A STUDY OF VITAMIN B_{12} REQUIREMENTS FOR LAYING PULLETS AND THEIR PROGENY

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INTRODUCTION

The isolation and crystallization of vitamin B_{12} by Rickes, Brink, Koniuszy, Wood and Folkers (1948) and Smith (1948 a) has opened a new field of nutritional research. It was soon demonstrated that this vitamin was required for the growth of chicks. It was also found that the improvement in growth obtained by the addition of animal protein to the chick diet could be explained in large part by its content of vitamin B_{12}. Furthermore it was shown that vitamin B_{12} was required for hatchability.

This study was undertaken to determine the vitamin B_{12} requirements of the pullet and her progeny, and the vitamin B_{12} content of the egg as affected by pullet diet.

REVIEW OF LITERATURE

History of Vitamin B_{12}

During World War II, simplified diets devoid of or very low in animal protein sources were fed to poultry from necessity. Poor growth, poor viability, and poor hatchability marked their use, indicating a deficiency of one or more growth factors required by the chick and the poult. Hammond (1944 a and b) found dried cow manure and dried rumen contents superior to alfalfa leaf meal for both the chick and the poult in such a ration when the diet was adequate in vitamin A and riboflavin. Further studies on this material revealed the presence of an unknown growth factor (Rubin and Bird, 1946 a). An excellent review of the work up to this point on the factor in cow manure was written by the editors in Nutrition Reviews, 1946. In 1948 Bird, Marsden and Kellogg reported a critical need in young turkeys for the cow manure factor.

Concurrent research in other fields (Shorb, 1947, Cary, Hartman, Dryden and Likely, 1946, etc.) beyond the scope of this paper, revealed that other animals and some microorganisms required this or a similar factor. Clinical work revealed the need for one or several unknown factors contained in liver for the relief of Addisonian pernicious anemia in humans (Minot and Murphy, 1927). With remarkable foresight Castle and Ham (1929) interpreted results on studies of this disease as indicating the existence of an intrinsic factor secreted by certain stomach glands, and an extrinsic factor which he described as an essential complementary factor found in meat, eggs, yeast, and liver. Hopes that the principal anti-pernicious anemia factor contained in these feedstuffs had been discovered with the isolation of folic acid were quickly dashed by the

reports of Bethell and Sturgis (1948) and Ross, Belding and Paegel (1948).

Finally, on April 16, 1948, Rickes, Brink, Koniuszy, Wood and Folkers (a) reported the isolation of biologically active crystalline material from liver concentrates clinically active in the treatment of pernicious anemia. Shorb (1948) confirmed the activity of this material, tentatively named vitamin B_{12}, as the LLD factor for Lactobacillus lactis Dorner, and West (1948) demonstrated its ability to induce favorable blood responses in patients suffering from pernicious anemia. On April 24, 1948, Smith (a) independently announced the purification of two amorphous forms of the anti-pernicious anemia factor from liver. Smith cited unpublished clinical studies by Ungley in England which demonstrated his material to be effective in stimulating blood regeneration and to be effective also in the treatment of three cases of subacute combined degeneration of the spinal cord associated with pernicious anemia.

Chemical and Physical Properties

Crystalline vitamin B_{12} is a garnet-red compound which contains cobalt (Smith, 1948 b, and Rickes, Brink, Koniuszy, Wood and Folkers, 1948 b) and PO, NH, and OH groups (Robinson, 1948). Acid hydrolysis of the vitamin yields 5:6-dimethylbenzimidazole. It is noteworthy that the 1:2-diamino-4:5-dimethylbenzene moiety appears in the above degradation product, in vitamin B_{12} itself, and in riboflavin, indicating a possible relationship between the two vitamins. The vitamin appears to be a six-group cobalt coordination complex with a molecular weight of 1,550 to 1,750. Analysis gave a composition typified by $C_{61-64}H_{86-92}N_{14}O_{13}PCo$. There are several weakly basic groups. Upon alkali fusion, vitamin B_{12}

formed products which react with Ehrlich's reagent. This and other tests are considered typical of certain cyclic five-membered nitrogen-containing compounds including pyrroles. The melting point was 300° C. or above. The microbiological activity was not affected by autoclaving at 121° C. for fifteen minutes, but it was inactivated upon standing at room temperature in dilute sodium hydroxide or hydrochloric acid (Rickes, Brink, Koniuszy, Wood, and Folkers, 1948 c, Brink, Wolf, Kaczka, Rickes, Koniuszy, Wood, and Folkers, 1949, Kaczka, Wolf, and Folkers, 1949, and Brink and Folkers, 1949).

