STUDIES ON THE INTERRELATIONSHIP OF VITAMIN B_{12} WITH METHYLATING COMPOUNDS AND AMINO ACIDS IN CHICK NUTRITION

by

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INTRODUCTION

Since the isolation and crystallization of vitamin B_{12} by Rickes *et al* (1948) and by Smith (1948) extensive studies dealing with this vitamin have been made with humans and certain other animals. In addition to preventing pernicious anemia in humans, vitamin B_{12} has been found to promote growth of young chicks, poults, rats, pigs, dogs and bacteria. Therefore the importance of vitamin B_{12} in practical nutrition has become well established.

To study further the role of vitamin B_{12} as it pertains to poultry nutrition, the experiments reported herein were conducted to determine the interrelationships of vitamin B_{12} and the biological methylating compounds, choline, betaine and methionine. In addition, a study has been made of the role of vitamin B_{12} in amino acid metabolism.

REVIEW OF LITERATURE

GENERAL

Discovery and Isolation of Vitamin B_{12}.

Several years before the isolation of vitamin B_{12}, it was recognized that the inclusion of animal protein supplements in rations for poultry and swine provided an unknown growth promoting substance not supplied by feedstuffs of plant origin. This unknown factor was studied in various laboratories and was given such names as "factor x", the "cow manure factor", "zoopherin" and "animal protein factor".

Cary et al (1946) reported that rats required an unidentified growth factor which they named factor x. Earlier studies with closely related, if not identical, factors had been conducted by other investigators engaged in poultry nutrition.

As early as 1927, Parkhurst reported that normal hatchability of eggs could not be obtained unless breeding rations contained some protein of animal origin. Subsequent studies with poultry led to the postulation of the "animal protein factor".

While the studies with rats and poultry were underway, another approach to the identification of this unidentified factor was made through microbiological studies. Shorb (1947), in an attempt to find a microorganism that would require the rat growth factor described by Cary et al (1946), noted that the organisms, Lactobacillus lactis, former strain, required the two unidentified factors for growth. One of these was a heat-stable factor found in highest concentration in liver extracts reported active for rat growth. Shorb (1947) showed that this factor was present in the refined liver extracts in almost linear relationship to the potency of

those extracts for effecting cures in pernicious anemia. ...e sug-
gested that ... factor might be the active principle ...ernicious
anemia.

Rickes et al, (1948) in this country and Smith et al, (1948) in England simultaneously and independently isolated the biologically active crystalline material from liver concentrates later found to be clinically active in the treatment of pernicious anemia. Shorb (1948), confirmed the activity of this substance, tentatively named vitamin B_{12}, as the factor for Lactobacillus lactis, Dorner strain. West (1948) also demonstrated its ability to stimulate blood regeneration and to be effective in the treatment of the neurological disturbance of patients suffering from pernicious anemia.

Properties and Chemical Composition of Vitamin B_{12}.

Vitamin B_{12} crystallises in the form of small red needles. It contains cobalt and phosphorus (Smith, 1948 and Rickes et al, 1948). The molecular weight by x-ray crystallography is 1550 to 1750. Spectrographic analysis of vitamin B_{12} appears to be a six group cobalt coordination complex. Vitamin B_{12a}, however, does not contain this group. The red color of vitamin B_{12} is apparently associated, at least in part, with its cobalt-complex character. Cobalt has been recognised as a nutritional essential in ruminants in which cobalt deficiency results in a typical anemia. It has been found, however, that injection of vitamin B_{12} does not cure cobalt deficiency in sheep. Nitrogen is also found to be present but sulfur is absent. Microbiological assay has shown that vitamin B_{12} in aqueous solution is stable by autoclaving at 121°C for 15 min. but it is not stable upon standing at room temperature or by autoclaving in dilute

sodium hydroxide or hydrochloric solution (... et al., 1948; Brink et al., 1949; Kaczka et al., 1949; ... Schei ... chweigert, 1950). Vitamin B_{12} also contains PO, NH and OH groups. It is levorotatory. The molecule is not ... peptide, since hydrolysis of the vitamin ... liberate d-amino acids. ... fusion of vitamin B_{12} forms ... ducts ... with p-dimethylaminobenzaldehyde characteristic of certain cyclic five-membered nitrogen containing compounds including pyrroles. Analysis gives ... sition typified by C_{61-64} H_{86-92} N_{14} $O_{13}PCo$ (Brink et al., 1949).

Vitamin B_{12} ... distinguished from the related vitamins of the vitamin B_{12} group solely by the presence of a cyano group, complex-bound to the cobalt atom (Kaczka et al., 1949) (Brink et al., 1950). During the oxidation of vitamin B_{12} in dilute sulfuric acid at 0° with potassium permanganate the characteristic odor of hydrogen cyanide is noted. Hydrogen cyanide is also liberated readily upon heating solution of vitamin B_{12} in hydrochloric acid or aqueous oxalic acid. No cyanide could be detected when pure vitamin B_{12a} is treated with oxalic acid solution (Brink et al., 1950). Treatment with cyanide reconverts vitamin B_{12a} to vitamin B_{12} (Brink et al., 1950; Kaczka et al., 1950; Veer et al., 1950). The extreme lack of toxicity of vitamin B_{12} reported by Minter and Rushett (1950) indicates that cyano group ... tightly bound within the coordination complex. Dose levels of 1,600 mg per kg, both intraperitoneally and intravenously in mice produced no deaths or toxic manifestations.

By means of paper strip chromatography when vitamin B_{12} is autoclaved with thiomalic acid, vitamin B_{12} is transformed into a new compound which is distinct from vitamin B_{12b}. The vitamin B_{12}-thiomalic acid reaction product is more potent microbiologically than vitamin B_{12}.

Vitamin B_{12b} is crystallized from liver [illegible] from Streptomyces aureofaciens. It was reported by Lichtman et al (1949) that vitamin B_{12b} (a pink fraction) has been separated from vitamin B_{12} by silicic acid chromatography and has been shown to have different ultraviolet and visible absorption spectra. Vitamin B_{12b} is found to be equally effective as vitamin B_{12} for rats and for humans suffering from Addisonian pernicious anemia and lower equal activity for the two compounds for the chick and Lactobacillus leichmannii (Stokstad et al, 1950). Vitamin B_{12b} is chemically less stable than vitamin B_{12}. On hydrolysis with hydrochloric acid, vitamin B_{12} yields 5:6-dimethylbenzimimazoles. The fact that riboflavin and vitamin B_{12} can thus be regarded as derived from a common chemical structure; it is possible, therefore, that riboflavin is involved in the formation of vitamin B_{12} in nature. When cyanide is added to vitamin B_{12} or vitamin B_{12b} a purple compound is produced by addition of free hydrogen cyanide. The terms "cyanocobalamin" and "hydroxy-cobalamin" have been proposed for vitamin B_{12} and B_{12b} respectively.

Vitamin B_{12} is converted to vitamin B_{12a} by reduction with hydrogen the presence of a platinum catalyst (Kaeske et al, 1949). Vitamin B_{12a} appeared to have less than half of the biological activity of vitamin B_{12} for rats, chicks, pernicious anemia patients and for bacteria (Lang and Chow, 1950). This loss of biological activity can be attributed to the reducing power of the agents, but is not necessarily related to the disappearance of the intensity of the red color.

Vitamin B_{12} is soluble in water, 80% acetone and in glacial acetic acid, but insoluble [illegible] ether, chloroform or non-polar solvents. (Smith, 1948).

3. Relationship of Vitamin B_{12} to Other Substances (Folic Acid, Thymine, Thymidine, Vitamin D, Unidentified Factors and Antibiotics) and Its Clinical Use.

Thymine has been reported to serve as a substitute for folic acid in the nutrition of lactic acid bacteria (Stokes, 1944) and in the treatment of human macrocytic anemia (Spies et al. 1946). Thymidine, the dexosyriboside of thymine (a pyrimidine compound) is a growth factor for some lactic acid bacteria and for Lactobacillus lactis, Dorner strain. Thymidine is able to replace the requirement for vitamin B_{12} (Wright et al. 1948). (Thymine, however, does not replace vitamin B_{12}). Because of the large amounts of thymidine required to replace vitamin B_{12}, the two are not considered identical. Wright et al. (1948) have interpreted the data to indicate that vitamin B_{12} acts as a coenzyme in the processes involved in the conversion of thymine (produced as a result of catalytic action by folic acid) to thymidine which is needed for the manufacture of nucleic acid for new cells, such as red blood cells and reticulocytes. The report of Popper et al. (1949) also suggested a possible role of vitamin B_{12} in the synthesis of ribonucleic acid in the liver of the rat. On the other hand, Shive et al.(1948) pointed out, conversely, that thymidine may be involved in the synthesis of vitamin B_{12}. Wright et al. (1948) postulated further that the primary biochemical defect in pernicious anemia may well be the inability to synthesize certain nucleosides, particularly, thymidine, from parent purines or pyrimidines. Stokes (1944), therefore, attributes the curative effects of folic acid in some cases of pernicious anemia to increase thymine synthesis, as confirmed by the clinical effectiveness of thymine as a substitute for folic acid therapy (Spies et al. 1946). The increased thymine resulting either from synthesis (due to folic acid) or from actual therapeutic administration, in turn is then believed to yield

more of the essential thymidine. Hypoxanthine deoxyribosides and guanine deoxyriboside [illegible] able to replace vitamin B_{12} as a growth factor for various bacteria (Kitay et al, 1949 and Hoff-Jorgensen, 1949). Still further interrelations among vitamin B_{12}, thymine, thymidine, purine, folic acid are discussed by Shive et al, (1948). They confirm the postulated role of vitamin B_{12} in the biosynthesis of thymine and thymidine. Therefore, as to the relation of these factors to pernicious anemia, folic acid is brought into direct interplay with vitamin B_{12} in the anemia. The key compound is apparently thymidine. Folic acid makes possible increased thymine synthesis. Increased thymine makes possible an increase in the synthesis of thymidine. This latter synthesis further aided by the catalytic action of vitamin B_{12} with the greatly increased synthesis of thymidine, basic material is available for making nucleic acids to be used in the manufacture of new cells to overcome the shortage characteristic of anemia.

When folic acid was given in patients suffering from pernicious anemia some improvement of some of the blood picture occurred but the neuralgic disease was not cured unless liver extract was administered. The basal deficiency, therefore, was that of some other material found in the liver extract. However, Nichol et al, (1949) obtained the result that liver extract alone did not influence the rate of hemoglobin formation. The combination of liver extract and folic acid caused a more rapid regeneration of hemoglobin than treatment with folic acid alone. Pure vitamin B_{12} completely replaced liver extract in stimulating the formation of hemoglobin in the presence of folic acid. Thus it appears that the component of liver extract causing the stimulation of hemoglobin regeneration in the presence of folic acid is vitamin B_{12}. This result indicates that vitamin B_{12} does not have optimum activity unless folic acid is present in adequate

amounts. Therefore, both folic acid and vitamin B_{12} are necessary for treatment of pernicious anemia. Schaefer et al, (1950) found folacin and vitamin B_{12} appeared capable of replacing each other for normal hemoglobin levels of chicks, but a slightly higher hemoglobin level was possible with a combination of vitamin B_{12} and folacin. Best (1948) noted that injection of vitamin B_{12} increased reticulocytes, red blood cells and hemoglobin of humans. Since the initial report by Best (1948) there has been ample evidence (Bethell et al, 1948; Hall and Campbell, 1948) showing that vitamin B_{12} relieves human pernicious anemia when administered parenterally to patients and is essentially ineffective when given by mouth. Orally administered vitamin B_{12}, however, has been shown effective when given in combination with human gastric juice (Berk et al, 1948; Hall et al, 1949) which indicates that vitamin B_{12} and Castles' extrinsic factor are either identical or closely related substances. Later Bethell et al, (1949) and Meyer et al, (1950) showed that the injection of an extract of desiccated swine duodenal mucosa and vitamin B_{12} are effective in pernicious anemia. They considered that their activity might be due to a combination of vitamin B_{12} and the intrinsic factor, and evidenced that vitamin B_{12} or a closely related substance and intrinsic factor constitute the effective hemopoietic agents in hog gastric and duodenal tissue. It has also been observed by Meyer et al, (1950) that the simultaneous oral administration of subminimal doses of folic acid and vitamin B_{12} to cases of pernicious anemia in relapse results in an excellent hematologic and clinical response. Castle and Ham (1939) interpreted these results on studies of pernicious anemia as indicating the existence of an intrinsic factor, a substance in normal gastric juice, and the extrinsic factor, a substance provided by certain foods. Thus, patients suffering from a deficiency of gastric juice, (and hence lack of intrinsic factor) fail to utilise the extrinsic

factor of foods and develo anemia.

ith the isolation of pure vitamin B_{12}, Berk et al, (1948) were able o demonstrate clinically a similar increase in activity of the pure vitamin when administered wit normal human gastric juice. They suggest, therefore, that the food (extrinsic factor) may be identical with vitamin B_{12}. Further, it is suggested that the function of the intrinsic factor (in gastric juice) is necessary for the complete absorption of vitamin B_{12}. Berk et al, (1948) reported large fecal losses of vitamin B_{12} in patients with untreated pernicious anemia. This would seem to confirm the hypothesis that normal gastric juice ai s the absorption of he vitamin or protects it from destruction in the upper gastrointestinal tract.

Furthermore, Berk et al, (1948) and Ungley in England (unpublished) indicated successful response of neurological symptoms to therapy with purified vitamin B_{12} which was found to be necessary for normal function of nervous - especially central nervous - cells.

The dietary requirement for vitamin B_{12} is also influenced by other vitamins. High levels of calcium pantothenate and pyridoxine fed to the chicks (Bird and Rubin, 1946) and high levels of riboflavin fed to rats in the presence of sulfasuxidine (Hartman et al, 1949) seemed to partially spare vitamin B_{12}. Extra vitamin B_{12} to a low vitamin diet increased the growth and the percentage of bone ash (Shorb and Cain, 1949). It was further noted that toxicity from a high level of folic acid when fed with vitamin B was eliminated by increasing the level of vitamin B or vitamin B_{12}. This serves to demonstrate a possible relationship among these vitamins. Vitamin B_{12} also seems to function as sparer of certain unidentified factors. Peeler (1951) demonstrated that when 4 to 6% dried whey was added to the soybean basal diet, a sparing effect of vitamin B_{12}

existed. This could possibly demonstrate a definite relationship between vitamin B_{12} and the unidentified factors. Combs et al, (1950) have presented data demonstrating the presence of four chick growth stimulating factors in fractions obtained from refined liver pa te dialysate. Two of these factors were shown by microbiological assay with Lactobacillus leichmanii to be different forms of vitamin B_{12}. The other two factors remained unidentified but were considered to be an integral part of the APF complex.

It was found that if an adequate amount of vitamin B_{12} was not included in the diet, antibiotics spared the level of vitamin B_{12} for the chick and rat by creating favorable conditions for a high rate of intestinal synthesis (Smith and Robinson, 1945; Oleson et al, 1950 and Stokstad and Jukes, 1951) and also for rats fed a purified synthetic diet (Cravioto-Munoz et al, 1951). They suggested that with a purified diet the antibiotic is able to favor those bacteria which can synthesise vitamin B_{12} required by the rat.

Vitamin B_{13} is fat soluble substance clearly different from vitamin B_{12} (Novak and Hange, 1948). Vitamin B_{14} while apparently closely related to both folic acid and vitamin B_{12} in function, is also an entirely separate entity (Morris and Majnarich, 1949). It is extremely active in cell proliferation in bone marrow cultures and was effective in curing experimentally induced anemia of rats.

4. Growth-Promoting Effect of Vitamin B_{12} and Its Identification with Cow Manure Factor, Animal Protein Factor and Factor X.

Several groups of workers (Hammond, 1942; Whitson et al, 1946; Rubin and Bird, 1946; Bird et al, 1948) found that cow manure improved the growth of chicks and turkey poults when added to plant protein basal

diets. Results were obtained by Nelson and Carby (1949) who found that sheep feces to be a potent source of the animal protein factor or vitamin B_{12} for poultry. Hind et al. (1946) found that the growth factor in cow manure was present in the droppings of hens but young chick droppings did not contain the factor. They also reported the same year that the feeding of concentrates prepared from cow manure stimulated the growth of chicks receiving a corn soybean oil meal basal diet. McGinnis and Carver (1947) found that the inclusion of 4.6% of fish meal in the maternal diet was sufficient to protect chicks from deficiency of growth factor, later shown to be vitamin B_{12}, during the first four weeks following hatching. It is assumed that the growth factors of cow manure, hen feces and fish meal are identical because of the similarity in growth response when these ingredients are added to the chick diet. It was also noted by McGinnis et al. (1949) that when the poultry manure was incorporated in all plant ration for chicks the fermented manure gave definite growth response whereas the droppings that were maintained frozen condition showed beneficial effect. Thus proof that vitamin B_{12} was produced in the droppings by bacterial fermentation.

It was reported by many groups of workers (Nichol et al, 1947; McGinnis and Carver, 1947; Combs et al, 1948; Robblee et al, 1948; Stokstad et al, 1949; Graham et al, 1949; Emerson et al, 1949) that liver extract and fish meal promote chick growth and prevent mortality. Later it has been shown that crystalline vitamin B_{12} can replace all or nearly all of the animal protein factor (Ott et al, 1948; Lillie et al, 1948; Nichol et al, 1949; Emerson et al, 1949 and Stokstad et al, 1949) and the concentrate or acid precipitate of cow manure factor (Lillie et al, 1948) for growth of chicks. Ott (1949) has reported further that depleted chicks under his conditions required at least 27 micrograms of vitamin B_{12} per kilogram

of ration fo[illegible] growth. [illegible]weigert (1949) concluded that unknown factors [illegible] the chick; but that vitamin B_{12} is capable replacing [illegible] active fractions f[illegible] manure, [illegible] extract an[illegible] clubbles either with a soybean [illegible]eal - corn or case [illegible]ration. He also concluded that the various animal protein factors may be attributable to one chemical entity or its conjugates, vitamin B_{12}.

[illegible]s been reported the [illegible]nal protein factor, fish meal, solubles or dried skim milk (McGinnis and Carver, 1947; Lindstrom et al, 1949), cow manure an[illegible] hen droppings (Whitson et al, 1946; Rubin et al, 1946 and 1947; Bird et al, 1946, and Rubin and Bird, 1947) an[illegible] AFF concentrates (Olcese et al, 1949; Carver and McGinnis, 1950) were added to the diet of breeder hens, hatchability was decidedly improved.

Olcese et al, (1949) found that when a sucrose-alpha soybean protein diet low in vitamin B_{12} was fed to a group of hens, egg production and hatchability decreased in from 3 to 6 weeks. The addition of AFF concentrate improved the egg production and hatchability of hens fed the purified diet. When a starch-soybean protein diet was used, egg production and hatchability were improved over those observed with comparable groups fed sucrose as a source of carbohydrate, indicating that starch promoted the intestinal synthesis of vitamin B_{12}. The work of Kennard and Chamberlin (1948) and Kennard et al, (1948) indicated that when hens were kept on built-up litter the eggs hatched much better. Chicks kept on built-up litter grew more rapidly when fed all vegetable type diets than those in which the litter was cleaned out regularly. Thus evidence showing that vitamin B_{12} is synthesized in the dropping of the hens after they are voided. This explains why some of the earlier investigators got better hatchability during summer months than they did during the winter, when the hens were kept on litter where they had access to their droppings.

Work with vitamin B_{12} has not been confined to poultry; many groups of investigators have worked with other animals. McIntire et al. (1949) and Curry et al. (1946) have found that the rat requires a factor present in liver extract for growth. Curry et al. (1946) called the material needed for rat growth "factor x". Tucker and Tucker (1946) were probably working with the same thing when the substance was known as zoopherin. Curry et al. (1946) and Tucker and Tucker (1948) confirmed the previous work and showed that an unidentified factor is essential for rat growth which apparently is identical to growth factors from new sources required by chicks. Tweed et al. (1949) indicated that the administration of vitamin B_{12} either orally or subcutaneously, to vitamin B_{12}-depleted rats, gave a marked growth response.

Hartman et al. (1949) and Stern et al. (1949) fed crystalline vitamin B_{12} in comparison with liver extract as a supplement to the rat x-deficient ration the rates of growth of the rats in both cases was markedly improved, and there was no significant difference between the effect of these supplements. This indicated that the only effective growth-promoting material in the liver extract was vitamin B_{12}. Stern et al. (1949) also obtained evidence which indicated that vitamin B_{12} promoted the formation of cytoplasmic basophilia in liver cells. O'Neil et al. (1951) found the addition of vitamin B_{12} concentrate to the diet or the injection of crystalline vitamin B_{12} in the dams during the early stages of gestation prevented this abnormality in young rats and increased their viability.

The source of calories plays a role in the requirement for vitamin B_{12}, as indicated by the studies with the growing mouse by Bernhardt et al. (1950). Using young, growing rats fed diets varying in fat, carbohydrate and vitamin B_{12} content, McCollum and Chow (1950) found that subcutaneous administration of vitamin B_{12} resulted in increased weight gains in both

sexes on all rations. The weight stimulating effect of vitamin B_{12} was more marked, however, in females fed high carbohydrate diets than in littermate sisters on diets of like protein content but with the calories provided by fat or a mixture of fat and carbohydrate. It also appeared that when the calories were derived primarily from carbohydrate, vitamin B_{12} exerted a greater effect on growth in young female rats than in littermate males. They suggested that vitamin B_{12} is involved in the conversion of carbohydrate into fat. Since females in general have a higher percentage of fat in proportion to total body weight than do males it might be expected that greater weight gains would result in females than in males upon the administration of any substance concerned with the conversion of carbohydrate into fat.

