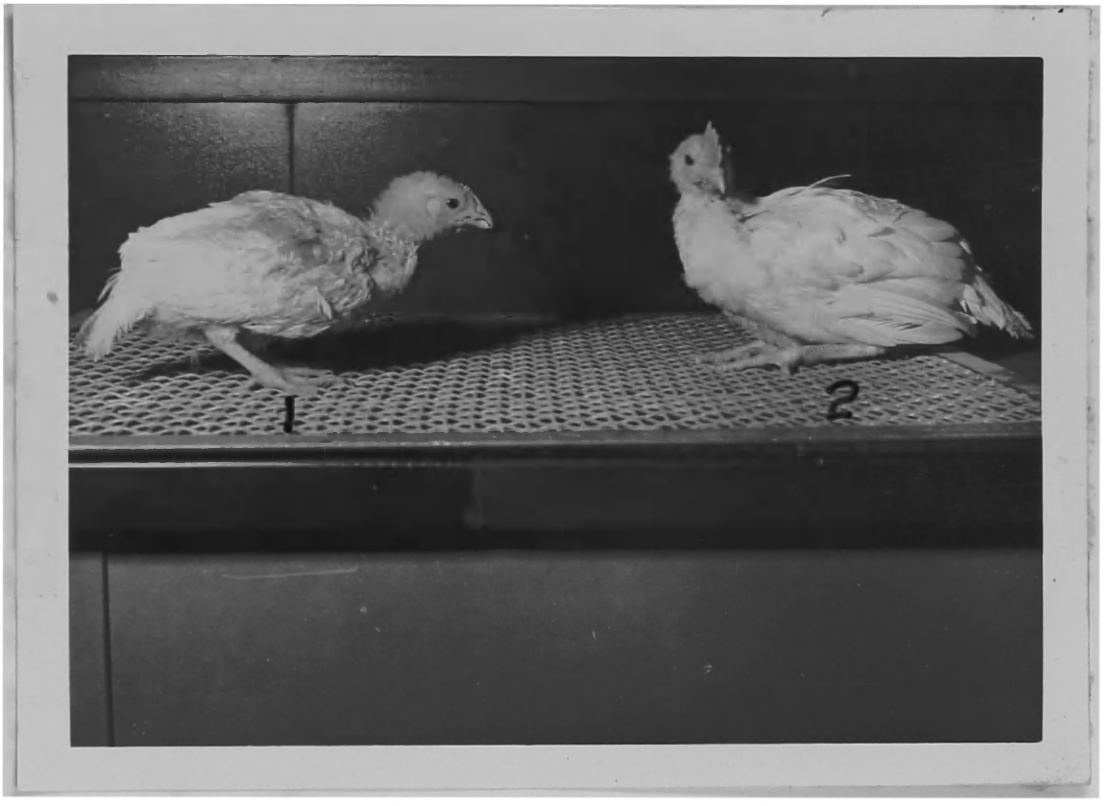
Bird 1

PGA Deficient Diet

Bird 2

Complete Diet

FIGURE III. The Effect of a Vitamin B-12 Deficiency on Chicks Infected with Ascaridia Galli.