When vitamin B_{12} was catalytically hydrogenated, a product was obtained which was called vitamin B_{12a}. It appeared to have less than half of the biological activity of vitamin B_{12} for rats, chicks, and pernicious anemia patients (Kaczka, Wolf, and Folkers, op. cit.).

Vitamin B_{12b} was crystallized by Pierce, Page, Stokstad and Jukes (1949) and showed substantial biological activity for chicks and for L. leichmannii 313. Fricke, Lanius, De-Rose, Labidus and Frost (1950) compared the potencies of vitamin B_{12} and vitamin B_{12b} for rats and for humans suffering from Addisonian pernicious anemia and found them equally effective. They concluded that the 2 compounds were apparently chemically almost identical. Stokstad, Jukes, Brockman, Pierce and Broquist (1950) found equal activity for the 2 compounds for the chick and L. leichmannii. Smith, Culbertson, Walker and Lees, (1950) found vitamin B_{12}, vitamin B_{12b}, and desoxyribosides of cytosine, guanine, hypoxanthine, adenine and thymine on partition chromatography of the vitamin B_{12} group of factors. They stated that pure vitamin B_{12} samples gave 2, sometimes 3, zones of growth due to artifacts produced presumably by oxidation on the paper.

Smith (1948 b, 1949) reported that the crystalline material was very soluble in water, soluble in nearly anhydrous alcohol, and in glacial acetic acid, but insoluble in ether, chloroform, or non-polar solvents.

Relationship with Other Growth Factors

Crystalline vitamin B_{12} has been shown to replace all or nearly all of the acid precipitate factor (the cow manure factor) (Lillie, Denton and Bird, 1948) and the animal protein factor for growth of chicks, (Ott, Rickes and Wood, 1948). It replaces nutrient X for rat growth and normal reproduction (Hartman, Dryden and Cary, 1949 a). It replaces anti-pernicious anemia liver extracts for pig growth (Johnson and Neumann, 1949) and is essential for growth of mice fed thyroprotein (Huff, 1949) as a "stress" factor. Children suffering from malnutrition and growth failure were fed daily oral doses of vitamin B_{12} with resultant heightened alertness and vigor, and a resumption of normal growth, indicating that it is a growth factor for humans (Wetzel, Fargo, Smith and Helikson, 1949). Although no confirmation has been published concerning the identity of vitamin B_{12} with "zoopherin", the growth factor for rats described by Zucker and Zucker, 1948 (a and b), the sources and effect of it indicate a very close relationship. It is probable that vitamin B_{12} is Castle's postulated extrinsic factor mentioned previously and that absorption of it is aided by the presence of the intrinsic factor of gastric juice (Berk, Castle, Welch, Heinle, Anker and Epstein, 1948, and Ternberg and Eakin, 1949). Although vitamin B_{12} accounts for at least a major portion of the activity of the animal protein factor, many groups of workers have presented evidence on the multiple nature

of the latter. Cravens (1950) has given an excellent review of the work on this question.

Results with microorganisms indicate a complicated interrelationship between desoxyribosides, reducing agents and vitamin B_{12}. A summary of much of the work in this field, which is beyond the scope of the present paper, is included in a review by Woods, 1949. Studies on the desoxyribosides have indicated slight if any activity on their part as substitutes for vitamin B_{12} for higher animals (Reisner and West, 1949, and Lewis, Register, Thompson and Elvehjem, 1949).

Some of the vitamins seem to bear a more direct relationship with the latter vitamin. High levels of calcium pantothenate and pyridoxine hydrochloride fed to chicks (Bird and Rubin, 1948) and high levels of riboflavin fed to rats in the presence of sulfasuxidine (Hartman, Dryden and Cary, 1949 b) seemed to partially offset a deficiency of vitamin B_{12}. The relationship of vitamin B_{12} to choline and methionine will be discussed in the section on the general functions of the vitamin.

Vitamin B_{13}, described by Novak and Hauge (1948), is a fat-soluble substance clearly different from vitamin B_{12}. Vitamin B_{14}, described by Norris and Majnarich (1949), while apparently closely related to both folic acid and vitamin B_{12} in function, is also apparently an entirely separate entity. It is extremely active in cell proliferation in bone marrow cultures and has been effective in curing experimentally induced anemia of rats. The authors believe that it, not vitamin B_{12}, is Castle's postulated extrinsic factor.