Several microorganisms also have been reported to require antipernicious anemia (APA) liver concentrates for growth. It was found later that vitamin B_{12} could replace APA as a growth factor for LLD (Shorb, 1947) for protozoan Euglena gracilis, (Hutner et al, 1949) and for Lactobacillus leichmannii (Skeggs et al, 1948; Hoffmann et al, 1948).

Therefore, vitamin B_{12} appears to be most important as a component of the "cow manure factor", "factor x" and the "animal protein factor" supplements which is responsible for their activity.

n nutrition there are many example .. onponent reduces the required intake of another necessary to maintain normal life. mong these examples two rrincipal clanses may be .tinguished: Class 1. Substances which act as precursors for physiologically required substance, ermittin synthesis of the required substance t. any extent necessary.

examples of thi class are; (a) the several vitamin A-active carotenoids which are precursors of vitamin A; (b) Methionine precursor of cystine; and (c) Dimethylethanolamine in replacing dietary choline. Class 2. Substances which may assume or diminish certain, but not all, functions of a required nutrient which not relate the required nutrient as a precursor or complete substitute. Examples c. class are: (a) the effect of fat in reducing the requirement for thiamin; (b) the similar effect of calcium and phosphorus on vitamin examples of class 2, the "sparing substance is able either to (1) partially reduce the need for, or (2) provide some, but not all, of the biological functions of the 'spared' compounds". It is the characteristic of these examples, in contrast to those of class 1, that the replacement does not continue to be effective as the intake of the spared compoun. decreases, but often becomes sharply limited. In other words, a significant quantity of the spared compound must be present in order that sparing action may become evident.

In the rat, choline can be synthesized from betaine or methionine, i.e. the known symptoms of choline deficiency may be relieved by using sufficient of these substitutes (du Vigneaud et al, 1939; Griffith, 1941; tetten, 1941). Although it was reported by Burke et al, (1951) that the methyl group from methionine can be used for the synthesis of choline

by the chick; on the other hand, it was reported by other workers that in the chick, choline synthesis is extremely limited. Methionine does not replace choline in the chick (Grau and Almquist, 1943; Jukes, 1940; Record and Bethke, 1942). A similar statement was made for betaine in the case of severe choline deficiency (Almquist and Grau, 1944; Jukes and Welch, 1942; McGinnis et al. 1944). Synthesis of choline in the chick may take place from dimethylaminoethanol which promotes growth and prevents perosis, but the total synthesis fails somewhere in the stages leading to this compound (Jukes and Oleson, 1945). Chicks grew very slowly on a purified diet which involved a combined severe choline and partial methionine deficiency (Almquist and Grau, 1944; Grau and Almquist, 1943). Upon the addition of methionine, the chicks made improved gains which reached a plateau at about two third normal rate. It is apparently indicated that the effect of methionine was definitely limited, and with respect to the choline requirement, probably consisted of the provision of methylating capacity but no extensive synthesis of choline. In the presence of sufficient choline and cystine and a partial deficiency of methionine in the basal diet, improved but limited growth rate was again observed. Almquist (1946) reported that with other sources of labile methyl (methionine and betaine) present in the diet not more than 0.02% choline was needed to support a two third optimal rate of gain.

McKittrick (1946) reported that the choline required by the chick may be divided into two parts called essential choline and replaceable choline. Essential choline is used in tissue formation and replaceable choline in transmethylation. Essential choline cannot be replaced by other methyl donors as betaine and methionine. The process of methyl transfer from one compound to another is called transmethylation. Methionine, choline and betaine have been shown to be suitable dietary sources of such methyl

groups. Therefore, replaceable choline can be replaced by betaine or methionine and essential choline cannot. It was also shown by McGinnis et al. (1944) that chick diet which is only mildly deficient in choline and approximately adequate in methionine and cystine may be improved for growth and perosis prevention by the addition of betaine or methionine. In such a diet the effect of methionine and betaine additions may be explained by assuming that both supplements assisted the choline in a methylating capacity, thereby, augmenting the supply of choline available for growth and perosis prevention.

Studies have shown that an interrelationship exists between the methylating agents (methionine, choline and betaine) and the "animal protein factor" (eventually identified as vitamin B_{12}). Long before the existence of the animal protein factor was conclusively established, many investigators in poultry nutrition had made the observation that methionine is effective in improving the nutritive value of both raw and heated soybean meal. This was the first in the chain of events which eventually were to link the animal protein factor to the phenomenon of transmethylation. Bird et al. (1947) showed that the addition of methionine to a high soybean diet produced as good growth in chicks as did the animal protein factor provided, however, that this factor had been present in the diet of the mother hens. Methionine was less effective when fed to chicks from hens whose diets had been deficient in the animal protein factor.

Gillis and Norris (1949) showed that certain types of practical chick rations are significantly improved by the addition of betaine or choline. However, when basal diet contained APF concentrate no further response was obtained by adding betaine or choline. It was evidenced that the APF concentrate exerted a sparing action on the chicks requirement for labile

methyl group indicating that at least one metabolic function of APF is concerned with transmethylation. Later the same workers (1951) obtained evidence that the effect of APF concentrate previously found to reduce the requirement for methylating compounds by the chick is due to its vitamin B_{12} content. They suggested that either transmethylation is more efficient in the presence of an adequate amount of APF or that partial deficiency of APF creates or stimulates metabolic processes which require excess methyl groups.

Immediately following the report by Gillis and Norris, their findings were confirmed by independent work conducted by Schaefer et al. (1949). In view of the relationship between methionine and choline, Schaefer et al. (1949) demonstrated the sparing effect of vitamin B_{12} on the dietary choline (labile methyl) requirement of rats and chicks. They were able to prevent "hemorrhagic kidney" in rats and promote growth in chicks with suboptimal amounts of choline and methionine provided vitamin B_{12} was added to the diet, but the addition of vitamin B_{12} to an adequate level of choline did not produce an increase in weight gain. Schaefer et al. (1949) also found that chicks responded to methionine homocystine or homocystine plus betaine only in the presence of vitamin B_{12}. These findings establish the existence of an interrelationship between vitamin B_{12} and choline or methionine and that the nutritional requirements for choline and vitamin B_{12} are interrelated. Schaefer et al. (1950) also reported that when vitamin B_{12} was included in the basal diet the level of choline required for growth was greater in the absence of added folic acid than in the presence. A similar finding was obtained recently by Jukes and Stokstad (1951) who reported that the choline requirement of the chick for maximum growth was greater in the absence of vitamin B_{12}

than in its presence. However, the amount of choline required for the prevention of perosis was not decreased by supplementing vitamin B_{12}. Betaine did not have a marked effect when added to diets containing an insufficient level of choline to prevent perosis. Schaefer et al. (1952) noted that the chick was able to utilize methylaminoethanol or dimethylaminoethanol plus betaine or methionine as replacement for dietary choline essential for growth and prevention of perosis only when vitamin B_{12} was added to the diet.

Peterson et al. (1948) had reported that the addition of fish meal to an all-vegetable chick ration apparently lowered the requirement for methionine. Jukes and Stokstad (1949) and Stokstad et al. (1950) obtained similar evidence indicating that vitamin B_{12} is involved in the methylation of homocystine to methionine in the chick. In a soybean meal diet, growth responses were obtained with either homocystine or methionine if vitamin B_{12} was also present in the diet. In the absence of vitamin B_{12} only methionine gave a response, while homocystine was completely ineffective. Ogasawara (1950) also found that the liver from vitamin B_{12}-deficient rats exhibit a lower ability to form methionine from homocystine or betaine, as compared with those from animals dosed with vitamin B_{12}. Briggs et al. (1950) reported that methionine has a strong sparing action on vitamin B_{12} and could completely replace the vitamin B_{12} requirement of chicks fed a corn-soybean oil meal diet with the samples of soybean oil meal studied. Choline had considerably less sparing activity than methionine.

Other workers had previously reported a stimulatory effect in chicks of methionine and choline when added to all vegetable protein rations before the discovery of vitamin B_{12} (Clandinin et al. 1946).

It is generally believed that the main function of choline in poultry nutrition is that of preventing perosis and promoting growth, and that betaine and methionine can partially replace choline in these functions.

Jukes (1940) has shown by feeding choline-deficient diet to chicks, that normal growth cannot be attained and that perosis will result even in the presence of adequate methionine. Later Jukes and Gleason (1945) found that monomethylaminoethanol reduced the incidence of perosis, but had no effect on the growth rate, while dimethylaminoethanol both reduced perosis and increased the growth rate. He also showed that ethanolamine was without any effect when added to this diet, thus showing that some methylation of mono- and di- methylaminoethanol might occur but not of ethanolamine. McGinnis et al, (1942) reported that betaine was effective in preventing perosis and promoting growth in chicks fed a simplified diet containing purified casein. However, Jukes and Welch (1942) found that betaine had growth promoting effect on chicks fed purified diet, but was without effect in preventing perosis. Almquist and Grau (1943) using purified diet containing isolated soybean protein, instead of casein and gelatin, found that betaine and methionine were as effective as choline in promoting growth. In later work, Almquist and Grau (1944), with a somewhat different basal diet, obtained a growth-promoting effect from betaine only when combined with cystine or homocystine. This effect, however, was not equal to that obtained with choline. The results of McGinnis et al, (1944), who explained this discrepancy, found that betaine and methionine were effective in preventing perosis and promoting growth in chicks when added to a simplified diet but ineffective when added to purified diet of higher methionine content. In contrast, choline prevented perosis and promoted growth on both diets. The results indicate that choline is required for the prevention of perosis and for growth and that betaine and methionine when added to the simplified diet enabled the chick to synthesize choline. When casein in the purified diet was replaced with an isolated soybean protein with resultant reduction in

methionine content, betaine and methionine failed to prevent perosis but promoted some growth. The growth response obtained with betaine and methionine in this instance is believed to be due to a deficiency of methyl groups only. Choline was again effective for both purposes.

Jukes (1940) first prevented perosis in turkey poults by adding .3% of choline to the diets. Later Jukes (1940) found that .1% of added choline was sufficient for growth, but was insufficient for the prevention of perosis. Addition of 0.2% of choline to the basal diet protected the poults against perosis. However, Evans et al, (1943) reported that perosis of turkey poults was not completely prevented by adding sufficient choline to raise the level to .2% in the diet. Therefore, another factor or factors appears to be necessary for the prevention of perosis in the poult.

Best (1932 and 1934) made the observation that choline prevented an accumulation of excessive amounts of fat in the livers of rats. Preliminary investigations with other species indicated that mice responded similarly, but inconclusive results were obtained with chickens and dogs. Abbott and DeMaster (1940) obtained limited data which indicated that choline supplements to a semipurified diet lowered the liver-fatty acids in pullets. Hegsted et al, (1941) confirmed, with chicks, the earlier observation made by Jukes (1940) with turkeys, that choline was essential for the prevention of perosis and the promotion of growth. They were, however, unable to demonstrate any lipotropic action of choline.

Some Canadian workers, Bernard and Demers (1949) have reported that choline has a lipotropic function in the nutrition of ducklings in addition to being required for growth and for prevention of perosis. They further reported that neither methionine nor betaine could completely replace choline in its lipotropic action. In view of the vast amount of work already done

on the effect of choline, betaine and methionine on the production of growth ' prevention cerosis, practically no reported data are available on possible lipotro function of these substances in the chick.

It was reported by Hall and Drill (1948) that in addition to choline, a crude liver extract exerted a lipotropic effect in rats with dietary induced liver injury. The effect of liver extract was not due to stimulation of the appetite with resultant increase in protein intake; nor did the lipotropic effect seem to be related to the choline content of the liver extract. It was further found by Drill and McCormick (1949) that a concentrate of vitamin B_{12} exerted a marked lipotropic effect when injected into rats receiving a high-fat-high-protein diet. This lipotropic effect was not due to the small amount of choline present in the concentrate. Gyorgy and Rose (1950) reported that vitamin B_{12} (0.5 microgram daily by mouth) has shown significant lipotropic activity in rats fed a low-protein-low-fat ration. Under the experimental conditions chosen, methionine was more effective than vitamin B_{12} in preventing fat deposition in the liver. low-protein-high-fat diet interfered with the lipotropic effect of vitamin B_{12} whereas a low protein and low fat diet, or, as in the experiments of Drill and McCormick (1949), a moderately high-protein-high-fat diet, will not interfere with the lipotropic reaction following the administration of vitamin B_{12}. It has also been reported that choline prevented hemorrhagic disease of the kidneys of the rat.

Not only in chicks, but also in rats and other animals, choline, methionine, betaine and related compounds may furnish labile methyl groups which are needed for growth. It was first postulated by du Vigneau et al. (1939) that the labile methyl group of choline might be transferred to homocystine to form the essential amino acid methionine. This effect shown by

Simmond et al, (1943) to be due to the utilization of the methyl groups of choline for the biosynthesis of methionine from homocystine. At this time, however, it was noted by du Vigneau et al, (1939 and 1940) that for some unexplained reason an occasional animal was able to grow slowly on the methionine-free diet even if choline were not supplied. The explanation of this discrepancy could not readily become apparent until nearly ten years later after the isolation of vitamin B_{12}.

Four years after the first report of du Vigneaud's group, further findings were reported by Toennies et al, (1943) at the Lankenau laboratory. They found that a large number of their animals consistently grew on a stock diet containing homocystine, but no methionine or choline. This in marked contrast to the findings of the Cornell group (du Vigneaud et al, 1939) growth of animals on a diet low in methyl groups or even entirely free of them had also been reported on occasion by other investigators. Brand (1938) had noted some growth of animals on a diet containing homocystine supplemented by milk and yeast concentrates. Similarly, White and Beach (1937-1938) had obtained growth of animals with homocystine when the diet was supplemented with B vitamins or liver extract as did Rose and Rice (1939) using milk concentrate as source of vitamins.

In a further study, Meden et al, (1944) made analysis of rat tissues and were able to demonstrate the actual synthesis of a small amount of the essential amino acid methionine, in rats on a diet containing homocystine and no dietary source of labile methyl groups. The synthesis of labile methyl groups, to a degree, was also demonstrated by du Vigneaud et al, (1945) through studies with isotopically labelled water.

Bennett (1949 and 1950) confirmed these findings showing that when growth had leveled on a labile-methyl-free diet containing adequate amounts of folic acid, addition of vitamin B_{12} promoted growth. It was suggested

that vitamin B_{12} functions in the biosynthesis of methyl groups. Coincident with Bennett's report, similar findings were reported by Tekol et al. (1950). They found that rats 30 days old or older are able to grow on a diet free of all the known labile methyl group donors, if vitamin B_{12} and homocystine or homocysteine were present. The test diet was complete with respect to all essential amino acids except methionine. Further, on a diet containing ample methionine (as the sole S-containing amino acid) but no vitamin B_{12}, poor growth was obtained. Addition of vitamin B_{12} gave good growth. It was suggested that vitamin B_{12} might function as an enzyme releasing methyl groups from methylated compounds in which the methyl group is not ordinarily labile and/or that vitamin B_{12} may function as part of an enzyme system involved in the synthesis of methyl groups in the body.

Further evidence for a mechanism of the first type seems to be indicated in recent studies by Schaefer et al. (1950). They noted that the effectiveness of methyl donors such as monomethylaminoethanol, betaine, methionine and choline (trimethylaminoethanol) was considerably improved in the presence of vitamin B_{12} with respect to the amounts required for prevention of renal damage and fatty livers in rats and growth and prevention of perosis in chicks. Further, on the basis of methyl content, monomethylaminoethanol and betaine are considerably less effective than dimethylaminoethanol and choline in the prevention of liver and kidney disorders in the rat. When vitamin B_{12} was added, all these compounds became equivalent to choline on a methyl basis. In these studies it would appear, therefore, that vitamin B_{12} might act to increase the lability of the methyl groups in methylated compounds which are less effective on a methyl content basis than choline.

Du Vigneaud et al, (1950) found that methyl groups were synthesized by the germ-free animals. The non-sterile animals on the same diet synthesized methyl groups to the same extent as did the germ-free animals. These studies established conclusively that synthesis of methyl groups did take place and that it occurred in the tissues. Further studies by Welch and Sakami (1950) confirmed this possibility. Rat liver slices were incubated in the presence of C^{14} - formate, homocysteine, dimethylaminoethanol, folic acid + vitamin B_{12}. Dl-methionine was detected and choline chloride was isolated from the rat liver slices. This evidence from *in vivo* studies with germ-free animals and *in vitro* studies on rat tissues, simultaneously and independently demonstrated the ability of animal tissues to synthesize methyl groups.

Stekol et al, (1950) studied the synthesis of methyl groups using pure crystalline vitamin B_{12} in place of the crude liver extract and either homocystine or homocysteine as the only sulfur-containing amino acids. They concluded that in the course of metabolic interconversion of certain amino acids, carbon units are elaborated which serve as the carbon source for the synthesis of the methyl groups of certain metabolites, including methionine and choline. Vitamin B_{12} and other dietary factors may play a decisive role in these interconversions, in the synthesis and utilisation of the labile methyl group. Many groups of workers found that serine and glycine are the precursors in the synthesis of labile methyl groups (Sakami, 1949; Welch and Sakami, 1950; Stekol and Weiss, 1950; Viekevits and Greenberg, 1950; du Vigneaud et al, 1950; Arnstein, 1951).

Cunha et al, (1949) reported that methionine was beneficial for growing pigs when added to the basal ration. However, when methionine was fed in addition to the AFF supplement, the rate of gain was no

different than when AFF supplement was fed alone. Methionine was also of no benefit when added to the basal ration and vitamin B_{12}. These data indicate that the addition of the AFF supplement to the basal ration relieved the need for supplementing the ration with methionine. It has also been established that the baby pig required choline (Johnson and James, 1948). However, Nesheim and Johnson (1950) found that the pig even at an early age was able to use methionine as a methyl donor in the synthesis of choline. The baby pig also did not require dietary choline when the synthetic milk diet contains 1.6% methionine. In this respect the pig resembles the rat (Stetten, 1941; Toennies et al, 1943; Bennett et al, 1944, 1945, 1946 and 1949; du Vigneaud et al, 1945; Treadwell, 1948; Stokal et al, 1950) and man (Simmonds and du Vigneaud, 1942) but may differ from the chick (Jukes, 1940; McKittrick, 1947).

B_{12} OR TOXICITY

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It has been shown that high levels protei inhibit growth of chicks (Rubin and Bird, 1947) and of rats (Cary et al, 1946; Hartman et al, 1949; Bosshardt et al, 1950) fed vitamin B_{12}-deficient diets. Vitamin B_{12} or liver extract tended to overcome this growth-inhibitory effect when fed as a supplement to such rations. Hartman et al, (1949) further showed that "x-deficient" rats fed 25% protein ha hyperthyroid kidneys. This condition was corrected by supplementation with liver extract. With all levels of protein tested, deficiency of vitamin B_{12} deleterious effect on growth; indicating that vitamin B_{12} is necessary in the utilization of protein by mammals. Furthermore, Zucker and Zucker (1948) reported that nonprotein nitrogen and urea values in rat blood were increased in zoopherin deficiency. It was also found by McGinnis et al, (1948) that the blood nonprotein nitrogen content in chicks fed a vegetable protein diet and deficient in "animal protein factor" was higher than in chicks fed the same diets supplemented with this factor. Subsequent work (Ott et al, 1948 and Lillie et al, 1948) indicated that vitamin B_{12} was identical with the factor contained in APF or liver extract. Charkey et al, (1950) demonstrated that the levels of nonprotein nitrogen and amino acids in the blood were higher in vitamin B_{12}-deficient chicks than in chicks fed vitamin B_{12} concentrates.

Monge and Combs (1950) reported that the growth of chicks was decreased when 1% and 4% glycine was added to vitamin B_{12}-deficient diets. The addition of vitamin B_{12} overcame the inhibitory effect of glycine. Stern and McGinnis (1951) demonstrated the alleviation of vitamin B_{12} of the toxicity

produced by administering a one gram portion of glycine by capsule to growing chicks and also found that the blood levels for nonprotein nitrogen and amino nitrogen after administration of glycine were higher in vitamin B_{12}-deficient chicks than in vitamin B_{12} injected chicks. Mashlin et al. (1951) also obtained the results indicating that the addition of 3%, 6% and 9% glycine to vitamin B_{12} low diets caused a growth depression and increased mortality in chicks. Vitamin B_{12} functions in counteracting this toxicity. Addition of 6% or 9% glycine to the diet increases blood uric acid levels. Vitamin B_{12} tended to increase the level of uric acid in the blood of young chicks.

Vitamin B_{12} has been demonstrated to counteract the harmful action of several substances administered in toxic quantities to rats and chicks. Thus, vitamin B_{12} has been shown to overcome the retardation of growth which follows administration of toxic amounts of thyroid powder or thyroprotein (Bethell and Lardy, 1949; Emerson, 1949; Meites and Nevland, 1950; and Sure and Easterling, 1950); diethylstilbestrol (Meites, 1950); thiouracil (Meites, 1950). Emerson (1949), Sure and Easterling (1950) and Register et al. (1949) reported that the growth of rats on a soybean meal diet was greatly retarded by the addition of thyroid powder. The addition of vitamin B_{12} resulted in a weight gain exceeding that of rats receiving the soybean meal without thyroid. This vitamin is equally effective when administered by the oral or the subcutaneous route. It is further indicated by Register et al. (1949) that pernicious anemia liver extract and crystalline vitamin B_{12} were equally effective in restoring the rat growth to more normal state. Michel et al. (1949) obtained evidence that crystalline vitamin B_{12} can replace the animal protein factor activity of condensed fish solubles or parenteral liver extracts in counteracting the thyrotoxic conditions produced in chicks by feeding a basal diet contain-

ing iodinated casein.