Bird 1

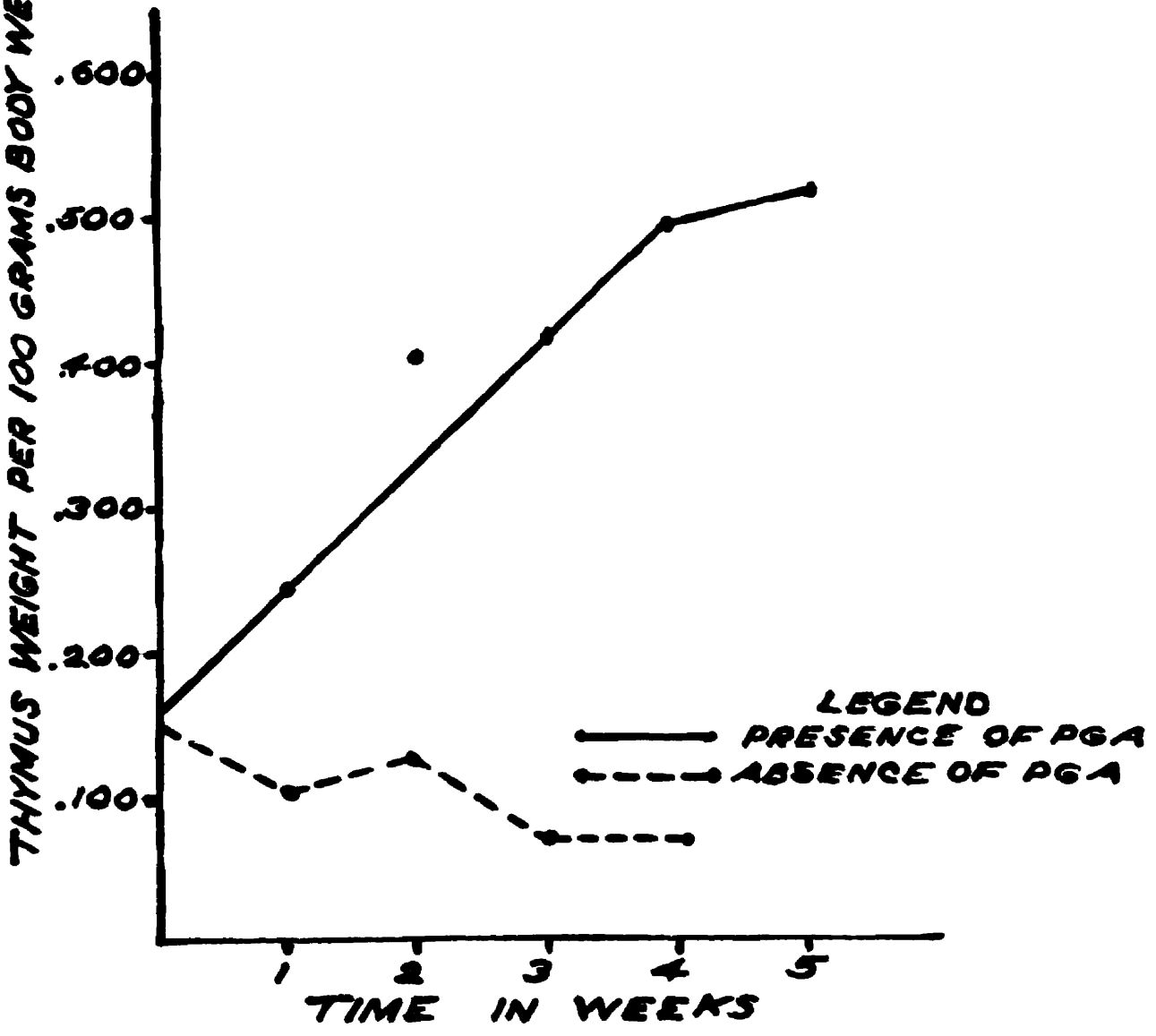
Vitamin B-12 Deficient Diet

Bird 2

Complete Diet

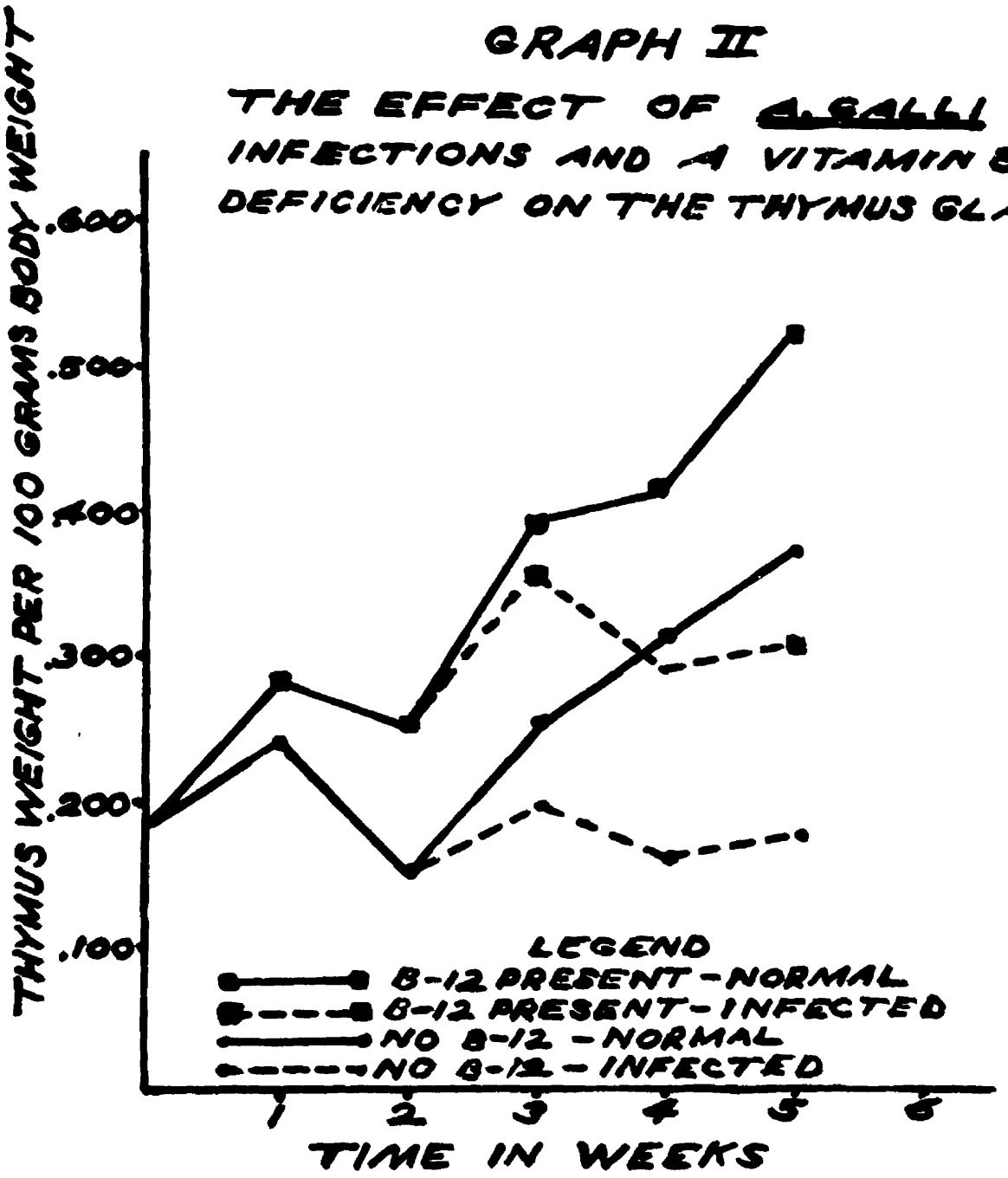
GRAPH I

THE EFFECT OF A PGA DIETARY DEFICIENCY ON THE THYMUS GLAND