It is possible that antibiotics may spare the level of vitamin B_{12} which must be supplied in the feed for the chick, by creating favorable conditions for a high rate of intestinal synthesis (Smith and

Robinson, 1945, and McGinnis, Stephenson, Levadie, Carver, Garibaldi, Ijichi, Snell and Lewis, 1949), but it is improbable that the actual physiological requirement for the chick is reduced.

Functions - General

Vitamin B_{12} functions in the efficient utilization of protein as shown by the intensification of the vitamin deficiency when unusually high levels of vegetable proteins are fed to chicks or rats (Rubin and Bird, 1947 a, and Hartman, Dryden and Cary, 1949 a).

The level of nucleoprotein in liver basophilia of dogs is coordinated with the level of vitamin B_{12}. Consequently it is believed a deficiency of the latter retards protein synthesis (Stern, Taylor, and Russell, 1949). This may be the function of the vitamin in preventing pernicious anemia. Vitamin B_{12} may be necessary to convert the thymine (produced as a result of catalytic action by folic acid) into thymidine which is needed for the manufacture of nucleic acid for new cells, such as red blood cells and reticulocytes (Wright, Skeggs, and Huff, 1948). The report of Popper, Koch-Weser and Szanto (1949) also suggested a possible role of vitamin B_{12} in the synthesis of ribonucleic acid in the liver of the rat. Reports indicate an interplay between choline, betaine, methionine and vitamin B_{12} in which the vitamin can reduce the requirement for the other factors for the chick (Gillis and Norris, 1949, Schaefer, Salmon, and Strength, 1949).

According to Emerson (1949) and Nichol, Dietrich, Cravens and Elvehjem (1949) vitamin B_{12} can counteract all thyrotoxicity, thus replacing liver and liver extracts for that purpose. Test animals used were, respectively, rats and chicks for the two studies.

Functions - In Poultry

Whitson, Titus and Bird (1946) reported that the feeding of the cow manure factor, in a diet high in soybean meal and containing no animal protein, significantly improved hatchability. There was no influence on hen body weight, egg production, or egg size. Rubin and Bird (1946 a) reported the cow manure factor necessary for chick growth. In 1947 (b), they reported that the factor was transmitted through the egg to the chick. They showed that the content of the factor in the eggs was directly related to the level of the factor in the hen's diet. Bird (1946) showed that chicks produced on diets devoid of the cow manure factor (vitamin B_{12}) suffered more than twenty percent greater mortality during the first week of life than undepleted chicks did. Nichol, Harper and Elvehjem (1949), presented evidence that vitamin B_{12} does have some influence on hemoglobin regeneration in chickens. Chicks fed on a folic acid-deficient diet and injected with phenylhydrazine hydrochloride were treated with folic acid injections alone, which restored hemoglobin values to normal. Vitamin B_{12} injections alone gave no benefit, but a combined injection of vitamin B_{12} and folic acid produced the most rapid regeneration. The effect could easily be an indirect action through improved utilization of folic acid.

Lillie, Denton and Bird (1948) reported better feathering of chickens when they were supplied with vitamin B_{12}. Lillie, Marsden, Groschke and Bird (1949) found that the apparent requirement of the growing chick for vitamin B_{12} dropped during the later stages of growth. Robblee, Nichol, Cravens, Elvehjem and Halpin (1949) cited work which indicated that even if the maternal diet contained supposedly adequate

levels of vitamin B_{12}, the progeny still required supplemental vitamin B_{12} in their diet to 4 weeks.

Nichol, Dietrich, Cravens and Elvehjem (1949) presented data indicating that 15 micrograms of crystalline vitamin B_{12} per kilogram of diet appeared to be the minimum effective dose when hyperthyroid chicks were used, although the exact level was not determined. Stokstad, Jukes, Pierce, Page and Franklin (1949) found that a minimum of 15 micrograms of crystalline vitamin B_{12} per kilogram of diet (when fed in diets containing seventy percent of soybean meal to depleted New Hampshire chicks) was as active in promoting growth as fish solubles and liver extracts.

Ott (1949) has reported that depleted chicks under his conditions required at least 27 micrograms of vitamin B_{12} per kilogram of ration for maximum growth.