Vitamin B_{12} has also been found to prevent the acute hepatic injury which results from administering carbon tetrachloride to rats (Popper et al. 1949), to prevent the disappearance of liver basophilia which occurs when soybean oil meal is the sole source of protein the diet of rats. (Stern et al. 1949) and to prevent the acute uremia observed in newborn rats, from mothers maintained on all-plant protein diets (Lieser and Schultze, 1950). These results and the findings of the growth response of vitamin B_{12} in animals described in previous sections indicate that vitamin B_{12} functions in the utilization of amino acids in protein metabolism.

PART I.

INTERRELATIONSHIP VITAMIN B_{12} AND METHYLATING COMPOUNDS (CHOLINE, BETAINE AND METHIONINE).

Experiments were conducted to determine the requirements of total and essential choline for promoting growth and preventing perosis and the effect of choline, betaine and methionine on hemoglobin level and liver fat in chicks.

Experimental Procedures

In all experiments New Hampshire chicks of mixed sexes were used. The vitamin B_{12}-deficient chicks were obtained from eggs produced by hens fed a ration deficient in vitamin B_{12}. In experiments 2 and 5 chicks were depleted at the first week by feeding vitamin B_{12}-deficient diet. During this preliminary period the weakest chicks were eliminated by natural mortality. In the remaining trial (experiments 1,3,4,6 and 7) day-old chicks were employed. At the beginning of each experiment the chicks were weighed individually, wing-banded and distributed into uniform weight groups following the elimination of the very rapid and very slow growing chicks. In experimental diets, choline chloride and betaine hydrochloride were added singly and in combination to a low-choline chick ration. The low choline synthetic basal diet containing α-soybean protein as the sole source of protein was used in all experiments except experiment 6 where the practical soybean-corn type diet was employed and contains a source of the whey factor. In this experiment choline and methionine were added singly and in combination to this low-choline practical chick ration. The composition of the basal

diets are shown in Tables 1 and 2. Substituents in these diets were made at the expense of cerelose. In experiment 7, the soybean oil was increased in the diet in order to increase the requirement for choline.

The chicks were maintained in electrically heated batteries with raised wire floors during the entire experimental period. Feed and water were fed *ad libitum*. The chicks were weighed and porosis records were taken individually at weekly intervals. Data on mortality was also noted. The experiments were terminated at 4 weeks of age. At the termination of each experiment the total feed consumption was determined.

Vitamin A and α-tocopheral acetate were given by dropper per week (2 drops per chick) in addition to that contained in the ration to insure that an adequate level of these vitamins was supplied. Thiamin hydrochloride was also administered by dropper weekly. Alpha-protein contains between 1.5% to 2% sodium sulfite which is known to destroy thiamin through cleavage and also increases oxidation of vitamin A and E. This practice was designed to insure an adequate supply of these vitamins and to compensate for probable losses that may occur as the feed remains in the hoppers prior to consumption. Alpha-protein (isolated soybean protein) was obtained from Glidden and Company, Chicago, Illinois.

Merck AFF concentrate #3 was added in experiments 1,6 and 7 to supply 30 micrograms of vitamin B_{12} per kilogram of diet and was added to experiment 3 to supply 20 micrograms of vitamin B_{12} per kilogram of diet. 0.3 micrograms of crystalline vitamin B_{12} was injected per chick per week in experiments 2 and 4. In experiment 5, 30 micrograms of crystalline vitamin B_{12} per kilogram of diet was added. The crystalline vitamin B_{12} was in the form of sodium chloride triturate containing 1 microgram of vitamin per mg and was obtained from Merck and Company. AFF concentrate #3, furnished by Merck and Company, [illegible] Fuller's earth adsorbate of vitamin B_{12}. The

TABLE 1

Composition of ynthetic Choline-low iets.

INGREDIENTS	Experiment Number			
	1,2,3	4	5	7
Alpha Protein	23.00	23.00	23.00	23.00
Cerelose	59.94	3.50	63.43	58.21
Starch	-	62.50	-	-
RufTex	5.00	-	-	-
Soybean oil	4.00	4.00	3.00	7.00
DL-methionine	0.30	0.30	0.30	0.30
L-cystine	0.30	0.30	0.30	0.30
Glycine	1.00	0.50	1.00	1.00
$CaCO_3$	1.50	1.50	1.50	1.50
K_2HPO_4	0.90	0.90	0.90	0.90
$Ca_3(PO_4)_2$	1.30	1.30	1.30	1.30
NaCl	0.38	0.60	0.50	0.50
Na_2HPO_4	0.73	0.73	0.73	0.73
$MgSO_4.7H_2O$	0.50	0.50	0.50	0.50
$Fe(C_6H_5O_7)_26H_2O$	0.14	0.14	0.14	0.14
$MnSO_4.4H_2O$	0.041	0.041	0.041	0.041
KI	0.004	0.004	0.004	0.004
$ZnCl_2$	0.002	0.002	0.002	0.002
H_3BO_3	0.0009	0.0009	0.0009	0.0009
$CoSO_4.7H_2O$	0.0001	0.0001	0.0001	0.0001
$CuSO_4$	0.0013	0.0013	0.0013	0.0013
"A" Activated Animal Sterol (2000 A.O.A.C. u/gm)	0.10	0.10	-	-
Vit. A pellets (5675 I.U./gm)	0.25	0.25	-	-
A & D Oil	-	-	0.50	0.50
H_3PO_4	-	-	.049g/kg	.049g/kg
Dried Whey	-	-	3.00	3.00
Biopar C	-	-	-	1.00
Protomone	-	-	-	0.03
APF #3	20 mcg/kg	-	-	30 mcg/kg
L-leucine	-	-	-	0.20
Vitamins *	0.10	0.10	0.10	0.10

*Vitamins: mg per 100 gm; thiamin HCL, 1.00; riboflavin, 1.00; calcium pantothenate, 2.00; niacin, 5.00; pyridoxine, 0.60; biotin, 0.02; folic acid, 0.30; p-amino benzoic acid, 0.20; Menadione, 0.50; alpha-tocopherol, 0.50; inositol, 100.00.

TABLE 2

Experiment 6

Composition of Practical Choline Low Basal Diet

INGREDIENTS	%
Ground Yellow Corn	59.1
Soybean oil meal (Solvent) Buckeye	33.5
Salt (iodized)	0.5
A and D Oil (400 units of D, 3000 units of A)	0.3
Manganese sulphate	0.025
Copper sulphate	0.001
Cobalt sulphate	0.0001
Ferrous sulphate	0.025
Limestone	0.50
Defluorinated Rock phosphate (14% P, 34% Ca)	3.00
Dried Whey	3.00
	mg/lb.
Riboflavin	1.20
Menadione	0.20
Niacin	8.0
Calcium pantothenate	1.0

activity as measured by LL" method is equivalent to that of 12.5 mg of vitamin B_{12} per lb.

The Biopar fraction "C" used in experiment 6 is the 70% alcohol insoluble portion of the hot water extract of liver. It was obtained from Armour laboratories, Armour and Company, Chicago, Illinois.

Protomone (iodinated casein) was used in experiment 7 as a "stress" mechanism to insure the sensitivity of the animal to a deficiency of this vitamin. Production of the hyperthyroid condition of the chick resulting in an increased metabolic rate would tend to increase the dietary requirements of the chick. The protomone used in experiment 7 was obtained from the Cerophyl Laboratories, Incorporated, Kansas City, Missouri. This material has a potency of approximately 3% thyroxine as determined by the manufacturer.

The dried whey used in experiments 5 and 7 is a product of the Western Condensing Company, Appleton, Wisconsin. It is a dried whey product with whey fermentation solubles (Ribolac).

The method for determining the severity of perosis for each group was used by the formula developed by Wilgus, Norris and Heuser (1937):

$$\frac{\text{Total H} + \text{total T} + \text{total S}}{\text{Number of chicks} \times 18} \times 100 = \text{percentage severity}$$

It was noted by the degree of hock enlargement (H), twisted or crooked tibia or metatarsus (T), and slipped tendon ( ) of each leg of each chick. A subnumber was used to denote severity: 1, designating slight; 2, moderate; and 3, severe. The maximum severity possible for each leg would therefore be $H_3T_3S_3$, or a total of 18 per chick.

Fat determination was done by means of a Soxhlet apparatus using ether. A dried finely ground sample of liver was extracted with the

anhydrous ether for 16 hours. The extract was dried at 100°C for 30 min., cooled in a desiccator and weighed until constant weight was obtained.

For determination of hemoglobin level 0.02 cc of the whole blood was delivered from a calibrated capillary pipette into 10 cc of distilled water in a test tube, one drop of concentrated ammonium hydroxide was added to each tube just before the reading was made. The content of the test tube was poured into a colorimeter tube and the reading was taken immediately with filter 540 using a blank tube containing distilled water for the initial adjustment of the galvanometer. Hemoglobin was determined in the Evelyn photoelectric colorimeter. The density of oxyhemoglobin was measured by the galvanometer reading.

The choline content of α-protein was analysed by the use of a microbiological assay with the mutant of cholineless mold Neurospora crassa according to Horowitz and Beadle's method (1943). The mutant strain of Neurospora crassa has lost the ability to synthesize choline on a simple medium and consequently requires choline for growth.

From this method the choline content of α-protein is 0.019%, which equivalent to 0.004% of basal diet containing 23% α-protein.

Effect of Vitamin B_{12} on Total Choline Requirement.

The results are given in Table 3. [illegible] riment 1, average weight gain of ch [illegible] ks fed vitamin B_{12}-deficient diets receiving 0.075% and 0.1% [illegible] ded choline were 163 gm and 189 gm respectively; the average gain of chicks fed vitamin B_{12}-supplemented diet receiving 0.075% and 0.1% added choline were 241 gm and 233 gm respectively. [illegible] results ind[illegible]cat[illegible] that in the vitamin B_{12}-deficient diet, 0.1% [illegible] added choline was required for best growth and 0.075% of added choline was required for prevention of perosis. In the presence [illegible] equate level of vitamin B_{12}, 0.075% of added choline [illegible] [illegible]equa[illegible] both for [illegible]owth and for perosis prevention.

In experiment 2, (see Table 3) the average gain of c[illegible] receiving [illegible] containing 0.1% and 0.125% a[illegible]ded choline and vitamin B_{12} was 268 gm and 276gm respectively. When vitamin B_{12} was not given, the average gain of chicks fed diets containing 0.1% and 0.125% of added choline was 227 gm and 252 gm, respectively. In this experiment, when no supplemental vitamin B_{12} was given, 0.1% of added choline was not enough for best growth or complete prevention of perosis, whereas this level of choline was adequate when vitamin B_{12} was supplied.

In experiment 4 (Table 3) the average gain of chicks fed diets containing 0.07%, 0.11% and 0.16% of added choline in the presence of vitamin B_{12} was 220 gm, 210 gm and 240 gm, respectively, while it was 165 gm, 176 gm and 234 gm, respectively, in the absence of vitamin B_{12}. Therefore, these results indicate that with adequate vitamin B_{12} 0.07% of added choline was sufficient to promote growth. However, it was not sufficient to completely prevent perosis. In the absence of vitamin B_{12} only the 0.16% level

TABLE 3

Influence of Vitamin B_{12} on Requirement for Choline

Exp. No.	Choline added % [3]	Ave. gain in 4 weeks (gm)		% increase in gain [5]	% severity of ataxia	
		$-B_{12}$	$+B_{12}$ [4]		$-B_{12}$	$+B_{12}$
1 [1]	0	-	113	-	-	53.1
	.075	163	241	47.8	0	0
	.10	189	233	23.2	1.1	0
	.125	194	244	26.3	2.5	0
	.15	-	234	-	-	0
	.175	-	231	-	-	0
2 [2]	0	99	136	37.4	75.0	90.0
	.10	227	268	18.0	11.9	0
	.125	252	276	9.5	1.3	
	.07	165	220	33.3	1.0	7.5
	.09	160	218	36.2	4.0	1.0
	.11	176	210	19.3	0	1.5
	.13	131	229	26.5	2.3	0
	.16	234	240	2.5	0	0

1/ 18 day-old chicks were distributed into groups in experiments 1 and 4.

2/ One week preliminary period; 11 chicks per group.

3/ .004% choline contained in basal diet.

4/ 30 mcg vitamin B_{12} (from Merck ADF #3) contained in experiment 1. 0.3 mcg vitamin B_{12} was injected per chick per week in experiments 2 and 4.

5/ The increase in gain obtained from the administration of vitamin B_{12} is expressed as a percentage of the average gain obtained with the respective control group which received the same diet except for vitamin B_{12}.

of added choline was adequate for growth and the prevention of perosis.

The results shown in Table also reveal that in these experiments, as the level of added choline increased, the percentage increase in gain between vitamin B_{12}-adequate and vitamin B_{12}-deficient chicks decreased. These data, therefore, show that choline exerts a sparing action on the requirement for vitamin B_{12}.

From the results of the above experiments it may be concluded that ca. 0.1% of added total choline is sufficient to support growth and prevent perosis even when an adequate level of vitamin B_{12} is supplied. In the absence of vitamin B_{12}, on the other hand, from 0.1% to 0.15% of total added choline is necessary. Since the choline contained in the basal diet was only .004%, the total choline levels required would be essentially the same.

The Sparing Effect of Betaine on Choline Requirement

The results of experiment 3 are presented in Table 4. The average gain of chicks fed the basal diet containing 0.05% betaine and 0.1% choline was 319 gm which is comparable with the average gain of chicks fed the basal diet plus 0.15% choline alone (315 gm). The average gain of chicks fed the diet which contained 0.05% choline plus 0.05% betaine was 287 gm. Chicks fed the diet supplemented with 0.1% added choline alone exhibited similar gains. However, 0.05% choline plus 0.05% betaine was not adequate for the prevention of perosis. Also 0.05% of added choline alone was not adequate for normal growth.

These results indicate that betaine had a supplementary effect on chick growth when it was added to the diet containing .104% choline but did not have a supplementary effect when it was added to the diet which

TABLE 4

(Experiment 3*)

Sparing Effect of Betaine on Choline Requirement

Choline added %	Betaine added, %			
	0	.05	.10	.15
	Average weight gain at 4 weeks (gm).			
0	157(79.4)**	167(60.0)	190(74.7)	164(60.5)
.05	265(23.1)	287(28.4)	271(13.6)	
.10	289(0)	319(2.7)		
.15	315(0)			

* 20 day-old vitamin B_{12}-deficient chicks per group. .004% choline contained in basal diet. Merck's "A-F" #3 was added to supply 20 mcg of vitamin B_{12} per kg of diet.

** o/o Severity of Perosis

contained only .054% choline. Therefore, betaine demonstrated a growth promoting action only when sufficient "essential" choline was supplied. Betaine alone did not improve growth under the conditions of this experiment. Neither did it prevent perosis. An adequate level of "essential" choline itself appears to be required for the prevention of perosis. In this study, even when an adequate level of vitamin B_{12} was supplied, more than .054% but not more than .10% essential choline was required for normal growth and the prevention of perosis.

he Requirement o" "ssential Choline in The Presence a 'beence of Vitamin B_{12} and Betaine

Results are given in Tables 5 and 6 and Figures 1 and 2. In experiment 1, when no vitamin B_{12} was fed, .087% of essential choline was required to support growth and prevent perosis(Table 6). In the presence of adequate levels of vitamin B_{12} more than .054% but less than .071% essential choline was adequate for growth and .08% essential choline was necessary for the prevention of perosis (Table 5).

In experiments 2 and 4, when sufficient vitamin B_{12} was supplied, .064% essential choline supported normal growth but was not adequate for the prevention of perosis even in the presence of betaine to spare the non-essential choline. From .074% to .084% choline was required to prevent perosis.

In experiment 2, .074% essential choline was adequate for growth but .034% essential choline was required to prevent perosis when no vitamin B_{12} was supplie In experiment 4, .084% essential choline was required for normal growth and prevention of perosis.

In experiment 5, the basal diet contained 3% dried whey in addition to 23% of α-protein. Dried whey and α-protein contained .005% and .004%

TABLE 5

Requirement for [illegible] Dial Choline in the Presence of Vitamin B_{12} and Betaine.

Exp. no.	Choline 5/ %	Betaine %	Average gain during 4 wks.		Porosis % severity
1/	.004	0	113	-	53.1
	.054	.10	204	(75.8) 6/	16.5
	.072	.083	227	(103.0)	7.4
	.087	.067	229	(96.8)	1
	.154	0	234	(100.2)	0
2/	.004	0	136	-	20.0
	.054	.10	250	(84.0)	5.4
	.064	.09	274	(97.0)	12.0
	.074	.08	274	(97.0)	11.0
	.154	0	286	(105.5)	0
3/	.004	0	137	-	45.0
	.004	.16	145	-	42.0
	.054	.11	217	(82.3)	6.2
	.064	.10	231	(97.3)	6.6
	.074	.09	229	(95.2)	0
	.084	.08	234	(100.1)	
	.164	0	240	(107.0)	
4/	.009	.15	130	-	67.4
	.049	.11	241	(85.5)	13.9
	.059	.10	259	(99.0)	12.5
	.069	.09	260	(99.5)	5.6
	.079	.08	266	(104.0)	4.8
	.159	0	257	(97.2)	0

1/ [illegible]ay-old chicks; Hereford #3 [illegible] to 30 mcg vitamin B_{12} per kg of diet.

2/ [illegible]eek-old chicks; .3 mcg vit. B_{12} was injected per chick per week.

3/ [illegible]ay-old chick; .3 mcg. vit. B_{12} was injected per chick per group.

4/ 16 day-old chicks were distributed into groups; 30 mcg. crystalline vit. B_{12} per kg of diet fed.

5/ % choline is equivalent to the % added plus % choline contained in basal diet.

6/ % response calculated by assigning a value of 100 percent to the gain in weight over the basal group when an adequate amount of choline was supplied. The response obtained from an adequate level of choline was based on an average of the plateaued groups.

Figure I (From Table 5). Requirements for Essential Choline in Presence of Vitamin B_{12} and Betaine

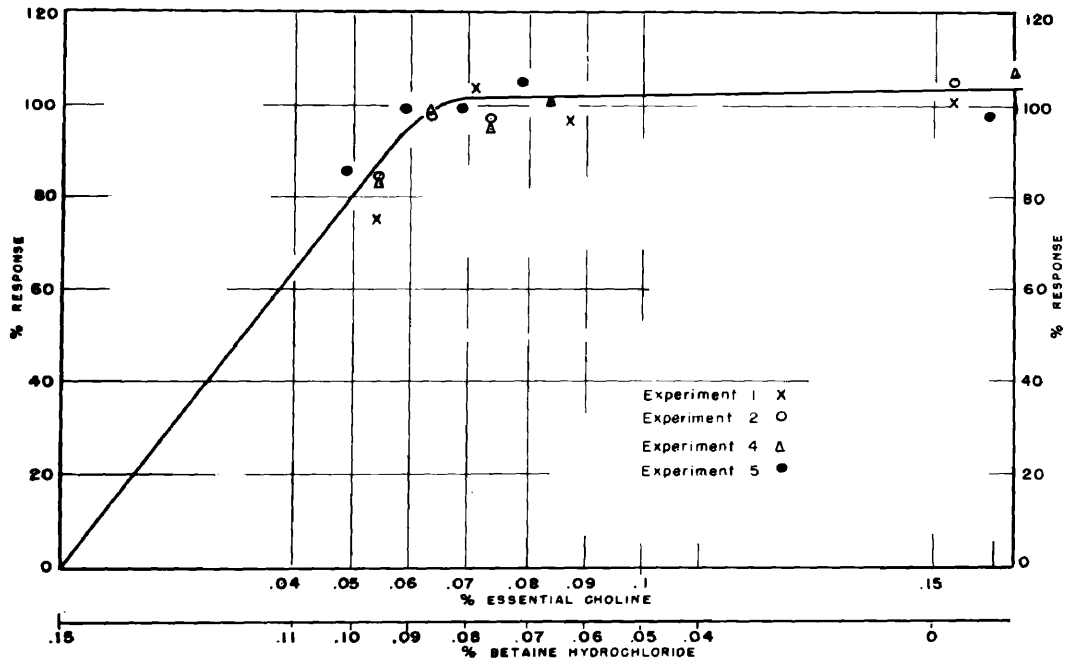


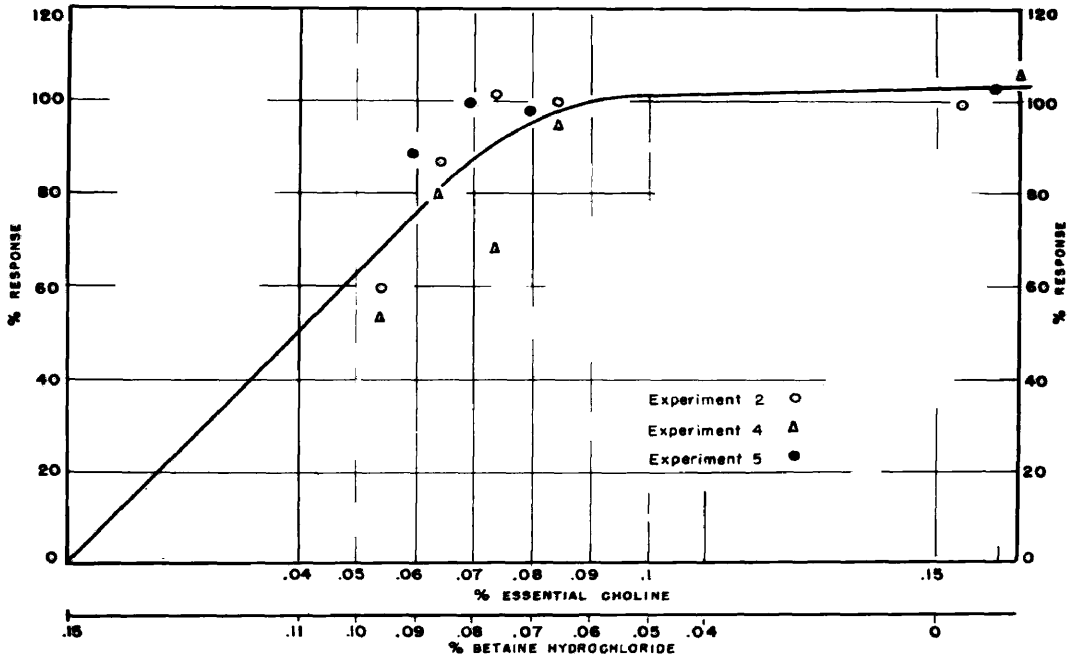
TABLE 6

Requirement for Essential Choline in Absence of Vitamin B_{12} but in Presence of Betaine

Exp. No.	Choline 5/ %	Betaine %	Average gain during 4 weeks		Perosis % Severity
1/	.054	.10	132	-	52.7
	.071	.083	162	(52.7)	3.2
	.087	.067	184	(91.1)	1.1
	.104	0	189	(100.0)	1.1
	.129	0	194	(109.0)	2.5
2/	.004	0	99	-	75.0
	.054	.1	183	(59.6)	21.0
	.064	.09	220	(86.0)	14.0
	.074	.08	242	(101.3)	9.0
	.084	.07	240	(100.0)	2.7
	.154	0	239	(99.3)	0.5
3/	.004	0	71	-	33.0
	.004	.16	122	-	60.7
	.054	.11	176	(53.0)	6.1
	.064	.10	200	(79.3)	6.6
	.074	.09	185	(67.8)	0
	.084	.08	219	(94.0)	0
	.164	0	234	(105.3)	0
4/	.009	.15	108	-	66.3
	.049	.11	117	-	25.3
	.059	.10	195	(88.2)	4.6
	.069	.09	206	(100.0)	0
	.079	.08	204	(98.0)	13.6
	.159	0	209	(103.3)	0

1/ 2/ 3/ 4/ 5/ 6/ - Same as in Table 5.