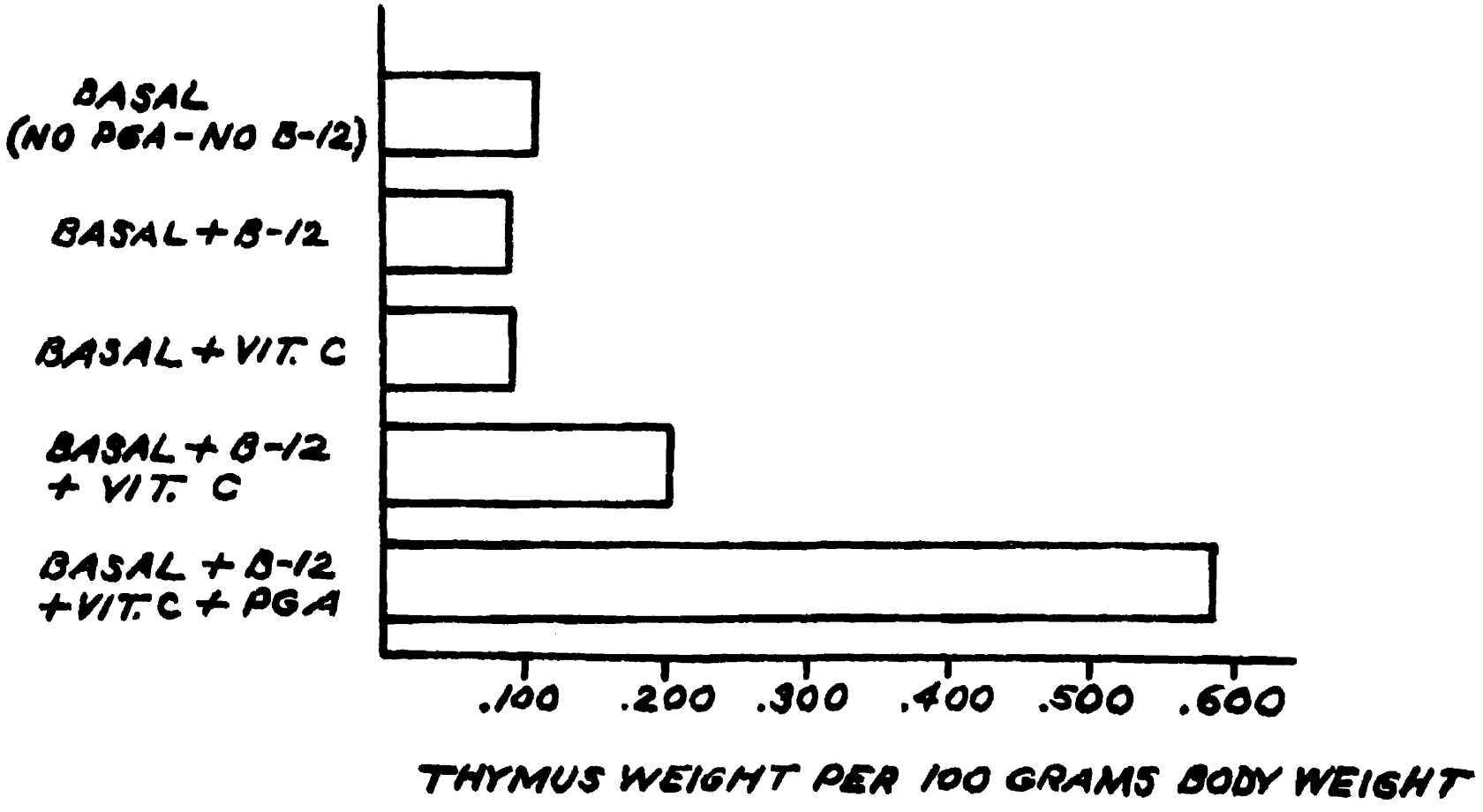
GRAPH II

THE EFFECT OF *A. GALLI* INFECTIONS AND A VITAMIN B-12 DEFICIENCY ON THE THYMUS GLAND

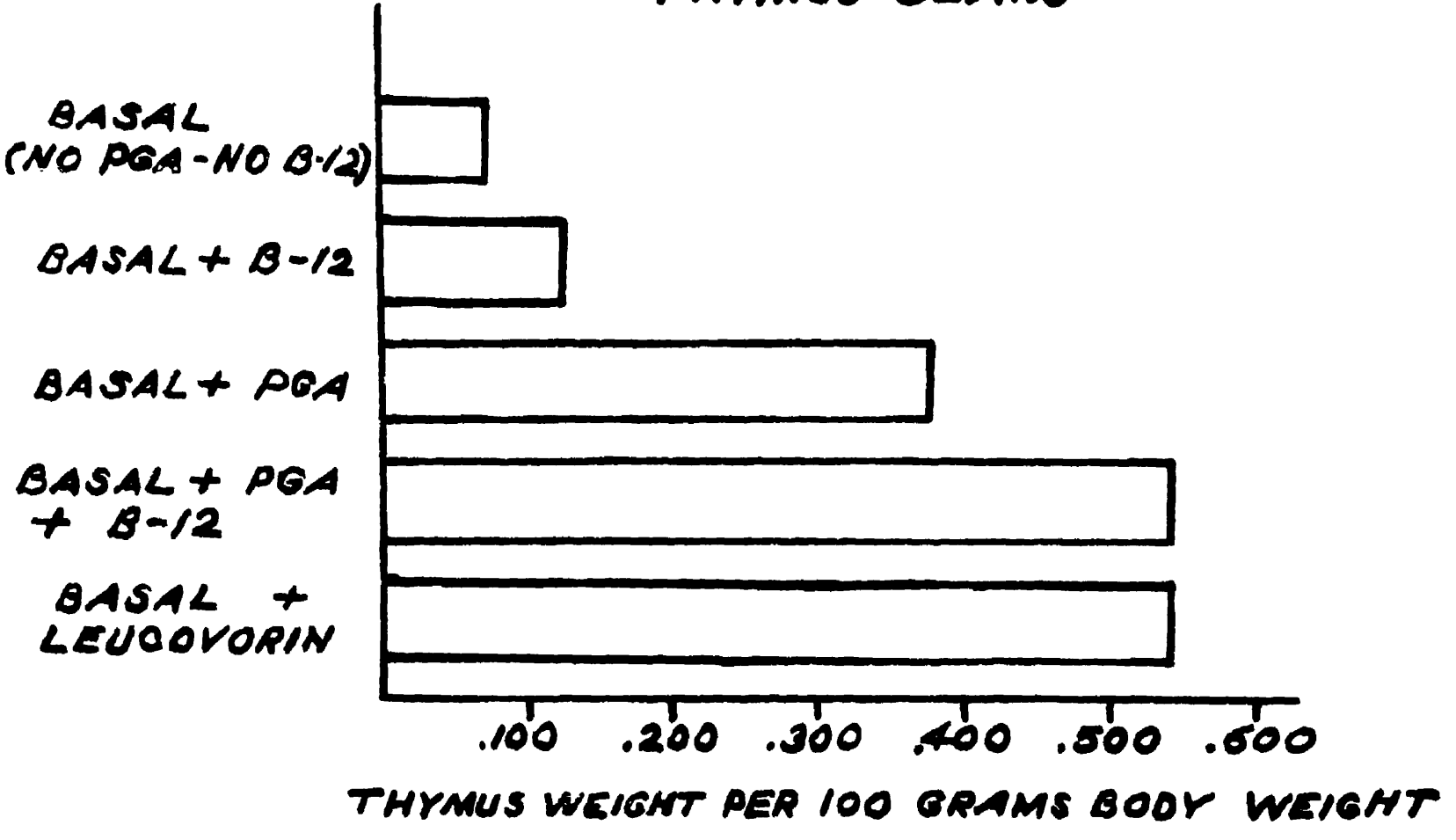


GRAPH III

THE EFFECT OF VITAMIN C ON THE THYMUS GLAND



GRAPH IX
THE EFFECT OF LEUCOVORIN ON THE THYMUS GLAND



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