If there is any need for vitamin B_{12} for egg production, the requirement must be low and at present is unknown. The quantitative requirements of the hen for the vitamin for normal hatchability, for viable offspring and for good growth of progeny fed various levels of vitamin B_{12} have not been clearly defined. Furthermore there is a genetical relationship to the vitamin B_{12} requirement of the chicken, which permits some birds to exist satisfactorily on levels quite deficient for others, (Bird, Rubin and Groschke, 1947).

Assay Methods

Both microbiological and macrobiological techniques have been employed for vitamin B_{12} assays. The first microbiological method, the LLD assay of Shorb (1947 b) proved to be difficult to use because of the

tendency of the species to disassociate. In the LLD assay method discussed by Greene, Brook, and McCormack in 1949, the oxygen tension is controlled, since it affects the assay.

Several assay methods employ L. leichmannii (Hoffmann, Stokstad, Hutchings, Dornbush and Jukes, 1949, Peeler, Yacowitz and Norris, 1949, and others). All of these assays have been complicated by the ability of thymidine (Shive, Ravel, and Harding, 1948) and other desoxyribosides (Kitay, McNutt and Snell, 1949) to replace or spare vitamin B_{12} under assay conditions.

Foster, Lally and Woodruff (1949) have outlined a cup assay for vitamin B_{12} which avoids some of the difficulties of standard turbidimetric or titrimetric procedures. The diffuse growth response of the test culture to desoxyribonucleic acid or to its constituent nucleosides is eliminated.

The use of the protozoan Euglena gracilus has been suggested for an assay since it apparently gives a quantitative response to vitamin B_{12} but is inactive to thymidine (Hutner, Provasoli, Stokstad, Hoffmann, Belt, Franklin and Jukes, 1949).

Macrobiological techniques involving the rat and the chick were already in use for the vitamin when it was isolated (Hartman, Dryden, and Likely, 1946, and Bird, Rubin and Groschke, 1948, respectively). Later (1949) Bosshardt, Paul, O'Doherty, Huff and Barnes employed a mouse assay. All of these methods involved a preliminary maternal depletion period prior to use of the progeny for assays. The above mouse assay, some rat assays (Frost, Fricke, and Spruth, 1949, Register, Ruegamer, and Elvehjem, 1949) and some chick assay techniques have utilized iodinated casein as a "stress" mechanism to increase the sensitivity of

the animal to a deficiency of this vitamin. The chick assay method described by Bird, Rubin and Groschke (1948) utilizes a maternal depletion period and a 2 week preliminary feeding period of a very high level of soybean meal to the progeny, followed by a 2 week test period. Growth is the criterion in all of these assays. No single assay, microbiological or macrobiological, is satisfactory in every respect.

Sources

Dried cow manure, fish meal, meat meal, meat scraps, condensed fish solubles and other animal protein supplements have been used as valuable sources of vitamin B_{12}. Indications that the dietary history of non-ruminants may have considerable effect on the value of their flesh as a source of vitamin B_{12} have been obtained by Register, Lewis, Thompson and Elvehjem (1949). Stephenson, McGinnis, Graham and Carver (1948) presented evidence further substantiated by Cunha, Burnside, Buschman, Glasscock, Pearson and Shealy (1949) that soil stimulated growth when added to vitamin B_{12} deficient diets.

Cereal grains and vegetable protein supplements are quite deficient in vitamin B_{12} (Whitson, Hammond, Titus and Bird, 1945, and McGinnis and Carver, 1947).

Various commercial crude concentrates of vitamin B_{12} produced from microbial fermentation have also become available (Stokstad, Page, Pierce, Jukes, Heinle, Epstein and Welsh, 1948, Ansbacker, Hill, Tieman, Downing and Caldwell, 1949, and Rickes, Brink, Koniuszy, Wood and Folkers, 1948 c).