Figure 2 (From Table 6). Requirement for Essential Choline in Absence of Vitamin B_{12} but in Presence of Betaine



choline respectively. Therefore, the basal diet contained .009% choline. In this experiment, when an adequate level of vitamin B_{12} was supplied, .059% essential choline was sufficient for growth and .069% essential choline was adequate to prevent perosis. In the vitamin B_{12}-deficient diet containing .069% essential choline was required for growth and perosis prevention. From these results, it is evident that betaine is not effective for prevention of perosis.

From these four experiments, chicks fed diets containing no vitamin B_{12} required from .064% to .087% of essential choline for growth and the prevention of perosis in the presence of betaine to replace the need for non-essential choline. When an adequate level of vitamin B_{12} was supplied more than .054%, but less than .067% of essential choline, was required for chick growth, but .069% to .087% of essential choline was necessary to prevent perosis. These results clearly show that even in the presence of vitamin B_{12}, betaine is unable to replace the essential choline requirement of the chick. It also appears that vitamin B_{12} supplementation increases the severity of perosis of chicks fed the choline-low basal diet. However, when choline was added to the diet, vitamin B_{12} supplementation did not effect the incidence of perosis.

Effect of Choline, Betaine and Vitamin B_{12} on Hemoglobin Level of Blood

The results presented in Table 7 reveal that the addition of choline or betaine to the basal diet reduced the hemoglobin level of the blood of chicks. This difference cannot be explained but is believed to have resulted because of differences in growth rate. The vitamin B_{12} content of the diet did not influence the hemoglobin level.

TABLE 7

Effect of Choline, Betaine and Vitamin B_{12} on Hemoglobin Level of Blood

Supplement		Hemoglobin gm per 100 cc blood		
		Experiment 2		Experiment 3
Choline added %	Betaine added %	Without B_{12}	With B_{12}	With B_{12}
-	-	8.22	8.26	9.25
.05	-	-	-	8.65
.10	-	7.84	7.12	8.49
.15	-	7.27	7.51	7.51
-	.05	-	-	9.35
-	.10	-	-	8.15
-	.15	-	-	6.88
.05	.05	7.53	7.86	7.85
.05	.10	7.49	7.29	-
.06	.04	7.34	7.14	-
.06	.09	7.37	7.16	-
.07	.03	7.35	7.57	-
.07	.08	7.34	7.51	-
.08	.02	7.65	7.63	-
.08	.07	7.83	6.27	-
.10	.05	-	-	7.69

Effect of Vitamin B_{12} Concentrate on Methionine and Choline Requirements.

The results of experiment 4 are summarized in Table 8. The average gain of vitamin B_{12}-deficient chicks receiving neither choline nor methionine was only 340 gm, whereas the average gain of vitamin B_{12}-deficient chicks fed diets containing either 1% methionine or 200 mg of choline per lb. was 383 gm, and 407 gm, respectively. This indicates that in the absence of vitamin B_{12} concentrate, addition of methionine or choline resulted in a marked growth response. When choline was fed in combination with added methionine in a vitamin B_{12}-low diet (diet 4) the average gain was improved to 426 gm. However, in the presence of the vitamin B_{12}-concentrate the addition of choline was not required in the diet if .1% methionine was included (diet 3). Likewise, the addition of methionine was not required when the vitamin B_{12}-adequate diet contained 200 mg per lb. choline (diet 2). This indicates that vitamin B_{12}, choline and methionine have a sparing action on each other.

Effect of Choline, Betaine and Methionine on Growth and Perosis of Chicks

In experiment 7, choline, betaine and methionine were added singly and in combination to a choline-low purified type chick ration. The "methyl group" equivalent supplied in the diet by choline, betaine and methionine, or the combination of these substances, were calculated to be the same for diets 3 to 7. The basal diet contained .005% choline. The levels of choline referred to herein represents the added choline plus choline contained in the basal diet.

The results of this experiment are presented in Table 9. Chicks fed diet 2, which contained .065% choline grew normally and did not exhibit

TABLE 8

Effect of Vitamin B_{12} Concentrate on Methionine and Choline Requirement

Exp. No.	Diet No.	Supplement[1]		Average gain[2] (gm) during 6 weeks.	
		Choline added mg/lb.	Methionine added %	With B_{12}[3]	Without B_{12}[4]
5				469	340
		200		430	407
				495	383
6		50		486	426

1/ Practical basal diet was used (Table 2).

2/ 15 day-old chicks per group.

3/ Vitamin B_{12} was added from Merck's "AFF" #3 to supply 30 mcg vitamin B_{12} per kg of diet on chicks hatched from eggs obtained from hens fed normal diet.

4/ "AFF" concentrate added on vitamin B_{12}-deficient chicks.

TABLE 9

(Experiment 7)*

Effect of Choline, Betaine and Methionine
on Growth and Perosis of Chicks

Diet No.	Supplement	Average gain** during 4 wks. (gm)	% Severity of Perosis
1	0	140	46.9
2	.06% choline	220	0
3	.06% choline + .168% methionine	196	
	.06% choline + .082% betaine	212	0
5	.15% choline	231	0
6	.136% betaine	158	54.3
7	.28% methionine	120	49.4
8	.15% choline + .136% betaine	209	0

.001% choline in Biopar C which is equivalent to .00001% choline in the basal diet. The amount is negligible. Therefore, .004% choline is in the basal diet containing 23% alpha-protein.

** 16 day-old normal chicks per group.

perosis. The addition of methionine or betaine (diets 6 and 7) were not effective for promoting growth and preventing perosis.

Effect of Choline, Betaine and Methionine on Fat Content of Liver in the Presence of Vitamin B_{12}.

The results given in Table 10 clearly indicate that the addition of choline lowered the fat content in the liver. Therefore, choline exerted a lipotropic effect. Methionine or betaine appeared to increase the fat content of the liver.

Influence of Vitamin B_{12} on Lipotropic Effect of Choline and Betaine

The results shown in Table 11 indicate that vitamin B_{12} lowers the fat content of the liver. Therefore, vitamin B_{12} also has a lipotropic action. This might be expected since vitamin B_{12} is concerned with transmethylation reactions, and spares the requirement for choline.

TABLE 10

Effect of Choline, Betaine and Methionine
on Fat Content of Liver in Presence of Vitamin B_{12}

Supplement			% Fat in Liver on ry Basis			
Choline added %	Betaine added %	Methionine added %	Experiment Number			
			2	3		6
-			13.17	13.19	13.60	11.27
.05			- - -	12.46	- - -	- - -
.06			- - -	- - -	- - -	10.48
.10			12.35	12.03	- - -	- - -
.15			- - -	11.67	- - -	11.16
.16			- - -	- - -	13.4	- - -
-	.05	-	- - -	13.75	- - -	- - -
	.10		- - -	14.78	- - -	- - -
	.136		- - -	- - -	- - -	12.07
	.15		- - -	16.57	- - -	- - -
	.16		- - -	- - -	14.45	- - -
.05	.05	-	13.50	12.95	- - -	- - -
.06	.04		13.35	- - -	- - -	- - -
.07	.03		13.07	- - -	- - -	- - -
.08	.02		11.61	- - -	- - -	- - -
.05	.1	-	13.56	13.67	- - -	- - -
.10	.05	-	- - -	13.07	- - -	- - -
.06	.08	-	- - -	- - -	- - -	11.68
.15	.136	-	- - -	- - -	- - -	11.62
.06	-	.168	- - -	- - -	- - -	11.33
		.28	- - -	- - -	- - -	12.22

TABLE 11

Influence of Vitamin B_{12} on Lipotropic Effect of Choline and Betaine

Supplement		% Fat in Liver			
Choline added %	Betaine added %	Experiment Number			
		2		4	
		With B_{12}	Without B_{12}	With B_{12}	Without B_{12}
-	-	13.17	12.36	13.60	14.79
.10	-	12.35	12.38	- - -	- - -
.16	-	- - -	- - -	13.40	13.42
0	.16	- - -	- - -	14.45	14.42
.05	.05	13.5	15.4	- - -	- - -
.06	.04	13.07	13.56	- - -	- - -
.08	.02	11.61	12.61	- - -	- - -
.05	.10	13.56	15.33	- - -	- - -
.06	.09	14.19	14.30	- - -	- - -
.07	.08	12.66	13.80	- - -	- - -
.08	.07	11.56	13.9	- - -	- - -

DISCUSSION

The results obtained with a purified type diet containing an adequate level of vitamin B_{12} clearly shows that .104% of total choline was sufficient to promote normal growth and prevent perosis in chicks. On the other hand, when no vitamin B_{12} was added higher level of total choline was required for growth and the prevention of perosis. The results also show that as the percentage of total choline in the diet was increased, the difference in weight between the vitamin B_{12} and vitamin B_{12}-deficient chicks was decreased. This indicates that choline exerted a sparing action on the requirement for vitamin B_{12}.

Using a soybean oil meal-corn practical type diet it was also found that methionine or choline markedly improved the growth of chicks fed diets containing no supplemental vitamin B_{12}. In contrast, when vitamin B_{12} and .1% methionine was supplied, the addition of choline was not required. Similarly the addition of methionine was not required when the diet contained 200 mg of choline per lb. This again indicates that vitamin B_{12}, choline and methionine have sparing action on each other.

The results als show that the choline requirement was lowered by the addition of vitamin B_{12} and betaine to the diet. The findings noted above clearly indicate the interrelationship of vitamin B_{12}, choline, methionine, and betaine. These results are in agreement with the work of other investigators. Schaefer et al, (1949) found that when vitamin B_{12} was provided in the diet, low levels of choline an methionine were able to prevent fatty liver, hemorrhagic kidney in rats and to promote growth in chicks but when vitamin B_{12} was added to a diet containing an adequate level of

choline no increase ight gain was observed. These workers also noted growth response in chicks to methionine, homocystine, or homocystine an. betaine only when vitamin B_{12} was added to the diet. These findings are confirme. by Gillis and Norris (1949) who reported th t vitamin B_{12} exerted a sparing action on the chick requirement for labile methyl groups (choline an' betaine) indicating that at least one metabolic function of vitamin B_{12} is concerned with transmethylation. Similarly it has been found that fish meal (Patton et al, 1946) and vitamin B_{12} (Briggs et al, 1950) lowered the requirement of the chick for methionine. Jukes and tokstad (1949 and 1950) also demonstrated that choline and methionine requirement in the chick for maximum growt' was greater when vitamin B_{12} was not contained in the diet. relationship between vitamin B_{12} and methionine had also been demonstrated in the pig by Cunha et al, (1949).

The results show that when diets contained no vitamin B_{12}, .064% to .087% of essential choline was required for growth if betaine was added. In contrast, when diets contained vitamin B_{12}, .054% to .067% of e.sential choline was adequate for promoting growth. These results indicate that under the condition of these experiments, choline used by the chick can ivided into essential and non-essential choline. Essential choline cannot be replaced by betaine. This finding is similar to those of McKittrick (1948) who found that the choline requirement of the chick for any given rate of growth on a homocystine supplemented diet was a roximately two-thirds replaceable by betaine. For optimal gains chicks required close to 0.06% betaine-irreplaceable choline and 0.14% replaceable choline in the diet. This figure of 0.06% irreplaceable choline is in close agreement to those presented herein and also is in agreement with an approximate figure of 0.05% found in the presence of surplus methionine or methionine plus betaine (Almquist an Grau, 1944).

However, results presented herein show that vitamin B_{12} influences the requirement for choline. Under the condition of these experiments choline cannot be synthesized in the body of the chick. However, Schaefer et al. (1951) demonstrated that vitamin B_{12} appeared to be involved in the synthesis of choline from ethylaminoethanol in the presence of methyl donors such as methionine or betaine. The workers explained the ability of the chick to utilize methylaminoethanol plus betaine or methionine as a replacement for the dietary choline essential for growth and prevention of perosis when the diet contained vitamin B_{12} as follows: (1) The diet contained sufficient methyl to methylate dimethylaminoethanol to choline and vitamin B_{12} is involved in this transfer; (2) Vitamin B_{12} functions in the utilization of dimethylaminoethanol as a replacement for choline; (3) Vitamin B_{12} stimulates the synthesis of methyl groups and functions in the synthesis of choline through transmethylation.

Furthermore, in the rat, choline synthesis from either betaine or methionine has been reported by other workers (du Vigneaud et al, 1939; Griffith, 1941; and Stetten, 1941).

Betaine had a supplementary effect on chick growth when it was added to the diet containing .104% choline but it did not have a supplementary effect when it was added to the diet containing only .054% choline. Therefore, betaine demonstrated a growth promoting action only when sufficient essential choline was supplied. This result is in agreement with the findings of McGinnis et al. (1944) who reported that the addition of betaine or methionine to the chick diet containing .08% choline improved chick growth. They assumed that both supplements assisted the choline in a methylating capacity, thereby increasing the choline supply available for growth. These workers also found that in other diets in which the choline level (.03%) was less than the level of essential choline, very little or

no improvement was noted from methionine or betaine addition. The addition of choline resulted in a strong response in every case.

Vitamin B_{12} increases the severity of perosis of chicks fed low-choline diets. Also, betaine did not prevent perosis. These findings were similar with those of Jukes and Stokstad (1951). The fact that betaine replaced non-essential choline for growth but was not effective for the prevention of perosis is also in agreement with the work of Jukes and Welch (1942).

The ineffectiveness of betaine and methionine in promoting growth and preventing perosis in choline deficiency indicates that the chick did not synthesize choline frommethionine or betaine to any measurable degree. Betaine may even be formed in the chick from choline since a choline sparing action on betaine is distinctly indicated. Similar results were reported by Jukes (1940) in that methionine was ineffective for the prevention of perosis. These findings are also in agreement with those of McGinnis et al. (1944) who found that betaine and methionine were ineffective in promoting growth and preventing perosis in the chick when added to purified diets but they were shown to be effective when added to a simplified diet. In contrast, choline prevented perosis and promoted growth on both diets. They, therefore, suggested that chicks were able to synthesize choline from betaine and methionine when the simplified diet was used.

Vitamin B_{12} did not influence the hemoglobin level. This is in apparent agreement with the observations of Nichol et al. (1949), who reported that vitamin B_{12} alone did not influence hemoglobin regeneration in the chicks. Furthermore, injections of folic acid alone restored the hemoglobin value to normal. These workers found that the combination of vitamin B_{12} and folic acid caused a more rapid regeneration of hemoglobin

than treatment with a similar dosage of folic acid alone. Moreover, Milligan (1950) reported that hemoglobin values of pullets were not reduced by the low dietary level of vitamin B_{12}.

Under the condition of these experiments, choline and betaine had little effect on the hemoglobin level of blood in the chicks. However, Engel (1946) demonstrated that choline chloride prevented anemia in dogs, rats and humans. This may be explained by the fact that methionine which is contained in the diet is essential for hemoglobin formation. Choline spares methionine for methylation functions and is not required for hemoglobin formation in the chick. Folic acid which is also contained in the diet is essential for hemoglobin formation. Vitamin B_{12} may stimulate bacterial synthesis of some folic acid.

Choline has a lipotropic effect by stimulating the rate of turnover of phospholipid formation in the liver. Similar results were obtained in rats (Best, 1932 and 1934) and in ducklings (Bernard and Demers, 1949), but limited data were reported from chick studies. The results also indicate that when diets contained methionine or betaine, the liver fat was increased. It may be assumed that methionine or betaine was anti-lipotropic. The result that methionine or betaine failed to replace choline in its lipotropic action in the chick is also similar to those of Bernard and Demers (1949) in ducklings.

Vitamin B_{12} decreased the fat content of the liver. This lipotropic effect of vitamin B_{12} for the chick is in agreement with the results obtained with rats (Drill and McCormick, 1949; and Gyorgy and Rose, 1950) and dogs (Burns and McKibbin, 1951). These findings may also be related to the results of Bernhardt *et al.* (1950) on the growth-promoting effect of vitamin B_{12} in mice receiving varying proportions of fat and protein in their diets.

SUMMARY

When an adequate level of vitamin B_{12} was present in the diet, .104% of total choline was sufficient for normal growth and the prevention of perosis in chicks. In contrast, when vitamin B_{12} was not present in the diet, .104% to .154% of total choline was required for growth and the prevention of perosis.

Betaine had a supplementary effect on chick growth when it was added to the diet containing .104% of choline. However, betaine did not have a supplementary effect when it was added to this diet containing only .054% choline. Therefore, betaine exerted a growth promoting action only when sufficient essential choline was supplied. Under the conditions of these experiments, betaine alone did not improve growth or prevent perosis. Choline itself was required for the prevention of perosis. Therefore, it appears that in the presence of an adequate level of vitamin B_{12} more than .054%, but not more than .067%, of essential choline is required for normal growth and the prevention of perosis.

When the diet contained no supplemental vitamin B_{12}, .064% to .067% of essential choline was required to promote growth and prevent perosis when betaine was included in the diet. When an adequate amount of vitamin B_{12} was supplied, more than .054%, but less than .067%, of essential choline was required for normal growth but .060% to .067% of essential choline was required to prevent perosis. When the basal diet contained no choline, vitamin B_{12} increased the severity of perosis of chicks. However, when choline was present vitamin B_{12} did not effect perosis.

When a practical diet containing no vitamin B_{12} was fed to chicks, the addition of methionine or choline resulted in a marked growth response. When choline was supplied with added methionine in the absence of vitamin

B_{12}, the growth response was greater. However, in the presence of vitamin B_{12} the addition of choline was not required in the diet which contained .1% methionine. In the presence of vitamin B_{12} the addition of methionine also was not required when the diet contained 200 mg of choline per lb.

In a purified diet, .065% choline supported normal growth and prevented perosis. The addition of methionine or betaine was not effective for growth promotion and the prevention of perosis. The vitamin B_{12} content of the diet had no effect on hemoglobin level.

Choline exerted a lipotropic effect while methionine or betaine appeared to increase the fat content in the liver of chicks. Vitamin B_{12} slightly lowered the fat content of the liver.

PART II.

ACTION OF VITAMIN B_{12} IN AMINO ACID METABOLISM.

These experiments were undertaken to investigate the effect of vitamin B_{12} on the growth inhibition and mortality of chicks fed high levels of several different amino acids and its influence on the blood levels of glucose and nitrogen containing compounds in the chick.

Experimental Procedures

New Hampshire chicks of mixed sexes from lams maintained on a wire floor were fed a vitamin B_{12}-deficient diet except as indicated. These chicks were reared in electrically heated batteries with raised wire floors. Feed and water were supplied *ad libitum*. In experiments 11, 13 and 18, day-old chicks were used. In the remaining experiments (3 to 10, 12, 14 to 17) a one-week preliminary depletion period was employed to further deplete the chicks and to standardise the effect of "carryover" of vitamin B_{12}. The composition of the vitamin B_{12}-deficient basal diets in various experiments is shown in Table 12. The percentage of crude protein and certain amino acids supplied by these basal diets was calculated to be as follows: crude protein, 21.26; arginine, 1.07; lysine, .83; methionine, .49; cystine, .31; tryptophane, .26; glycine, 2.28; histidine, .476; tyrosine, 1.05; phenylalanine, 1.20; threonine, .83; leucine, 2.81; isoleucine, .94; valine, .92. During the one week preliminary period the chicks were maintained on the respective vitamin B_{12}-deficient basal diet. At the end of the depletion period, the chicks were wing-banded, individually weighed and distributed into uniform groups on the basis of body

TABLE 12

Composition of Vitamin B_{12}-Deficient Basal Diets.

Ingredients	%		
	Experiment Number *		
	8	10, 11, 12, 18	13 - 17
Ground Yellow Corn	60.00	62.20	62.20
Corn gluten meal	10.00	7.50	7.50
Soybean oil meal (44% protein)	15.00	-	-
Soybean oil meal (50% protein)	-	25.00	25.00
Cotton Seed Meal	10.00	-	-
Limestone	1.25	1.25	1.25
Bone meal	3.25	3.25	3.25
Iodized Salt	0.50	0.50	0.50
A and D Oil (400 D and 3000 A)	0.300	0.30	0.30
$MnSO_4$	0.0125	0.025	0.025
Lysine	0.50	-	-
Methionine	0.05	-	-
Protomone	0.03	0.03	0.03
	mg 1 lb.		
Riboflavin	1.20	3.00	1.60
Niacin	8.00	33.00	10.00
Calcium pantothenate	2.50	5.00	5.00
Choline Chloride	3.00	250.0	250.0
Menadione	0.20	0.20	0.20
Folic Acid	-	0.95	0.35
Biotin	-	0.045	0.045
Pyridoxine		2.60	1.60

* Basal diet in experiment 9 was the same as that used on experiment 8, except for the addition of 2.5 mg of pyridoxine and 3.5 mg of additional calcium pantothenate per lb.

weight. Excessively large and small chicks were discarded to eliminate the very rapid and very slow growing chicks.

In experiment 8, yolk sacs were removed in order to deplete vitamin B_{12} contained in the yolk on the first day from progeny of hens fed normal diets. They were then fed the vitamin B_{12}-deficient diet for the first week. The method used in removing the yolk sac was reported by Menge et al. (1950). The number of chicks used in each experiment is noted under each table of results. In these experiments, nine different amino acids were added to the basal diets at a level of 4% as indicated. Zein was also used as supplement in two experiments. In each trial, one group of chicks received the basal diet containing a single added amino acid or zein. corresponding group received the same diet plus supplementary vitamin B_{12}. In experiments 13 to 18 each vitamin B_{12}-supplemented chick received subcutaneous injections of 1.2, 1.8 and 2.4 mcg of crystalline vitamin B_{12} at the beginning of the second, third and fourth week of age, respectively. In experiments 11, 13 and 18, 0.6 mcg of vitamin B_{12} was also injected in each vitamin B_{12}-supplemented chick at the first day of age. In experiment 8 to 10 and 12 to 13, the vitamin B_{12}-supplemented chicks received crystalline vitamin B_{12} in the diet at a level of 3 mcg per 100 gm of diet. Protomone was also used in each experiment in order to increase the dietary requirement for vitamin B_{12}. Weighing and other observations were made at weekly intervals during the experimental period. 11 experiments were terminated when the chicks were 4 weeks of age.

For blood determination in experiments 12 to 15, the 4 week old chicks were fasted for approximately 12 hours at the end of the study and a 2.5 ml of blood was removed by heart puncture from each of eight chicks per group. The blood was pooled and a group sample was used in making the determination.

In experiment 16 and 17, glycine, methionine and zein were also administered by capsule to 4 week old vitamin B_{12}-deficient chicks and corresponding controls. Following 12 hours of fasting, in experiment 16, 0.5 gm of either glycine or methionine was force-fed to each chick. In experiment 17, .2 mg of either glycine or zein was similarly administered per 100 gm of body weight. Each chick which received glycine, methionine or zein by capsule had previously received the corresponding amino acid or zein in the diet during the first 4 weeks. This procedure was adopted in an effort to magnify the changes in the levels of the various blood constituents measured. In both experiments, 2.5 ml of sample blood was taken from each bird of eight per group by heart puncture at 0, 2 and 4 hours after the administration of the capsule.

In experiments 14 and 15, a glucose tolerance test was also performed following a 12 hour period of fasting. Three gm of glucose per kg of body weight was administered orally as a 30% aqueous solution. A blood sample was taken just prior to the administration of glucose, another, one hour later, and a third 2.5 or 3 hours later as indicated. Blood was with withdrawn from 4 different chickens for each sample.

In all experiments, .1 ml of 10% potassium oxalate per 5 to 10 ml of blood was used as the anticoagulant. The protein-free blood filtrates were prepared from the pooled sample and analyzed for glucose, non-protein nitrogen, urea nitrogen and uric acid and creatinine. The protein was precipitated with 2.5% zinc sulfate and .15 N barium hydroxide for the samples used in glucose tolerance determination in experiments 14 and 15. The tungstic acid method was used in the remaining determinations. This protein-free blood filtrate was prepared by the method of Haden's modification (1923) as described in Hawk, Oser and Summerson(1947). The method of Somogyi (1945) was used for the determination of glucose. The method

of Koch and McMeekin for nonprotein nitrogen; Brown for uric acid; Folin and Wu for creatinine and Sanielson for amino nitrogen as modified by Hawk, Oser and Summerson (1947) were used. Urea nitrogen was determined by the method outlined by Kibrick and Skupp (1950). For nonprotein nitrogen and amino nitrogen determination, a few modifications were made. In the determination of nonprotein nitrogen, 2 ml of filtrate and 3 ml of water were used in each sample instead of 5 ml of the blood filtrate. Samples were chilled in the ice bath for 10 min. before adding Nessler's reagent. Two drops of gum ghatti solution was added just before adding Nessler's reagent and after chilling.

In amino nitrogen determination, 2 ml of the blood filtrate and 3 ml of water were used in each sample instead of 5 ml filtrate. The final colored solution was diluted to a volume of 35 ml. In all determinations after the color was developed, its density was determined by a Leitz Rouy-Photometer.

Effect of Vitamin B_{12} on Chick Growth and Blood Levels of Various Nitrogen Containing Compounds and Glucose

The results are given in Table 13. The average weight gain of vitamin B_{12}-deficient chicks was 153 gm and those of chicks receiving an adequate amount of vitamin B_{12} was 244 gm. The difference between average weight gain of the chicks receiving no supplemental vitamin B_{12} and those receiving an adequate amount reveals that a marked vitamin B_{12}-deficiency existed. It is evident from the data that the blood level of nonprotein nitrogen was significantly higher in vitamin B_{12}-deficient chicks than in the normal controls. Moreover, the blood levels of amino nitrogen, urea nitrogen and creatinine were also consistantly higher in vitamin B_{12}-deficient chicks than in those receiving an adequate level of vitamin B_{12}. The differences noted were statistically significant. Also a slight but consistant difference existed between the levels of blood glucose in vitamin B_{12}-deficient chicks and the normal controls. This difference was also statistically significant. The differences of average weight gain, nonprotein nitrogen and amino nitrogen between vitamin B_{12}-deficient and the normal controls were statistically significant to a 1% level and the differences of urea nitrogen and creatinine and glucose between them were statistically significant to a 5% level. The uric acid level, however, revealed no consistant differences, being little affected in general as result of vitamin B_{12} administration.

Effect of Vitamin B_{12} on Growth Depressing Action of Glycine and L-Leucine

The average gain and mortality observed in chicks are presented in Tables 14 and 15. The relative average gain of the various groups of

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Effect of Vitamin B_{12} on Chick Growth and Blood Levels of Various Nitrogen Containing Compounds on Glucose

	Exp. no. 1/	Ave. Gain during exp. period (gm)	Blood Analysis mg %					
			NPN	Amino N	Urea N	Uric Acid	Creatinine	Glucose
No Supplemental Vitamin B_{12}	12	174	46	21.0	12.0	4.10	.80	177.5
	13	183	45	22.5	6.0	3.10	.65	190.0
	14	117	40.0	25.0	9.0	2.60	.65	192.5
	15	145	45.0	23.5	9.25	1.85	.43	190.0
	16	128	47.0	22.0	3.75	3.70	.62	180.0
	17	168	46.0	23.5	18.5	4.20	.90	180.0
	Av.	153	44.3	22.9	9.8	3.30	.68	185.0
Supplemental Vitamin B_{12} 2/	12	244	41.0	20.0	7.9	3.80	.50	170.0
	13	267	35.0	21.5	5.5	3.10	.50	177.0
	14	231	36.7	24.0	6.0	2.40	.60	172.0
	15	230	40.0	21.0	6.1	1.85	.40	185.0
	16	252	37.5	20.5	3.4	3.50	.50	175.0
	17	239	42.0	21.5	15.75	6.00	.60	175.0
	Av.	244	37.9	21.4	7.8	3.88	.52	175.7
Average Difference		9.1**	6.2**	1.5**	2.0*	.58	.16*	9.3*

1/ In experiment 13, day-old chicks were used and a 4-week experimental period was employed. In the remaining experiments, a 1-week preliminary period followed by a 3-week experimental period was used.

2/ Crystalline vitamin B_{12} was injected in all experiments except experiment 12, where it was administered orally.

* Difference statistically significant to the 5% level.

** Difference statistically significant to the 1% level.

Table 14

Effect of Vitamin B_{12} on Growth Depressing Action of Glycine

Exp. No. [1]	Glycine added %	Average gain during experimental period (gm)		Liver storage	
		With B_{12} [2]	Without B_{12}	With B_{12}	Without B_{12}
8 [3]	0	264 (100)	249 (100)	5.5	22.0
	4	236 (89.4) [4]	186 (74.7)	5.5	22.0
9	0	273 (100)	237 (100)	5.5	27.5
	4	274 (100.4)	230 (97.0)	5.5	33.0
10	0	265 (100)	176 (100)	0	35.7
	4	245 (92.4)	137 (77.8)	11.7	43.0
13A	0	258 (100)	183 (100)	0	55.0
	2	269 (104.2)	143 (78.2)	0	65.0
	4	247 (95.7)	113 (61.8)	5.0	65.0
	6	230 (89.2)	145 (79.2)	35.0	55.0
13B		267 (100)	183 (100)	5.0	55.0
		223 (85.4)	143 (78.2)	5.0	65.0
		247 (92.5)	113 (61.8)	0	65.0
		219 (82.1)	145 (79.2)	20.0	55.0
16	0	252 (100)	128 (100)	0	28.0
	4	246 (97.6)	108 (84.4)	11.1	38.9
17	0	239 (100)	168 (100)	0	16.7
	4	234 (98.0)	125 (74.0)	0	61.1
Average	0	260 (100)	187 (100)	2.3	34.3
	4	247 (95.1)	144 (76.2)	6.2	46.9

1/ 18 chicks per group were used in experiments 8, 9, 16 and 17. 20 chicks per group were used in experiments 13A and 13B and 10 chicks were distributed into groups in experiment 10. Experiments 13A and 13B were day-old chicks at start. The remaining experiments were week-old chicks.

TABLE 14
continued

Effect of Vitamin B_{12} on Growth
Depressing Action of Glycine

2/ 30 mcg crystalline vitamin B_{12} per kg of diet were fed in experiments 8, 9, 10 and 13A. The rest of the experiments (13B, 16 and 17) were injected with vitamin B_{12} weekly as described in Table 13.

3/ Yolk sacs were removed at first day from chicks hatched from hens fed normal diets. They were on vitamin B_{12}-deficient diets at the first week and then distributed on the basis of weight at the second week.

4/ These numbers in parenthesis represent the relative gain expressed as a percentage of that obtained with the corresponding control chicks fed the same diet without the added amino acid.

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Effect of Vitamin B_{12} on growth depression caused by excess dietary L-leucine.

Exp. No.	L-leucine added %	Average gain during experimental period (gm)		Mortality	
		With B_{12}	Without B_{12}		B_{12}
2/	0	273 (100)	237 (100) 1/	5.5	27.5
	4	277 (101.5)	184 (77.6)	5.5	17.0
13/	0	191 (100)	141 (100)	0	55.6
	4	204 (106.6)	111 (78.7)	0	22.2
14/	0	230 (100)	145 (100)	0	50.0
	4	223 (97)	109 (75)	0	23.0
15/	0	242 (100)	131 (100)	5.3	63.2
	4	234 (96.7)	135 (74.5)	0	26.3
Average	0	234 (100)	176 (100)	2.7	49.1
	4	235 (100.4)	134 (76.1)	1.4	23.4

1/ Table 14 - note 4/.

2/ Vitamin B_{12}-deficient chicks hatched from Beltsville were put on basal (vit. B_{12}-deficient) diet for one week and then they were distributed into 15 chicks per group.

3/ 15 two-day-old vitamin B_{12}-deficient per group start.

4/ 18 week-old vitamin B_{12}-deficient chicks were distributed into experimental diets. They were fed vitamin B_{12}-deficient basal diet at the first week.

5/ 15 day-old vitamin B_{12}-deficien

6/ Same as in Table 14 - note 4/

chicks, expressed as a percentage of that obtained with the corresponding control chicks fed the same diet without the added amino acids is also given in these tables.

In Table 14, the average gain of vitamin B_{12}-deficient chicks was 144 gm and those of vitamin B_{12}-supplemented chicks was 247 gm when they were fed on the diet containing 4% added glycine. The relative average gain obtained in 7 experiments in the presence of vitamin B_{12} was 95.1% an in the absence of vitamin B_{12} was 76.2%. These results clearly show that in all experiments the addition of 4% glycine to the diet inhibits the growth and increases mortality of chicks fed vitamin B_{12}-deficient diets. The toxicity of glycine was overcome by the administration of vitamin B_{12} either by injection as in experiments 13B, 16 and 17, or by feeding as in experiments 3, 9, 10 and 13A.

Similar results are given in Table 15. The average gain of chicks fed diets containing 4% added leucine administered with vitamin B_{12} was 234 gm and those given no vitamin B_{12} was 134 gm. The average relative gain with vitamin B_{12} was 100.4% an without vitamin B_{12} was 76.2%. These data show that growth of vitamin B_{12}-deficient chicks was inhibited markedly by the addition of 4% leucine to the diet. However, the growth inhibitory action of leucine was completely overcome by the administration of vitamin B_{12}. The mortality of chicks fed the vitamin B_{12}-deficient diet containing 4% added leucine was less than that of chicks fed the same diet without the added amino acid. Administration of vitamin B_{12}, however, markedly reduced the mortality regardless of the leucine included in the diet.

Effect of Vitamin B_{12} and Several Other Individual Amino Acid Imbalances on Chick Growth.

The results are given in Table 16, 17 and 18 an Figure 3. The relative average gain of the various groups of chicks, expressed percen-

TABLE 16

Effect of Vitamin B_{12} on Toxicity of Some Amino Acids.

Exp. No.	Supplement	Average Gain during experimental period (gm)		% Mortality	
		With B_{12}	Without B_{12}	With B_{12}	Without B_{12}
12/	0	273 (100)	237 (100)	5.5	27.5
	4% L-glutamic acid	273 (100) 1/	216 (91.1)	5.5	27.5
	4% DL-lysine	244 (89.4)	183 (77.2)	11.0	16.5
13/	0	231 (100)	145 (100)		56.3
	4% L-glutamic acid	224 (97.0)	136 (93.8)		50.0
	4% DL-lysine	193 (98.6)	143 (83.5)	0	18.8
		244 (100)	174 (100)	^	53.0
	4% DL-lysine	256 (104.2)	176 (100.9)	-	23.5
	0	230 (100)	145 (100)	^	50.0
	4% L-tyrosine	185 (80.4)	108 (74.4)	0	11.1
	0	252 (100)	128 (100)	^	28.0
	4% DL-aspartic acid	169 (67.1)	76 (59.4)	22	55.0

1/ Same as in Table 14 - note 4/.

2/ as in Table 15.

3/ 13 week-old vitamin B_{12}-deficient chicks were distributed into experiment diets in experiments 12 to 16. They were on basal vitamin B_{12}-deficient diets at the first week.

TABLE 17

Effect of Vitamin B_{12} on Toxicity of Some Amino Acids.

Exp. No.	Supplement	Average gain during experimental period (gm)		% Mortality	
		With B_{12}	Without B_{12}	With B_{12}	Without B_{12}
3/	0	264 (100)	249 (100)	5.5	22
	4% DL-α-alanine	160 (60.6)	166 (66.6)	22	22
	0	244 (100)	174 (100)	0	53
	4% DL-α-alanine	186 (70.0)	155 (91.3)	5.9	53
	4% L-cystine	160 (70.9)	135 (82.1)	23.5	58.8
14	0	231 (100)	145 (100)	0	56.3
	4% DL-α-alanine	168 (72.7)	113 (81.4)	12.5	43.8
	0	230 (100)	145 (100)		50
	4% L-cystine	145 (64)	111 (77)	5.6	39
2/	0	273 (100) 1/	237 (100)	5.5	27.5
	2% L-cystine	239 (87.6)	237 (100)	0	22
16	0	252 (100)	128 (100)	0	28
	4% DL-methionine	69 (27.4)	73 (57.0)	11.1	50.6

1/ Same as in Table 14 - note 4/.

2/ Same as in Table 15.

3/ Same as in Table 14.

TABLE 18

Effect of Vitamin B_{12} and Amino Acid Imbalances on Chick Growth (Summary)

Supplement	No. of trials	Average gain during experimental periods (gm)		% Mortality	
		With B_{12}	Without B_{12}	With B_{12}	Without B_{12}
0	2(37)*	240	175	2.6	40.0
6-8% zein		232 (96.7)**	122 (69.8)**	2.6	45.3
0	4(53)	234	176	2.7	49.1
4% L-leucine		235 (100.4)	134 (76.2)	1.4	23.4
-	7(128)	260	187	2.3	34.3
4% glycine		247 (95.1)	144 (76.2)	6.2	46.9
0	3(55)	249	188	3.6	49.0
4% L-glutanic acid		240 (96.4)	177 (94.1)	1.8	41.6
0	3(54)	249	185	1.8	45.6
4% L-lysine		231 (92.8)	167 (90.3)	3.7	19.6
0	1(18)	230	145	0	50.0
4% L-tyrosine		185 (80.4)	108 (74.5)	0	11.1
-	1(18)	252	128		20.0
Aspartic Acid		169 (67.1)	76 (59.4)	22	55.0
0	3(54)	246	189	1.8	43.8
4% L-α-alanine		171 (69.5)	146 (77.3)	13.5	39.6
0	3(54)	249	185	1.8	43.5
4% L-cystine		181 (72.7)	161 (87.2)	9.7	39.9

TABLE 13
continued

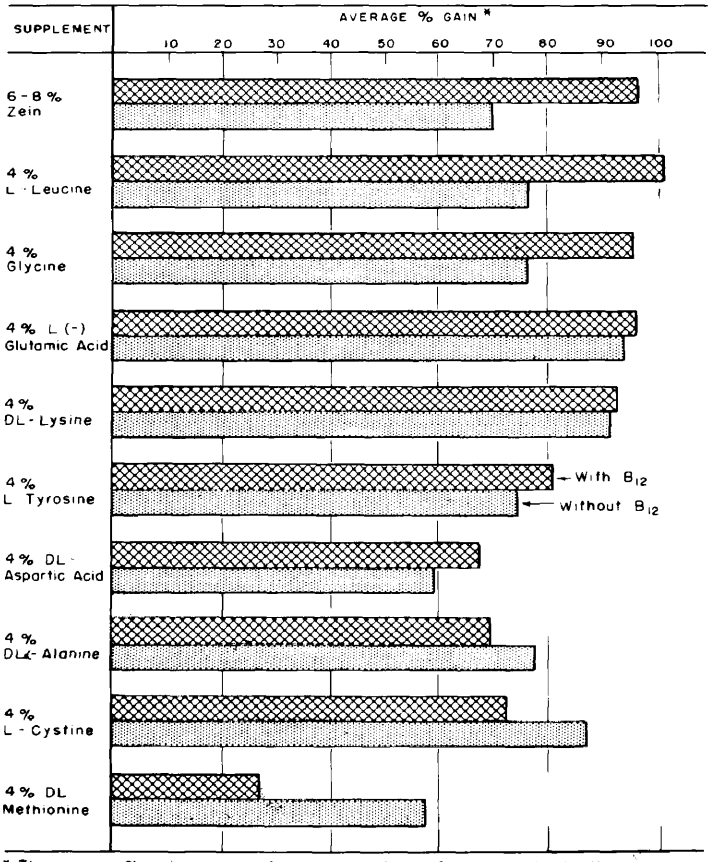
Effect of Vitamin B_{12} and Amino Acid Imbalances on Chick Growth (Summary)

Supplement	No. of trials	Average gain during experimental periods (gm)		% Mortality	
		With B_{12}	Without B_{12}	With B_{12}	Without B_{12}
0	1(18)	252	128	0	28.0
4% DL-methionine		69 (27.4)	73 (57.0)	11.1	50.6

Refer to number of total chicks used per treatment.

** These numbers represent the relative gain expressed as a percentage of that obtained with the corresponding control chicks fed the same diet without the added amino acid.