EXPERIMENTAL PROCEDURE AND RESULTS

A. Studies with Laying Pullets

1. General procedure. The studies with laying pullets involved a preliminary period and two experimental periods. During the preliminary period records of hatchability were obtained which served as a basis for formation of equalized groups for the later work. Records on egg production and fertility were also obtained. Basal diet 1, shown in table 1, was used to systematically deplete the pullets of any stores of vitamin B_{12}, during this period. Durations of periods were August 27, 1949 to November 8, 1949, November 8, 1949 to April 8, 1950 and April 8, 1950 to July 27, 1950, respectively, for the preliminary period, experiment 1 and experiment 2. Sixty New Hampshire pullets, 22 weeks of age, were housed in individual laying cages at the start of the preliminary period. They had been reared on conventional starting and growing rations. Selections were made primarily on the basis of sexual maturity, although consideration was also given to body weight and other qualifications. At the same time New Hampshire males were housed in batteries. They had been reared on the same diet as the pullets. Pullets found to be unsatisfactory during the preliminary period were replaced by others held in reserve on fresh litter in laying pens under otherwise similar conditions. After termination of the preliminary period no further replacements were made. The pullets were artifically inseminated with pooled semen twice weekly throughout the entire study. Eggs were collected daily and stored at 50° F. until incubated. Settings were made at two-week intervals. During the preliminary period and first 4 weeks of experiment 1, the eggs were candled on the sixteenth day of incubation. Thereafter they were candled on the

TABLE 1

Composition of Pullet Basal Rations

Ingredients	Basal Diet 1 %	Basal Diet 2 %
Ground yellow corn	44.975	46.975
Ground wheat	30.000	30.000
Expeller soybean meal	18.000	7.000
Dried brewers yeast	—	4.000
Menhaden fish meal	—	4.000
Unextracted liver meal	—	1.000
Ground limestone	3.000	3.000
Defluorinated rock phosphate	3.000	3.000
Iodized salt	0.500	0.500
$MnSO_4.4\ H_2O$	0.025	0.025
A & D feeding oil (2250 A, 400 D)	0.500	0.500
Total	100.000	100.000
Supplemental ingredients	mg./lb.	mg./lb.
Calcium pantothenate	0.75	0.75
Riboflavin	1.00	1.00
Menadione	0.20	0.20
Choline chloride	150.00	150.00
Analysis		
Crude protein by assay	14.90%	15.10%
Methionine	0.26%	0.33%
Cystine	0.25%	0.26%
Choline	609.00 mg./lb.	637.50 mg./lb.

eighth day to avoid excessive fragility of the vitelline membrane. This difficulty arose in all the eggs produced on diet 1 described in table 1, regardless of supplementation with vitamin B_{12}. Observations by Olsen at Beltsville, Maryland, indicate that a tendency towards fragility of the membrane occurs when the eggs are produced on soybean meal as the sole protein supplement. All apparently infertile eggs were broken out to accurately determine fertility. The few eggs which were pipped but which failed to hatch by the twenty-second day were included as dead germs. The chicks which hatched were used in experiments to be described later.

Observations were made on the physical condition of the pullets during the three periods and on their reproductive performance. Birds were weighed five times, as indicated in table 3, during the preliminary period and experiment 1. Hemoglobin values were obtained during experiment 1 by use of the method described by Evelyn (1936). Mortality, egg production, fertility and hatchability data were recorded during all three periods.

At the end of the preliminary period the pullets were divided into five experimental groups, A, B, C, D and E. Groupings were made principally on the basis of equalized hatchability. The groups were left unchanged except by natural causes for both experiments.

Feed and water were supplied ad libitum. The composition of the basal diets used for the pullet studies is listed in table 1. Both basal diets contain added choline and on the basis of the limited data available on requirements of mature stock, are believed to be adequate in methionine and choline. Basal diet 1 was fed without supplementation to all pullets during the preliminary period. It was an all-mash breeding

ration composed of practical feedstuffs and was considered to be adequate in all known nutrients, except vitamin B_{12}. The vitamin B_{12} content of such a diet may be assumed on the basis of universal experience to be at best very low. There are no reliable assays for the vitamin B_{12} content of its ingredients. Basal diet 2 includes natural sources of vitamin B_{12} and unidentified nutritional factors. Average figures for the vitamin B_{12} potencies of liver meal and fish meal were obtained by Lillie, Denton, and Bird (1949). According to their results with chick assays these feedstuffs may furnish a total of about 15 micrograms of vitamin B_{12} per kilogram of basal diet 2. Variability in the results of individual assays by these workers indicate that these values are only approximations.