Figure 3 (From Table 18) Effect of Vitamin B_{12} and Amino Acid Imbalances on Chick Growth (Summary)



* The average % gain expressed as a percentage of that obtained with the corresponding control chicks fed the same diet without the added Amino Acid

tage of that obtained with the corresponding control chicks fed the same diet without the added amino acids is also given in these tables. The relative average gain of chicks fed diets containing 4% added glutamic acid and lysine in presence of vitamin B_{12} was 96.4% and 92.8% respectively, and in the absence of vitamin B_{12} was 94.1% and 90.3% respectively. Therefore, the addition of glutamic acid or lysine exerted only a slight growth inhibitory action even in the absence of an adequate intake of vitamin B_{12}. Furthermore, vitamin B_{12} administration was of little or no benefit to the diets containing the added amino acid. The relative average per cent gain of chicks fed diets containing 4% added tyrosine and aspartic acid with vitamin B_{12} were 80.4% and 67.1% respectively, and without vitamin B_{12} were 74.9% and 59.4% respectively. The amino acids, tyrosine and aspartic acid depressed chick growth when added singly at a level of 4% to the vitamin B_{12}-deficient diets. The administration of vitamin B_{12} to diets containing these amino acids exerted only a slight effect in overcoming this growth depression. The addition of 4% added alanine, methionine and cystine, the average relative per cent of vitamin B_{12}-deficient chicks were 77.3%, 57.0% and 87.2% respectively, and those of chicks administered vitamin B_{12} were 69.5%, 27.4% and 72.7% respectively. These data show that alanine, methionine and, to a lesser extent, cystine exerted growth inhibitory effects but the administration of vitamin B_{12} was of no value in alleviating this condition.

Except for aspartic acid, alanine, methionine and cystine, the addition of high levels of each amino acid to the diets did not appreciably affect the per cent mortality when vitamin B_{12} was supplied. The addition of 4% aspartic acid, methionine or 6 - 8% zein to the vitamin B_{12}-deficient basal diets increased the mortality while the addition of glutamic acid, alanine or cystine had little effect. On the other hand, the mortality

of chicks fed the vitamin B_{12}-[illegible] lot containing either 4% added lysine or tyrosine [illegible] less than [illegible] for the same diet without the added amino acid. [illegible]nistration vitamin B_{12}, however, markedly reduced [illegible] mortality regardless [illegible] acid included diet.

Effect of Vitamin B_{12} on Growth Inhibitory Action of Zein, L-leucine and L-glutamic acid.

Since leucine was found to exert a marked growth inhibitory effect which could be overcome by administration of vitamin B_{12}, two experiments were performed to determine the effect of zein on chick growth. Zein contains high levels of leucine and glutamic acid and, therefore, the addition of zein to the diet would greatly affect the levels of these amino acids fed. In the first trial (experiment 17) zein was fed at a level of 6% while in the second, (experiment 18), 8% zein was used. In experiment 18, leucine and glutamic acid were added singly to the diet at a level of 4%, also, one group of chicks received 1.6% added leucine plus 2.4% added glutamic acid. These quantities of leucine and glutamic acid are comparable to those supplied by the addition of 8% zein. Vitamin B_{12} was administered by injection in these two trials. The results obtained in these two experiments are given in Table 19. When either zein or 8% zein was added, the relative per cent gain of vitamin B_{12}-deficient chicks was 74% or 66.3% respectively, and those of chicks administered with vitamin B_{12} was 100% and 93.4% respectively. These results show that the addition of either 6% or 8% zein to the diet of vitamin B_{12}-deficient chicks depressed growth in a manner similar to that obtained from the addition of leucine. Likewise, the administration of vitamin B_{12} was effective in counteracting this growth inhibition. The addition of

Effect of Vitamin B_{12} on Growth Promoting Action of Zein, L-leucine L-glutamic acid.

Exp. No.	Supplement	Average gain during experimental period (gm)				% Mortality	
		With B_{12}		Without B_{12}		With B_{12}	Without B_{12}
17 2/	0	239	(100)	168	(100)	0	16.7
	6% Zein	238	(100) 1/	125	(74)	0	22.2
18 3/	0	242	(100)	181	(100)	5.3	63.2
	8% Zein	226	(93.4)	120	(66.3)	5.3	68.4
	1.6% L-leucine + 2.4% L-glutamic acid	256	(105.7)	122	(67.4)	5.3	26.3
	4% L-glutamic acid	222	(91.7)	178	(98.3)		47.4
	4% L-leucine	232	(95.8)	135	(74.5)		26.3

1/ Same as in Table 14 - note 4/.

2/ 18 week-old vitamin B_{12}-deficient chicks were distributed into experimental diets. They were on basal vitamin B_{12}-deficient diets for one week.

3/ 10 day-old vitamin B_{12}-deficient chicks were distributed into groups.

tamic acid alone had only a slight effect on the rate of gain regardless of the amount of vitamin B_{12} supplied. However, when leucine and glutamic acid were included in the vitamin B_{12}-deficient diet at levels comparable to that supplied by 8% zein, growth depression resulted which overcome by the administration of vitamin B_{12}. The imbalance produced by the addition of zein appe explained primarily on the basis of the increased leucine content of the diet. These findings indicate that the amino acid balance may important factor in determining the vitamin B_{12} requirement of growing chicks.

Effect of Different Levels of Vitamin B_{12} on Glycine and L-leucine Toxicity.

In experiments 10 and 11, different levels of vitamin B_{12} were fed to chicks receiving diets containing no added amino acids and diets containing either 4% glycine or leucine. The chicks used were from dams which have received 1.5 mcg of vitamin B_{12} per lb. of ration for a period of 2 and 4 weeks respectively. The results are shown in Tables 20 and 21. The data given in Table 20 indicates that the growth inhibitory action of glycine was overcome by either 1, 5 or 30 mcg of vitamin B_{12} per kg of diet. One mcg vitamin B_{12} per kg of diet overcame the glycine toxicity partially, 5 mcg vitamin B_{12} per kg was even more effective, and 30 mcg vitamin B_{12} counteracts the toxicity almost completely. Therefore, more than 5 mcg, but probably less than 30 mcg vitamin B_{12} per kg of diet was necessary for counteracting the glycine toxicity. It can be seen from the data in Table 21 that vitamin B_{12} also overcame the toxicity of leucine even at low level. Five micrograms vitamin B_{12} per kg diet counteracts the growth inhibitory action of leucine almost as effectively as 30 mcg of vitamin B_{12} per kg of diet. The vitamin B_{12} requirement was greater, therefore, when

TABLE 20

Effect of Different Levels of Vitamin B_{12} on Glycine Toxicity

Exp. No.	Supplement Vitamin B_{12} added mcg/kg diet	Average gain during 4 weeks (gm)			% Mortality	
		Without Glycine	With Glycine		Without Glycine	With Glycine
	none	176	137	(77.8)*	35.7	42.9
	1	185	161	(87.0)	7.7	21.4
10**	5	245	211	(86.1)	12.5	0
	30	265	245	(92.4)	0	7.7

Same as in Table 14 - note 4/.

16 week-old vitamin B_{12}-deficient chicks were distributed into groups. They were on preliminary vitamin B_{12}-deficient diet at the first week.

TABLE 21

Effect of Different Levels of Vitamin B_{12} on L-leucine Toxicity.

Exp. No.	Supplement Vitamin B_{12} added mcg/kg diet	Average gain during experimental period (gm)		% Mortality	
		Without 4% L-leucine	With 4% L-leucine	Without 4% L-leucine	With 4% L-leucine
11**	0	141	111 (78.7)*	55.6	16.7
	2	192	167 (87.0)	16.7	11.1
	5	190	194 (102.1)	22.7	0
	30	191	204 (106.8)	0	

* Same as in Table 14 - note 4/.

** 18 day-old vitamin B_{12}-deficient chicks per group were distributed into experimental diets.

the added amino acid was present in the diet. Also it should be noted that the relative growth inhibition caused by the addition of either of these amino acids was progressively lessened as the vitamin B_{12} content of the diet was increased.

Effect of Feeding and Injecting Vitamin B_{12} on Growth Inhibitory Action of Glycine.

This experiment was conducted to determine whether the effect of vitamin B_{12} on glycine toxicity at 2, 4% and 6% was due to bacterial synthesis of other substances or to the action of vitamin B_{12} itself. Vitamin B_{12} was administered either by feeding at a level of 30 mcg per kg of diet or by weekly injections of vitamin B_{12} to a total of 6 mcg per chick for the 4 week experimental period. The results are given in Table 22. It appears evident that all levels of glycine were toxic to the vitamin B_{12}-deficient chicks fed the basal diet. Administration of vitamin B_{12} either orally or by subcutaneous injection as described was equally effective in overcoming the growth inhibitory action of 2%, 4% or 6% glycine. The blood nitrogen containing compounds and sugar, except uric acid, are higher in vitamin B_{12}-deficient chicks than in chicks receiving adequate amounts of vitamin B_{12} either by injection or orally. These differences were observed either in the presence or absence of glycine. The addition of glycine increased the blood nonprotein nitrogen, urea nitrogen and glucose levels. The amino nitrogen levels were little affected or lowered by the glycine supplement when vitamin B_{12} was supplied. The blood creatinine of vitamin B_{12}-deficient chicks fed diets containing added glycine was lower than that of chicks fed the unsupplemented basal diets. However, the addition of glycine to the diet of

TABLE 22

Effect of Feeding and Injecting Vitamin B_{12} on Growth Depressing Action of Glycine at Different Levels and Their Blood Glucose and Various Nitrogen Containing Compounds

Exp. No.				% Glycine Added 0	4	6
13			Ave. gain (gm)	183	113	145
Without Vit. B_{12}	Blood Analysis mg%		NPN	45.0	49.6	49.0
			Amino N	22.5	22.0	22.5
			Urea N	6.0	6.3	6.25
			Uric Acid	3.1	3.45	3.50
			Creatinine	.65	.65	.60
			Glucose	190	200	200
			Ave. gain (gm)	258	247	230
Feeding* Vit. B_{12}	Blood Analysis mg%		NPN	35.0	37.0	41.0
			Amino N	20.0	19.0	20.0
			Urea N	5.75	6.25	5.4
			Uric Acid	3.00	2.35	3.55
			Creatinine	.50	.64	.60
			Glucose	177	195	190
			Ave. gain (gm)	267	247	219
Injecting** Vit. B_{12}	Blood Analysis mg%		NPN	30.0	36.0	34.0
			Amino N	21.5	21.5	20.5
			Urea N	5.5	5.9	5.75
			Uric Acid	3.1	3.85	3.35
			Creatinine	.50	.55	.50
			Glucose	177	-	192

* 30 mcg crystalline vitamin B_{12} per kg of diet.

** Vitamin B_{12} was injected as in previous tables.

chicks receiving an adequate vitamin B_{12} increased the blood creatinine level. Only slight changes were observed in the levels of uric result of [illegible] lysine.

Effect of Vitamin B_{12} and Amino Acid Imbalances on Certain Blood Constituents: Nonprotein Nitrogen, Amino Nitrogen, Urea Nitrogen, Uric Acid, Creatinine and Glucose.

The blood levels of nonprotein nitrogen, amino nitrogen, urea nitrogen, uric acid, creatinine and glucose determined in these experiments are presented in Tables 23 to 28. In order to facilitate their presentation, the values obtained in different trials involving the same amino acid supplement are given as averages as shown in Figures 5 to 10 which are obtained from Tables 33 to 38 included in the Appendix. These values are also indicated as relative percentage of the respective average value for the corresponding controls which received an adequate intake of vitamin B_{12} with no supplemental amino acid or zein.

The results in Tables 23 and 33 and Figure 4 reveal that the addition of single amino acids or zein consistantly increased the blood nonprotein nitrogen level in the presence of vitamin B_{12}. Similar increases were noted when no vitamin B_{12} was given except in chicks receiving additional cystine or methionine. Despite the marked effect of vitamin B_{12} in lowering the abnormally high nonprotein nitrogen level of the blood in vitamin B_{12}-deficient chicks it should be noted that the increases in nonprotein nitrogen resulting from the addition of the amino acids fed are not appreciably effected by the addition of vitamin B_{12}. The nonprotein nitrogen value of blood from chicks receiving vitamin B_{12} and 4% lysine, or 4% cystine in experiments 12 and 15, respectively, are similar to those values from chicks receiving corresponding amino acids without vitamin B_{12}.

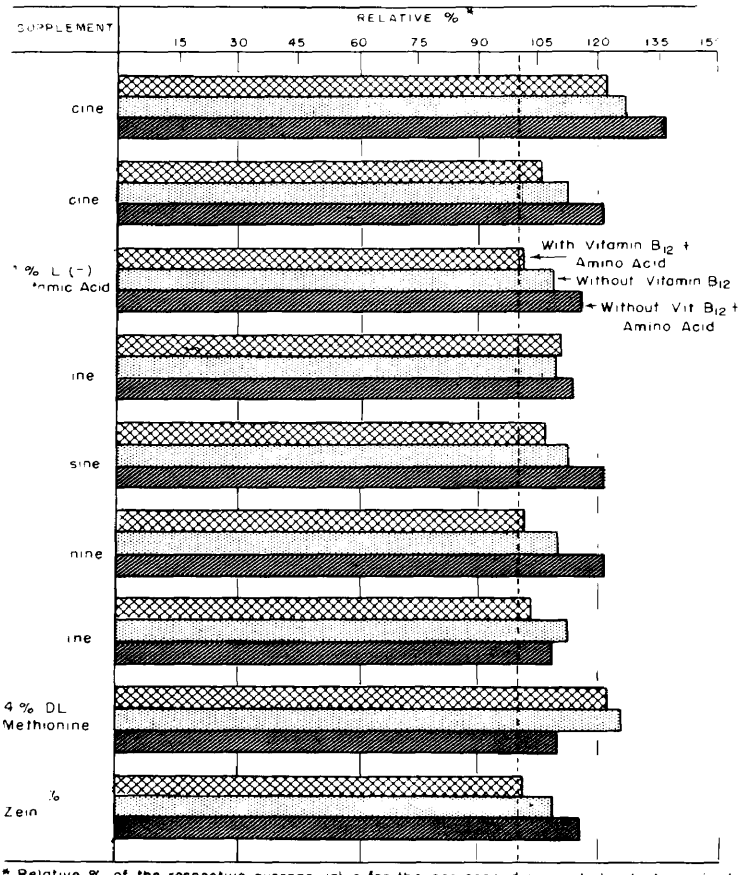
TABLE 23

Effect of Vitamin B_{12} on Blood NPN of Chicks Fed Excesses of Various Amino Acids or Zein.

Exp. No.	Supplement	mg % With B_{12}	mg % Without B_{12}
13A	0	35.0	45.0
	4% glycine	37.0	49.6
	6% glycine	41.0	49.0
13B	0	30.0	45.0
	4% glycine	36.0	49.6
	6% glycine	30.0	49.0
16	0	37.5	47.0
	4% glycine	46.5	50.0
	4% DL-methionine	46.0	41.0
17	0	42.0	46.0
	4% glycine	42.5	48.5
	6% zein	43.0	49.0
15	0	40.0	45.0
	4% L-leucine	42.7	48.3
	4% L-tyrosine	43.0	48.3
	4% L-cystine	44.0	45.0
12	0	41.0	46.0
	4% L-cystine	40.0	43.0
	4% DL-lysine	45.0	46.0
	4% DL-α-alanine	42.0	44.0
14	0	36.7	40.0
	4% DL-α-alanine	-	50.0
	4% DL-lysine	41.9	43.3
	4% L-glutamic acid	37.6	42.7

* Crystalline vitamin B_{12} was injected as described in previous tables in all experiments except experiments 12 and 13A where it was administered by feeding 30 mcg per kg of diet.

Figure 4 (From Table 33 in Appendix) Effect of Vitamin B_{12} on Blood NPN of Chicks Fed Excess of Various Amino Acids or Zein (Summary)



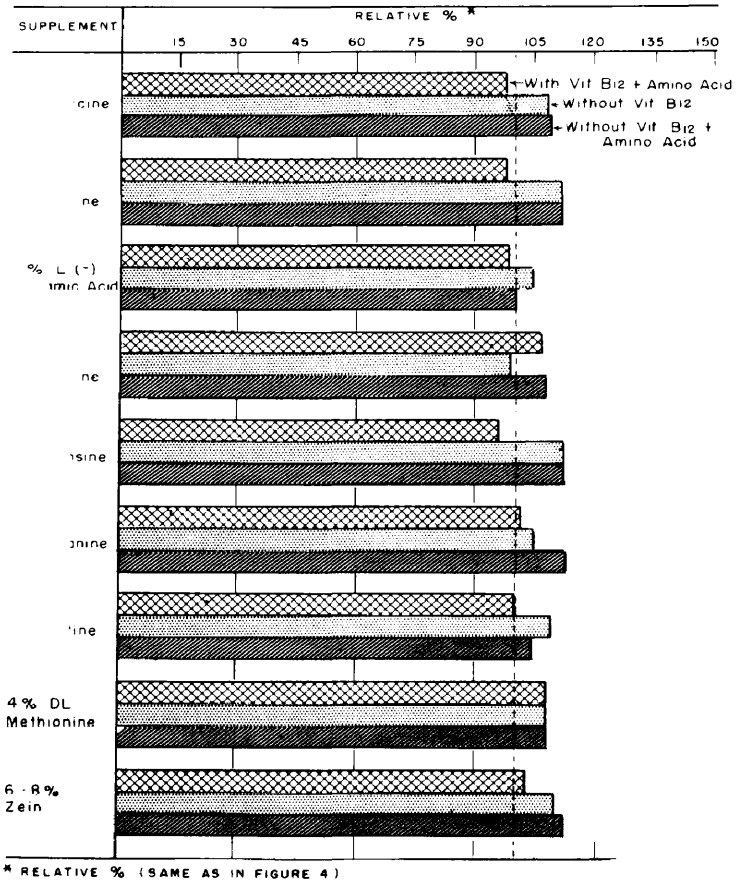
* Relative % of the respective average value for the corresponding controls which received adequate intake of Vitamin B_{12} with no supplemental Amino Acid or Zein

TABLE 24

Effect of Vitamin B_{12} on Blood Amino Nitrogen of Chicks
[illegible]

Exp. No.	Supplement	% mg	
		With B_{12}	Without B_{12}
13A	0	20.0	22.5
	4% glycine	19.5	24.5
	6% glycine	20.0	21.5
13B	0	21.5	22.5
	4% glycine	21.5	22.5
	6% glycine	20.5	21.5
	0	20.5	22.0
	4% glycine	19.5	22.5
	4% DL-methionine	22.0	22.0
	0	21.5	23.5
	4% glycine	21.0	23.5
	6% soin	22.0	24.0
	0	21.0	23.5
	4% L-leucine	20.5	23.5
	4% L-tyrosine	20.0	23.5
	4% L-cystine	21.0	22.5
	0	20.0	21.0
	4% L-cystine	19.5	20.5
	4% DL-lysine	21.5	22.0
	4% DL-α-alanine	20.5	22.1
	0	24.0	25.0
	4% DL-α-alanine	24.0	27.5
	4% DL-lysine	25.5	25.5
	4% L-glutamic acid	23.5	24.0

Figure 5 (From Table 34 in Appendix) Effect of Vitamin B_{12} on Blood Amino Nitrogen of Chicks Fed Excesses of Various Amino Acids or Zein (Summary)



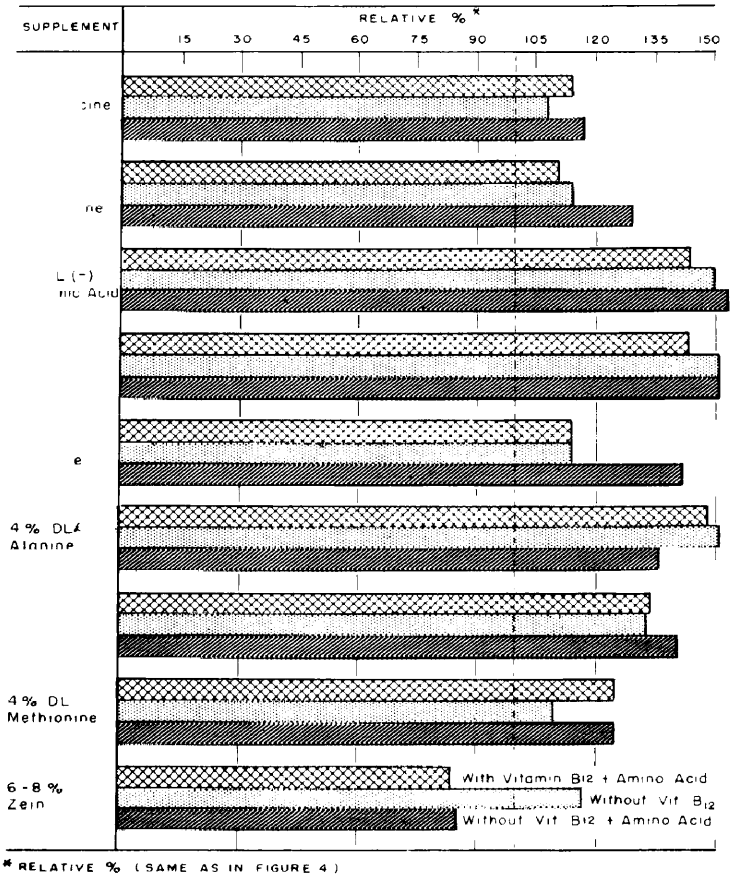
* RELATIVE % (SAME AS IN FIGURE 4)

TABLE 25

Effect of Vitamin B_{12} on Blood Urea Nitrogen of Chicks Fed Excesses of Various Amino Acids or Lein.

Exp. No.	Supplement	mg % With B_{12}	mg % Without B_{12}
13A	0	5.75	6.00
	4% glycine	6.25	6.30
	6% glycine	5.40	6.25
13B	0	5.50	6.00
	4% glycine	5.90	6.30
	6% glycine	5.75	6.25
	0	3.40	3.75
	4% glycine	4.55	4.60
	4% DL-methionine	4.25	4.25
_7	0	15.75	18.50
	4% glycine	16.25	18.50
	4% DL-methionine	13.25	15.50
	0	8.10	9.25
	4% L-leucine	9.00	10.50
	4% L-tyrosine	9.25	11.50
	4% L-cystine	10.00	11.00
	0	7.90	12.00
	4% L-cystine	11.50	11.50
	4% DL-lysine	12.00	12.75
	4% DL-α-alanine	11.30	11.30
	0	6.00	9.00
	4% DL-α-alanine	9.20	7.75
	4% DL-lysine	8.00	8.25
	4% L-glutamic acid	8.60	9.20

Figure 6 (From Table 35 in Appendix) Effect of Vitamin B_{12} on Blood Urea Nitrogen of Chicks Fed Excess of Various Amino Acids or Zein (Summary)



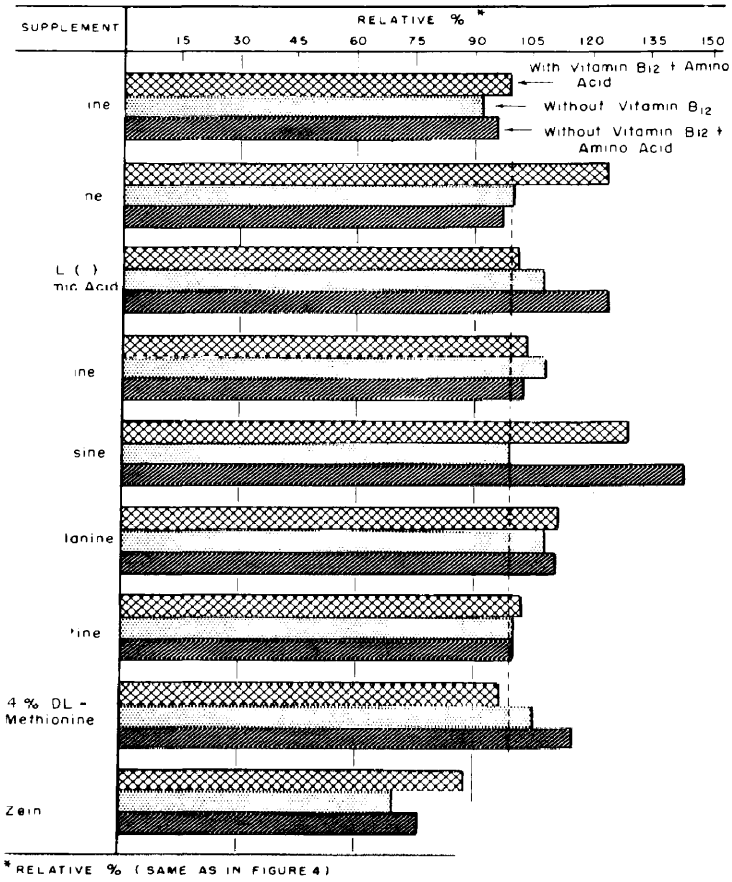
* RELATIVE % (SAME AS IN FIGURE 4)

Table 26

Effect of Vitamin B_{12} on Blood Uric Acid of Chicks Fed Excesses of Various Amino Acids or Zein.

Exp. No.	Supplement	mg % With B_{12}	mg % Without B_{12}
13A	0	3.00	3.10
	4% glycine	2.85	3.45
	6% glycine	3.55	3.70
13B	0	3.10	3.10
	4% glycine	3.85	3.45
	6% glycine	3.35	3.50
16	0	3.50	3.70
	4% glycine	3.90	3.90
	4% DL-methionine	3.40	4.05
17	0	6.00	4.20
	4% glycine	3.90	3.90
	6% zein	5.30	4.60
	0	1.85	1.85
	4% L-leucine	2.30	1.80
	4% L-tyrosine	2.40	2.65
	4% L-cystine	2.05	2.25
	0	3.80	4.10
	4% L-cystine	3.30	3.50
	4% DL-lysine	3.30	3.60
	4% L-α-alanine	4.10	4.12
	0	2.40	2.60
	4% DL-α-alanine	2.85	2.80
14	4% DL-lysine	2.65	2.75
	4% L-glutamic	2.45	2.98

Figure 7 (From Table 36 in Appendix) Effect of Vitamin B_{12} on Blood Uric Acid of Chicks Fed Excess of Various Amino Acids or Zein (Summary)



*RELATIVE % (SAME AS IN FIGURE 4)

TABLE 27

Effect of Vitamin B_{12} on Blood Creatinine Level of Chicks Fed Excesses of Various Amino Acids or Zein

Exp. No.	Supplement	γ mg %	
		With B_{12}	Without B_{12}
13A	0	.55	.65
	4% glycine	.64	.65
	6% glycine	.50	.60
13B	0	.50	.65
	4% glycine	.45	.65
	6% glycine	.50	.60
	0	.50	.62
	4% glycine	.60	.62
	4% DL-methionine	.63	.63
	0	.60	.90
	4% glycine	.75	.90
	6% zein	.76	.80
	0	.40	.48
	4% L-leucine	.40	.54
	4% L-tyrosine	.45	.54
	4% L-cystine	.45	.48
	0	.50	.80
	4% L-cystine	.45	.55
	4% DL-lysine	.58	.60
	4% DL- -alanine	.60	.55
	0	.60	.65
	4% L- -alanine	.60	.65
	4% DL-lysine	.42	.45
	4% L-glutamic acid	.56	.60

Figure 8 (From Table 37 in Appendix) Effect of Vitamin B_{12} on Blood Creatinine Level of Chicks Fed Excess of Various Amino Acids or Zein (Summary)

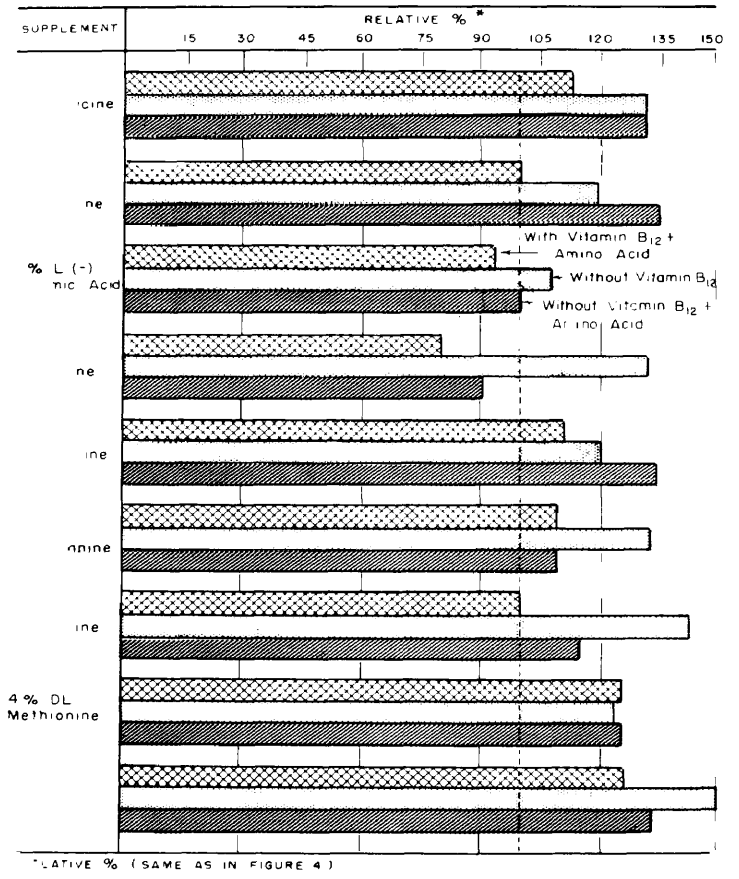
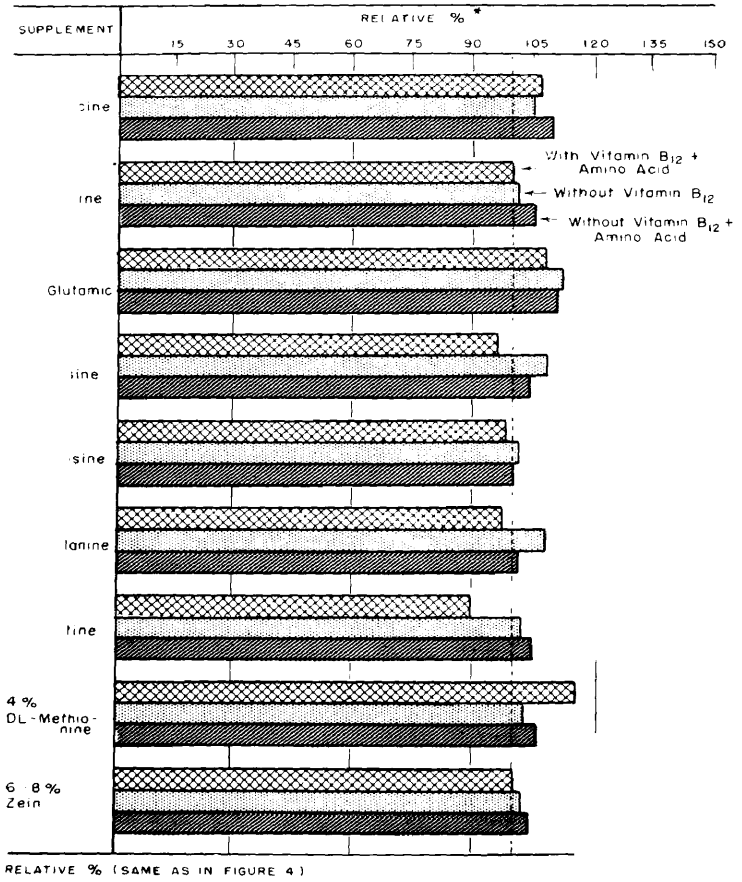


Table 3

Effect of [illegible] in B_{12} on blood glucose lev l of [illegible] on Excess o. Various Amino cids or Protein

Exp. No.	Supplement	% Hr With B_{12}	% Hr Without B_{12}
	0	177.0	190.0
	4% glycine	195.0	200.0
	6% glycine		
	0	177.0	190.0
	4% glycine	-	200.0
13B	6% glycine		
	0	175.0	190.0
	4% glycine	185.0	195.0
16	4% DL-methionine	202.0	195.0
	0	175.0	185.0
	4% glycine	184.5	183.0
17	6% zein	174.0	182.0
	0	185.0	190.5
	4% L-leucine	185.0	195.0
	4% L-tyrosine	182.5	185.0
	4% L-cystine	165.0	192.5
	0	170.0	177.5
	4% L-cystine	155.0	-
	4% DL-lysine	152.5	175.0
	4% DL-alanine	157.5	165.0
	0	172.0	192.5
	4% DL-alanine	177.0	181.0
	4% L-lysine	177.5	182.5
	4% L-glutamic acid	187.0	192.0

Figure 9 (From Table 38 in Appendix) Effect of Vitamin B_{12} on Blood Glucose Level of Chicks Fed Excess of Various Amino Acids or Zein (Summary)



These findings indicate that vitamin B_{12} counteracts the toxicity of glycine, leucine and zein almost completely but does not overcome the growth depression caused by toxicity of methionine, cystine or lysine. The blood nonprotein nitrogen level of chicks was elevated in a vitamin B_{12}-deficiency. This difference was observed either in the presence or absence of the individual amino acids added.

The results obtained from Tables 24 and 34 and Figure 5 indicate that the amino nitrogen levels were little affected by the amino acid supplements when vitamin B_{12} was supplied except for lysine and methionine where the level was slightly raised. The addition of these experiments to the vitamin B_{12}-deficient diet did not appreciably affect the amino nitrogen level except in the case of alanine where it was slightly increased. Vitamin B_{12} consistantly lowered the amino nitrogen content of blood obtained from chicks receiving diets supplemented singly with various amino acids or with no supplemental amino acids.

Data presented in Tables 25 and 35 and Figure 6 show that the level of blood urea was increased in almost all cases where the amino acid supplements were fed. The addition of zein, however, resulted in a decrease in the blood urea level both in the presence and absence of vitamin B_{12}. In all cases except when 4% alanine was added vitamin B_{12} correspondingly decreased the urea nitrogen value.

Only slight changes were observed in the levels of uric acid (Tables 26 and 36 and Figure 7) as a result of feeding high levels of amino acids. When adequate vitamin B_{12} was administered, leucine and tyrosine supplementation resulted in a slight increase. When no vitamin B_{12} was supplied, glutamic acid and tyrosine feeding also produced slightly higher blood uric acid levels. Zein, on the other hand, decreased the uric acid level when

added to the diet. Vitamin B_{12} not consistantly effect the blood uric acid level of chicks.

The results obtained in Tables 27 and 37 and Figure 8 show that the blood of vitamin B_{12}-deficient chicks fed diets containing added methionine, glycine, alanine or zein was no higher in creatinine than that of similar chicks fed the unsupplemented basal diets. However, the addition of these amino acids or zein to the diet of chicks receiving an adequate level of vitamin B_{12} increased the blood creatinine level. Feeding high level of glutamic acid or lysine decreased the blood creatinine level regardless of the intake of vitamin B_{12}. Addition of vitamin B_{12} to the diet containing various amino acids except alanine and methionine, in experiments 12 and 16, respectively, decreased blood creatinine value quite consistantly.

The results presented in Tables 28 and 38 and Figure 9 indicate that the level of blood glucose of chicks receiving vitamin B_{12} was increased when glycine, glutamic acid or methionine were added to their diets. The addition of 4% cystine, lysine, tyrosine and alanine, however, decreased the blood glucose level slightly in the presence of adequate vitamin B_{12}. The other amino acid supplements had little or no effect on the blood glucose level. In all cases, except when methionine was used, vitamin B_{12} correspondingly decreased the blood glucose level of chicks.

Effect of Vitamin B_{12} and Amino Acid Imbalance on Blood Glucose Tolerance Test.

Glucose tolerance tests were done with vitamin B_{12}-deficient chicks and chicks receiving vitamin B_{12} previously fed either the basal diet or the basal diet plus 4% of a single added amino acid. The results are given in Table 29. It was observed that vitamin B_{12}-deficient chicks consistantly had higher blood glucose levels during the test than chicks

TABLE 29

Effect of Vitamin B_{12} on Blood Glucose Tolerance Test of Chicks Fed with Various Amino Acids.

Exp. No.	Supplement	% mg					
		0 hr.*:	0 hr.*:	1 hr.:	1 hr.:	2.5-3hr:	2.5-3hr
		+ B_{12}	- B_{12}	+ B_{12}	- B_{12}	+ B_{12}	- B_{12}
14	none	172.0	192.5	221.0	230.0	210.0	225.0
	4% DL-lysine	177.5	182.5	210.0	276.0	225.0	224.0
	4% DL- -alanine	177.0	181.0	246.0	276.0	223.0	257.0
	4% L-glutamic acid	187.0	192.0	266.0	230.0	241.0	240.0
15	none	185.0	190.0	193.0	205.0	195.0	203.0
	4% L-cystine	185.0	192.5	200.0	220.0	217.5	206.0
	4% L-leucine	170.0	190.0	-	240.0	200.0	220.0
	4% L-tyrosine	182.5	185.0	220.0	240.0	210.0	238.0

The glucose (dextrose) was given at weight as a 30% solution in water.

which received vitamin B_{12}. Moreover, vitamin B_{12}-deficient chicks which previously been fed rations containing 4% added alanine, lysine, tyrosine or leucine higher blood glucose levels one three hours following the administration glucose than chicks fed diet without vitamin B_{12}. The blood glucose of chicks which previously received diets containing added alanine or tyrosine and supplemental vitamin B_{12} remained slightly higher during the glucose tolerance test than that of chicks not fed excess amounts of these amino acids.

Effect of Vitamin B_{12} and DL-methionine, Glycine or Zein Imbalance on Blood Levels of Various Nitrogen Containing Compounds and Glucose in Chicks.

presented in Tables 30 to 32. When capsules containing methionine were given orally to 4 week old vitamin B_{12}-deficient chicks, previously fed diets containing 4% methionine, increases in the blood levels of uric acid, nonprotein nitrogen, amino nitrogen, urea nitrogen and glucose were observed 4 hours following the administration of the capsules. These changes in blood levels were also noted when vitamin B_{12} was supplied. However, a greater increase in the uric acid level and the creatinine level was observed in chicks given vitamin B_{12} than in chicks not receiving the vitamin.

In the experiments where glycine and zein were administered in a similar manner to chicks fed diets containing 4% of glycine or zein, respectively, the differences in the levels of the different blood constituents following their administration due to the administration of vitamin B_{12} were very similar to those previously observed in the feeding trials. In all cases, the levels of nonprotein nitrogen, amino nitrogen, urea nitrogen and glucose were somewhat higher in vitamin B_{12}-deficient chicks than in those

TABLE 30

Effect of Vitamin B_{12} on Blood Levels of Various Nitrogen Containing Compounds and Glucose of Chicks Fed with DL-methionine

Exp. No.	Blood Determination	Supplemental vit. B_{12}	No added methionine	4% added DL-methionine				
				0 hr.*	hrs.		4 hrs.	
16	NPN	+	37.5	46.0	60.3	(131.1)**	57.5	(125.0)
		-	47.0	41.0	41.0	(100.0)	51.0	(124.4)
16	Amino N	+	20.5	22.0	33.5	(152.3)	30.0	(136.4)
		-	22.0	22.0	31.5	(143.2)	27.0	(122.7)
16	Urea N	+	3.4	4.3	6.3	(146.5)	6.8	(158.1)
		-	3.8	4.3	-		7.0	(162.8)
16	Uric Acid	+	3.5	3.4	7.0	(205.9)	5.5	(161.8)
		-	3.7	4.1	-		8.7	(212.2)
	Creatinine	+	.50	.63	.68	(107.9)	.63	(100)
		-	.62	.63	.64	(101.6)	.64	(101.6)
6	Glucose	+	175.0	202	255.0	(126.2)	230.0	(133.6)
		-	190.0	185	-		250.0	(135.1)

Following 18 hours of fasting, 0.5 gm of methionine capsule was force-fed to each chick which received methionine in the diet during experimental period.

** Relative %, of the respective value for the corresponding controls which received an adequate intake of vitamin B_{12} with 4% added methionine before giving capsule.

Table 21

Effect of Vitamin B_{12} on Blood Levels of Various Nitrogen Containing Compounds and Sugar of Chicks Fed with Glycine

Blood Determination (mg.%)	Suppl. Vit B_{12}	Exp. No.	No glycine added	4% added glycine 4 hr.*	4 hrs.	24 hrs.	
Amino Nitrogen		16	37.5	46.5	51.0	46.0	
		17	42.0	42.5	50.2	45.0	
		av.	39.8	44.5	50.6 (113.7)**	45.5	(95.5)
	+	16	47.0	50.0	55.0	45.0	
		17	46.0	48.5	56.0	50.0	
		av.	46.5	49.3	55.5 (112.6)	47.6	(95.6)
Amino Nitrogen		16	20.5	19.5	25.5	23.5	
		17	21.5	21.0	28.0	25.0	
		av.	21.0	20.3	26.3 (129.6)	24.3	(119.7)
	+	16	22.0	21.0	29.0	25.0	
		17	23.5	23.8	30.0	26.0	
		av.	22.8	22.4	29.5 (131.7)	25.5	(113.8)
Urea Nitrogen		16	3.40	4.75	6.50	6.10	
		17	15.75	18.50	18.25	18.50	
		av.	9.60	11.60	12.38(106.7)	12.30	(106.0)
	+	16	3.75	4.00	6.25	6.50	
		17	18.50	18.25	18.25	20.00	
		av.	11.10	11.30	12.25(108.4)	13.25	(117.3)
Uric Acid		16	3.50	3.90	6.50	7.55	
		17	6.00	3.70	7.30	5.80	
		av.	4.80	3.80	6.90	4.70	
	+	16	3.70	3.90	6.80	4.20	
		17	4.20	3.70	6.50	4.00	
		Av.	3.95	3.80	6.70	4.10	

TABLE 31
continued

Effect of Vitamin B_{12} on Blood Levels of Various Nitrogen Containing Compounds and Glucose of Chicks Fed with Glycine

Blood Detrm. (mg %)	Suppl. Vit B_{12}	Exp. No.	No Glycine	4% added glycine: 0 hr.*	2 hrs.		4 hrs.	
Creatinine		16	.50	.60	.55		.55	
		17	.60	.75	.90		.74	
		av.	.55	.68	.73	(107.4)	.65	(95.6)
		16	.62	.62	.65		.64	
		17	.90	.90	.72		.80	
		av.	.76	.76	.69	(90.8)	.72	(94.7)
Glucose		16	175.0	185.0	215.0		210.0	
		17	175.0	187.5	177.5		177.0	
		av.	175.0	186.3	196.3	(105.4)	193.5	(103.9)
		16	180.0	195.0	220.0		235.0	
		17	180.0	185.0	185.0		177.5	
		av.	180.0	190.0	202.5	(106.6)	206.3	(108.6)

In experiment 16, each chick was force-fed with 0.5 gm of glycine capsule after being fasted for about 12 hours. In experiment 17, the amount of glycine capsule used was according to body weight (0.2 mg per 100 gm of body weight) after being fasted for 12 hours. In both experiments, 2.5 ml of blood was taken from each bird of six of each group by heart puncture at 0 hr., 2 hrs., and 4 hrs., after feeding capsule.

Relative %, of the respective value for the corresponding controls which received an adequate intake of vitamin B_{12} with 4% added glycine before giving capsule.