The males were fed a normal breeding ration including sources of vitamin B_{12} throughout the study. During both experimental periods, basal diet 1 was fed to groups A, B, C and D, while basal diet 2 was fed to group E. Consequently group E functioned as the positive control. Table 2 presents the variations in supplements for the different groups of pullets during the three periods. The source of supplemental vitamin B_{12} for all groups except E was Rubramin, manufactured by Squibb and Sons. The latter product was a highly purified protein-free solution of vitamin B_{12} derived from Streptomyces griseus. According to determination with Lactobacillus leichmannii by the manufacturer, it contained 30 micrograms of vitamin B_{12} per cubic centimeter and was added to basal diet 1 as a highly dilute aqueous solution. During experiment 2 group E was fed basal diet 2 supplemented with Merck and Co. experimental APF concentrate #5. According to the manufacturer, this concentrate contained 27.5 milligrams of vitamin B_{12} per kilogram when measured with the LLD assay.

Neither one of the vitamin B_{12} concentrates was fortified with any antibiotic.

TABLE 2

Basal Diets and Supplements Fed the Pullet Groups

Group	Basal Diet	Supplements per Kilogram		
		Preliminary Period	Experiment 1	Experiment 2
A	1	—	—	3 mcg. of B_{12}
B	1	—	4 mcg. of B_{12}	4 mcg. of B_{12}
C	1	—	8 mcg. of B_{12}	—
D	1	—	16 mcg. of B_{12}	—
E	2*	—	—	0.1% APF Concentrate #3

* This group fed basal diet 1 during preliminary period.

For the sake of brevity the phrase "level of supplemental vitamin B_{12} per kilogram of feed" will be replaced in most instances by "level of vitamin B_{12} fed", or a similar phrase, throughout the remainder of the paper. It will be understood that the actual total level of vitamin B_{12} fed would also include the content in the basal diets.

2. Results of pullet experiment 1. Experiment 1 was undertaken to study the effect of graded levels of vitamin B_{12} on the physical condition and on the reproduction of laying pullets. Table 3 presents the data obtained during the first experiment on the physical condition of the pullets.

TABLE 3

Experiment 1: Effect of Vitamin B_{12} on Physical Condition of Pullets

Group	Average Pullet Weight in Kilograms					Gm. Hemoglobin per 100 cc. of Blood		% Mortality Nov. 5, 1949 to April 8, 1950
	August 27, 1949	November 5, 1949	December 24,1949	February 13, 1950	April 8, 1950	November 5, 1949	April 8, 1950	
A	2.328(7)*	2.542	2.698	2.752	2.728	8.10	8.65	16.7
B	2.261(8)	2.438	2.719	2.867	2.726	8.17	8.45	0.0
C	2.212(5)	2.440	2.685	2.863	2.577	8.39	8.70	8.3
D	2.226(9)	2.456	2.694	2.796	2.605	8.19	8.84	0.0
E	2.147(8)	2.430	2.624	2.967	2.792	8.60	9.00	0.0

* Number in parenthesis indicates number of surviving birds from preliminary period which were used for the experimental periods. Remaining birds in the groups for the latter 2 periods were replacements for whom no weights were available for the date of August 27, 1949.
Each group contained 12 birds at the beginning of experiment 1.

Body Weight of Pullets

During experiment 1 average weights increased generally in the early months of the test, followed by a slight decline toward the end of the test. The final weights were still above the starting weights in every group. There was no indication of any effect by vitamin B_{12} on the average weight.

Hemoglobin Values

The average hemoglobin values rose in all 5 groups during the experiment. Group A, fed the unsupplemented basal diet 1, showed the second highest increase, but differences between groups were small and all of the hemoglobin averages were within a normal range.

Mortality

Mortality was highest in group A, fed the unsupplemented basal diet 1. Mortality also occurred in group C, fed 8 micrograms of vitamin B_{12}. The results did not indicate any definite need for supplemental vitamin B_{12} to prevent excessive mortality in mature stock.

Egg Production

Table 4 presents the average percentage egg production of each group on a hen-day basis. The differences in initial production among the groups, during the preliminary period, are also shown in table 4 to permit calculation of the relative decline during the first experiment. Egg production declined for all groups, but the drop lessened as the level of vitamin B_{12} fed increased. The egg production of the group fed

TABLE 4

Experiment 1: Effect of Diet upon Egg Production on Hen-Day Basis

Group	Hen-day Total		Total Eggs Laid		Average % Egg Production per Hen		Decline in Average % Egg Production During Experiment 1
	Preliminary Period	Experiment 1	Preliminary Period	Experiment 1	Preliminary Period	Experiment 1	
A	702	1731	492	726	70.1	41.9	28.2
B	687	1836	436	672	63.5	36.6	26.9
C	686	1792	389	627	56.7	35.0	21.7
D	714	1856	384	717	53.8	39.1	14.7
E	719	1836	454	822	63.1	44.8	18.3

basal diet 2 was intermediate between the average productions of the pullets fed 8 micrograms and 16 micrograms of vitamin B_{12}. Results in experiment 1 indicate that the level of egg production was affected by the level of vitamin B_{12} fed.