TABLE 32

Effect of Vitamin B_{12} on Blood Levels of Various Nitrogen Containing Compounds and Glucose of Chicks Fed with Zein

Blood Petrn.	Suppl. Vit B_{12}	No added zein	6% added zein				
			0 hr.*	2 hrs.		4 hrs.	
NPN	+	42.0	43.0	45.0	(104.7)**	43.5	(101.2)
	-	46.0	49.0	50.0	(102.0)	45.0	(91.8)
Amino Nitrogen	+	21.5	22.0	23.0	(104.5)	20.5	(93.2)
	-	23.5	24.0	24.0	(100.0)	22.0	(91.7)
Urea Nitrogen	+	15.8	13.25	13.3	(100.4)	16.3	(123.0)
	-	13.5	15.50	16.0	(103.2)	18.5	(119.4)
Uric Acid	+	6.00	5.3	7.0	(132.1)	5.3	(100.0)
	-	4.20	3.6	4.8	(133.3)	5.9	(163.8)
Creatinine	+	.60	.76	.60	(78.9)	.60	(78.9)
	-	.90	.80	.68	(85.0)	.60	(75.0)
Glucose	+	175.0	174.0	174.0	(100.0)	170.0	(97.7)
	-	180.0	182.0	174.0	(95.6)	175.0	(96.2)

Each chick was force-fed with zein capsule after fasting for 12 hrs. The amount of zein used was according to body weight (.2 mg per 100 gm of body weight).

The numbers in parenthesis are relative % of the respective value for the corresponding controls which received an adequate intake of vitamin B_{12} with 6% added zein and before giving capsule (at 0 hr.).

receiving this vitamin. In general, the magnitude of difference was little effected by the administration of the capsule during the 4 hours test period. An increase in the uric acid level did result from the administration of glycine or sein which was more pronounced in the chicks receiving vitamin B_{12}. After 2 hours when glycine was given a slightly higher creatinine was also observed in chicks which had received vitamin B_{12} than in the vitamin B_{12}-deficient controls.

DISCUSSION

The growth inhibitory effect of 4% added glycine or leucine in vitamin B_{12}-deficient chicks confirms the observations of Menge and Combs (1950) and Machlin *et al.* (1951) who reported glycine toxicity in similar chicks. The fact that crystalline vitamin B_{12} was effective in overcoming this growth inhibition when administered by subcutaneous injection as well as by oral administration indicates that vitamin B_{12} is necessary for the metabolism of excess amounts of these amino acids. The growth depression resulting from the feeding of the excess amino acids appears to be due to an imbalance of the amino acid for protein synthesis. Because vitamin B_{12} functions in the correction of the amino acid imbalance the author suggests that vitamin B_{12} might also be concerned in transamination. Since glutamic acid did not inhibit growth when fed at the same level the apparent increase in the requirement for vitamin B_{12} cannot be explained on the basis of an increased nitrogen intake alone. The results of Menge and Combs (1950) also support this conclusion. The growth inhibitory action of zein may be explained largely, if not entirely, on the basis of its leucine content. These findings reveal that the levels of certain amino acids exert a considerable influence on the dietary requirement for vitamin B_{12}. Practical poultry rations which include large amounts of corn and soybean oil meal contain relatively high levels of both leucine and glycine. Since these amino acids have been shown to increase the need for vitamin B_{12}, it follows that such rations should contain greater amounts of this vitamin for best results than would be required with rations containing a more favorable amino acid balance. These experiments confirm the reports of Groschke *et al.* (1948) that by use of an amino acid

mixture simulating zein, pellagra symptoms in chicks caused by the feeding of zein were due to the cumulative action of the amino acid constituents of this protein. Excess levels of certain other amino acids in the diet of vitamin B_{12}-deficient chicks also exerted growth inhibitory effects which were partially counteracted by vitamin B_{12}. Therefore, the levels of these amino acids would also be expected to influence the requirement for this vitamin. A similar observation was reported by Ott (1949), who found that the vitamin B_{12} requirements were increased by inclusion of dried whey, alfalfa meal and certain other natural products into the diet. Hartman et al, (1949) also showed that the vitamin B_{12} requirement was increased as the protein level of the diet was increased.

This finding that the vitamin B_{12} requirement is influenced by the levels of certain amino acids in the diet is similar to that of Groschke and Briggs (1946); Groschke et al, (1948) and Anderson et al, (1951), who found the need for niacin to be increased when chicks were fed a diet containing high levels of either glycine, alanine, leucine, arginine, aspartic acid, glutamic acid or threonine. When these amino acids were fed singly at a level of 4% to chicks receiving a niacin-low diet growth inhibition resulted which could be overcome by the inclusion of niacin in the diet. These workers further demonstrated that the growth depressing action of zein or gelatin in chicks fed niacin-low diets could be duplicated by feeding combinations of the amino acids which they contained. Groschke and Briggs (1946) demonstrated that glycine was highly "pellagragenic" when fed to chicks receiving a niacin-low diet. Anderson et al, (1949) similarly showed that the chick growth was depressed by the addition of certain amino acids to a low-pyridoxine diet. On the contrary, if sufficient pyridoxine was present, growth was increased by the addition of the amino acid. The ability of certain vitamins to

overcome growth inhibitory action of excess amounts of individual amino acids in rats has been reported. Binning et al, (1949) reported that the growth of rats fed diets containing 10% glycine was greatly reduced as compared with control group. Supplementation of the high glycine diet with pteroylglutamic acid and with pteroylglutamic acid plus liver extract resulted in a marked improvement in growth rate. Page and Gingras (1946) found that high levels of glycine depressed growth of pyridoxine deficient rats, but that the supplementation of 1 mg % of pyridoxine to the diet overcame this depression.

Martin (1946) demonstrated that cystine, tryptophane, tyrosine, histidine, glycine and glutamic acid were all more toxic if fed to rats in a riboflavin-deficient diet than they were when the same diets were supplemented with riboflavin. These indicate that vitamin B_{12}, niacin, pyridoxine, folic acid and riboflavin are required in the metabolism of amino acids or protein.

The observation that vitamin B_{12}-deficiency results in an increase in blood nonprotein nitrogen, amino nitrogen, creatinine, urea and glucose level in agreement with those of Charkey et al, (1950) who found that blood of the birds receiving vitamin B_{12} contained less nonprotein nitrogen and less of each of the amino acids measured than did the blood from birds deprived of vitamin B_{12} and that the birds given vitamin B_{12} grew more rapidly. Since better growth was obtained at lower blood levels of amino acids, they suggested that one function of vitamin B_{12} is to enhance utilisation of certain amino acids to form fixed tissue proteins.

It was also found that the addition of individual amino acids, except glutamic acid, at a level of 4% resulted in growth depression and at the same time increases, with few exceptions, in the nonprotein nitrogen and urea levels of the blood. This observation is comparable with that of

Andersen et al. (1949) who found that addition of 4% different amino acids to pyridoxine low diet increased the levels of nonprotein nitrogen. Administration of vitamin B_{12} to chicks fed diets containing high levels of single amino acids was also effective in reducing the blood levels of nonprotein nitrogen, amino nitrogen, urea nitrogen, creatinine and glucose a s compared with control chicks not given vitamin B_{12}. Nevertheless, the blood levels of nonprotein nitrogen and urea in chicks fed the amino acid supplements were still higher than those of chicks not receiving the added amino acids even though vitamin B_{12} was administered.

Since the amino nitrogen level is not consistantly increased as a result of feeding high levels of individual amino acids either in the presence or in the absence of vitamin B_{12} it appears that the amino nitrogen level of the blood was determined more by the vitamin B_{12} given than by the increased amount of the different amino acids fed.

It is of interest to note that the creatinine level in the blood of vitamin B_{12}-deficient chicks was not increased when 4% added glycine or methionine were included in their diets, while it was increased upon feeding high levels of these amino acids when vitamin B_{12} was supplied. This might be expected since glycine and methionine are normally involved in creatinine formation. Nevertheless, it must be remembered that the creatinine level is increased in a vitamin B_{12}-deficiency regardless of amino acid fed. However, it is possible that vitamin B_{12} is concerned in the synthesis of creatinine under certain conditions.

Mechlin et al. (1951) reported that the level of blood uric acid was usually increased by the administration of vitamin B_{12} to chicks fed a vitamin B_{12} low and folic acid low diet. These workers also found the inclusion of 6% additional glycine in the diet also increased the uric acid level of the blood particularly in the vitamin B_{12}-deficient chicks. In the present study the administration of vitamin B_{12} to chicks fed

vitamin B_{12}-deficient diets containing high levels of glycine, leucine or zein increased the amount of blood uric acid as compared with the amount found in the blood of chicks fed the same diet without supplemental vitamin B_{12}. Since these supplements, i.e. glycine, leucine and zein, also exhibited growth inhibitory effects which were overcome by vitamin B_{12} administration, these results suggest that vitamin B_{12} may be concerned in the formation of uric acid, in the chick. Moreover, it has been shown that glycine is a direct precursor of uric acid in the bird (Buchanan et al, 1948 and Karlsson et al, 1949)and can evidently be utilized intact for uric acid synthesis. The corresponding decrease in the amino nitrogen levels of the blood of chicks receiving high levels of leucine, glycine and zein resulting from vitamin B_{12} administration, lends support to the hypothesis that vitamin B_{12} may be concerned in the deamination of these amino acids or the utilization of the amino acid nitrogen to form uric acid. Machlin et al, (1951) have also suggested that vitamin B_{12} may function in this manner.

The very consistant increase in blood glucose level of the chick resulting from a vitamin B_{12} deficiency is of particular interest and suggests that vitamin B_{12} is involved in glucose utilization. This is also indicated by the higher blood sugar levels noted during the glucose tolerance tests in vitamin B_{12}-deficient chicks fed diets containing high levels of certain individual amino acids as compared with those of chicks receiving vitamin B_{12} and fed the same diets. Also the blood glucose levels of vitamin B_{12} adequate chicks was increased slightly more as a result of feeding high levels of glycine, glutamic acid or methionine than it was in vitamin B_{12}-deficient chicks fed the same diet. This also suggests that vitamin B_{12} might function in the conversion of certain glucogenic amino acids into glucose. Furthermore, it has been reported

by Rossbardt *et al.* (1950) that the source of energy in the diets exerts a marked effect on the requirement of mice for vitamin B_{12}. These workers found that the need for vitamin B_{12} was increased as the percentage of the total energy intake supplied by carbohydrate was raised at the expense of energy from fat. These workers estimated two possible functions of vitamin B_{12}: (1) vitamin B_{12} may, like thiamin, play an essential role in catabolism of carbohydrate or carbohydrate like residues of protein; (2) vitamin B_{12} may have a function in the conversion of intermediates formed in the metabolism of carbohydrate and protein to fats (other than fatty acids) that are required for optimum growth. That the deleterious effects of a high protein or amino acids diet may overcome by vitamin B_{12} suggests a further role of vitamin B_{12} in protein or amino acid metabolism other than conversion of carbohydrate-like residues of fat. In similar studies with rats, McCollum and Chow (1950) obtained results which suggested that vitamin B_{12} is involved in the conversion of carbohydrate into fat.

The present findings, as well as those of others, reveal that when vitamin B_{12} is supplied it is possible to use plant protein concentrates to a far greater extent in rations for poultry and other animals. This has also been made possible in decreasing the amount of the more expensive animal protein concentrates previously needed in these rations. Thus vitamin B_{12} has served the purpose of stretching the available supply of fish meal, meat scraps or other animal protein concentrates which has always been short. Therefore, vitamin B_{12} is of great importance in practical poultry rations.

SUMMARY

The growth of vitamin B_{12}-deficient chicks was inhibited by the addition of 4% leucine, 4% glycine or 6% to 8% zein to their diet. The administration of vitamin B_{12} by subcutaneous injection or by oral administration counteracted this growth inhibition. The growth depressing action of zein can be attributed to its leucine content. Tyrosine and aspartic acid fed at the same level exerted similar growth inhibitory effects although vitamin B_{12} was only partially effective in overcoming the growth depression. Excess of alanine, methionine or cystine also depressed growth even when vitamin B_{12} was supplied. Glutamic acid and lysine, however, exerted only a very slight growth inhibitory effect in the presence or absence of vitamin B_{12}.

The addition of 4% glycine, aspartic acid, methionine or 6% to 8% zein to vitamin B_{12}-deficient basal diets increased the mortality while the addition of 4% glutamic acid, alanine or cystine had little effect. On the other hand, the mortality of chicks fed the vitamin B_{12}-deficient diet containing either 4% added leucine, lysine or tyrosine was less than that of chicks fed the same diet without the added amino acid. Administration of vitamin B_{12}, however, markedly reduced the mortality regardless of the amino acid included in the diet. When vitamin B_{12} was supplied, the addition of high levels of each amino acid to the diets did not appreciably affect the per cent mortality except for aspartic acid, alanine, methionine and cystine. These amino acid additions increased the mortality.

The chick blood levels of nonprotein nitrogen, amino nitrogen, urea nitrogen, creatinine and glucose were significantly elevated in a vitamin B_{12}-deficiency. These differences were also generally observed in chicks receiving high levels of individual amino acids except for methionine.

The addition of different amino acids at a level of 4% to chicks diets resulted in an increase in the level of nonprotein nitrogen and urea nitrogen with a few exceptions. In the presence of vitamin B_{12}, the feeding of 4% additional glycine or methionine resulted in an increase in the blood level of creatinine. When vitamin B_{12} was administered to chicks fed diets containing high levels of glycine, leucine or zein, the blood amino nitrogen was decreased while the blood uric acid was correspondingly increased. Although the blood glucose level of vitamin B_{12}-deficient chicks was higher almost without exception than in chicks receiving vitamin B_{12}, the increase in blood glucose resulting from the feeding of 4% glycine, methionine or glutamic acid was greater when vitamin B_{12} was supplied. Possible ways in which vitamin B_{12} may function in the metabolism of amino acids and glucose are discussed.

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TABLE 33

Effect of Vitamin B_{12} on Blood NPN of Chicks Fed Excesses of Various Amino Acids or Protein (Summary)

Supplement	Number of trials	mg % With B_{12}		mg % Without B_{12}	
0		36.1	(100)	45.8	(126.9)
4% Tyrosine		40.5	(122)*	49.4	(136.8)
0		40.0	(100)	45.0	(112.5)
4% L-leucine		42.7	(106.8)	48.3	(120.8)
0		36.7	(100)	40.0	(109.0)
4% L-glutamic acid		37.6	(102.5)	42.7	(116.3)
0		38.9	(100)	43.0	(110.5)
4% DL-lysine		43.4	(111.6)	44.6	(114.7)
0		40.0	(100)	45.0	(112.5)
4% L-tyrosine		43.0	(107.5)	48.3	(120.8)
0		38.9	(100)	43.0	(110.5)
4% DL- -alanine		40.0	(102.8)	47.0	(120.8)
0		40.5	(100)	45.5	(112.3)
4% L-cystine		42.0	(103.7)	44.0	(108.7)
0		37.5	(100)	47.0	(125.3)
4% DL-methionine		46.0	(122.7)	41.0	(109.3)
0		42.0	(100)	46.0	(109.5)
6% Protein		43.0	(102.4)	49.0	(116.7)

The numbers in parenthesis are relative % of the respective average value for the corresponding controls which received an adequate intake of vitamin B_{12} with no supplemental amino acid or protein.

			12		12
		20.9 [illegible].4	(100) (97.0)	[illegible]	(10[illegible].1) [illegible].1)
0 [illegible]ersine	[illegible]	[illegible] 20.5	[illegible]) [illegible]7.5)	[illegible] [illegible]3.5	(111.0) (111.0)
0 [illegible]l[illegible]tar[illegible] acid	1	24.0 3.5	(100) (97.0)	25.0 [illegible].0	(104.[illegible]) 10[illegible].0)
0 [illegible]	2	22.0 3.[illegible]	(100.0) (100.0)	23.0 [illegible]	(104.5) (10[illegible].2)
 [illegible]tyrosine	1	[illegible]1.0 20.0	[illegible]) (95.0)	[illegible] 23.5	(1[illegible]) (111.9)
0 [illegible]	2	22.0 [illegible]	(100) [illegible]1.4)	23.0 [illegible]	(104.5) (11[illegible].7)
0 [illegible]tine	1	20.[illegible] 20.3	(100) (97.0)	[illegible] 21.[illegible]	[illegible].0) (104.0)
0 [illegible]	1	20.5 12.0	(100) [illegible]7.[illegible]	[illegible].0 [illegible]	(107.3) (1[illegible].3)
6:	[illegible]	[illegible].5 22.0	([illegible]) (10[illegible].3)	[illegible] [illegible].0	[illegible]) 111.[illegible])

[illegible]

Supplement	Number of trials	[illegible] B_{12}		[illegible] + B_{12}	
0		4.05	(100)	4.40	(108.6)
[illegible]		4.63	(114.3)*	4.76	(117.5)
0	1	8.10	(100)	[illegible].25	([illegible]14.2)
4[illegible] L-leucine		9.00	(111.1)	1[illegible].50	([illegible]9.6)
0	1	6.[illegible]	(100)	[illegible].0[illegible]	(150.0)
[illegible]-glutamic acid		[illegible].60	(143.3)	9.20	([illegible]53.3)
0	2	6.95	(100)	10.50	(151.1)
[illegible]-lysine		1[illegible].[illegible]	([illegible].9)	1[illegible].50	(151.1)
0	1	8.10	(100)	[illegible].25	(114.2)
4[illegible] L-tyrosine		[illegible].25	([illegible].2)	11.50	([illegible].0)
0	2	6.95	(100)	10.50	(151.1)
4[illegible] L-[illegible]		10.30	(14[illegible].[illegible])	[illegible].55	([illegible].[illegible])
0	1	8.00	(100)	10.63	(132.8)
4[illegible] L-cystine		1[illegible].75	(13[illegible])	[illegible]	([illegible])
0	[illegible]	3.40	(100)	3.7[illegible]	(110.3)
[illegible]		4.25	(125.0)	4.25	(125.0)
0	1	15.75	([illegible])	[illegible].50	([illegible]7.[illegible])
[illegible]		[illegible]	([illegible].1)	[illegible]	([illegible].7)

Table 32

Effect of Vitamin B_{12} on Blood Uric Acid of Chicks Fed Excesses of Various Amino Acids or Protein (Summary)

Supplement	Number of trials	mg % With B_{12}		mg % Without B_{12}	
0		3.83	(100)	3.52	(100)
4% glycine		3.80	(99.2) *	3.63	(96.1)
0	1	1.85	(100)	1.85	(100)
4% L-leucine		2.30	(124.3)	1.68	(97.3)
0	1	2.40	(100)	2.58	(106.3)
4% L-glutamic acid		2.45	(102.1)	2.93	(124.2)
0	2	3.10	(100)	3.35	(106.1)
4% DL-lysine		3.23	(104.2)	3.30	(103.2)
0	1	1.85	(100)	1.85	(100)
4% L-tyrosine		2.40	(129.7)	2.65	(143.2)
0	2	3.10	(100)	3.35	(106.1)
4% DL-α-alanine		3.43	(112.3)	3.47	(111.9)
0	1	2.83	(100)	2.86	(101.7)
4% L-cystine		2.93	(103.5)	2.88	(101.7)
0	1	3.50	(100)	3.70	(105.7)
4% DL-methionine		3.40	(97.1)	4.05	(115.7)
0	1	6.00	(100)	4.20	(70.0)
6% zein		5.30	(88.3)	4.60	(76.6)

* 1 of 33.

Table 37

Effect of Vitamin B_{12} on Blood Creatinine Level of Chicks Fed Excesses of Various Amino Acids or Oil (Assay)

Supplement	Number of trials	mg %	
		With B_{12}	Without B_{12}
0	1	.54 (100)	.71 (131.5)
4% glycine		.61 (113.0)*	.71 (131.5)
0	1	.40 (100)	.43 (130.0)
4% L-leucine		.40 (100)	.54 (135.0)
0	1	.60 (100)	.65 (108.3)
4% L-glutamic acid		.56 (93.3)	.60 (100)
0		.55 (100)	.72 (132.7)
4% L-lysine		.44 (80.0)	.50 (90.9)
0	1	.40 (100)	.43 (130.0)
4% L-tyrosine		.45 (112.5)	.54 (135.0)
0	-	.55 (100)	.73 (132.7)
4% DL-alanine		.60 (109.1)	.60 (109.1)
0	2	.45 (100)	.64 (142.2)
4% L-cystine		.45 (100)	.52 (115.6)
0	1	.50 (100)	.62 (124.0)
4% DL-methionine		.63 (126.0)	.63 (126.0)
0	1	.60 (100)	.90 (150.0)
6% soin		.76 (126.7)	.80 (133.3)

Table 33.

Table 33

Effect of Vitamin B_{12} on Blood Glucose Level of Chicks Fed Excesses of Various Amino Acids or Protein (Summary)

Supplement	Number of Birds	mg %			
		Without B_{12}		With B_{12}	
0	4	176.0	(100)	185.0	(105.1)
4% Glycine		179.0	(107.4)*	195.0	(110.8)
0	1	185.0	(100)	180.0	(102.7)
4% L-leucine		185.0	(100)	195.0	(105.4)
0	1	172.0	(100)	192.5	(111.9)
4% L-glutamic acid		167.0	(102.7)	192.0	(111.6)
0	2	171.0	(100)	185.0	(108.2)
4% L-alanine		165.0	(95.5)	178.0	(104.6)
0	1	185.0	(100)	190.0	(107.7)
4% L-tyrosine		182.5	(98.6)	185.0	(100.0)
0	2	171.0	(100)	185.0	(108.2)
4% L-methionine		167.5	(97.8)	171.0	(101.8)
0	2	185.0	(100)	190.0	(107.7)
4% L-cystine		165.0	(89.2)	192.5	(104.1)
0	1	175.0	(100.0)	180.0	(102.9)
L-arginine		201.0	(115.4)	185.0	(105.7)
0	1	175.0	(100)	180.0	(102.9)
6% zein		174.0	(99.4)	182.0	(104.0)

Table 33.