Following a delay to permit the nutritional status of the pullets to come to equilibrium after the dietary changes, data on fertility and hatchability were collected. The period, representing experiment 1, started December 30, 1949 and terminated March 16, 1950.

Fertility

As shown in table 5 there was a general decline in fertility of the eggs except in the case of those produced by group C, where it increased somewhat. The level of supplemental vitamin B_{12} fed had no measurable influence on it.

Hatchability of Fertile Eggs

The data on hatchability during the first experiment is also included in table 5. Hatchability was maintained or improved in every case except in group A, fed the unsupplemented basal diet 1. Group A showed a decided drop in hatchability. The results indicated that a level of 4 micrograms of supplemental vitamin B_{12} would adequately maintain hatchability.

TABLE 5

Experiment 1: Fertility and Hatchability

Group	No. Eggs Set		% Fertility		% Hatchability of Fertile Eggs	
	Preliminary Period	Experiment 1	Preliminary Period	Experiment 1	Preliminary Period	Experiment 1
A	294	237	61.9	50.2	81.5	62.2
B	268	242	77.6	76.0	80.9	83.7
C	244	183	79.1	88.0	80.7	87.6
D	283	257	75.3	67.7	80.6	80.5
E	286	314	71.7	66.2	80.3	87.0

Vitamin B_{12} Content of Eggs Produced During Experiment 1

Chick assays and microbiological assays for vitamin B_{12} activity were conducted on the eggs produced between March 17, 1950 and April 8, 1950. The chicks were housed in electrically heated metal batteries with raised wire floors. Feed and water were fed ad libitum. The composition of the basal diets used is listed in table 6. Diet 3 is composed of practical feedstuffs deficient in vitamin B_{12} and contains a source of the whey factor, described by Hill, Scott, Norris and Heuser (1944). It furnishes a total of 24.9 percent protein by assay. Only the principle ingredients of diet 4 are detailed in table 6. The remainder are included in table 1 in the appendix. Diet 4 is a vitamin B_{12}-deficient purified ration containing both 35 percent protein and iodinated casein as "stress" factors. (All factors which increase the sensitivity of the test animals toward a vitamin B_{12} deficiency are included under this term.) It is fortified with five times the level of thiamine recommended by the National Research Council because of the destruction of the vitamin in rations containing alpha soybean protein. Alpha soybean protein contains from 1.5 to 2.0 percent sodium sulfite which destroys thiamine and also increases oxidation of vitamins A and E. The chicks fed diet 4 received 1,200 International Units of additional vitamin A per 100 grams of diet and 0.50 milligram of alpha-tocopheral acetate per 100 grams of diet. These vitamins were administered weekly by dropper. They also received an additional supplement of 10 micrograms of thiamine per chick by dropper when 4 weeks old. The source of the vitamin B_{12} for the standard growth curves produced with either diet was crystalline vitamin B_{12} sodium chloride triturate, containing 1 microgram of vitamin per milligram.

TABLE 6

Rations Employed in Egg Assays with Chicks

Ingredients	Diet 3	Principle Ingredients	Diet 4
Ground yellow corn	54.875	Alpha soybean protein	38.000
Expeller soybean meal (41% protein)	40.000	Cerelose (including vitamin mix*)	46.870
Ground limestone	2.000	Salt mix*	6.000
Dicalcium phosphate	2.000	Soybean oil	5.000
Iodized salt	0.500	DL-methionine	0.600
$MnSO_4.4\ H_2O$	0.025	Iodinated casein	0.030
DL-methionine	0.100	A & D feeding oil (2250 A, 400 D)	0.500
A & D feeding oil (2250 A, 400 D)	0.500	Ruffex	5.000
Total	100.000	Total	100.000
Supplemental Ingredients		Protein content	35.000
Dried whey	3.000		
	mg/lb.		
Calcium pantothenate	3.00		
Riboflavin	1.20		
Menadione	0.20		
Nicotinic acid	8.00		
Choline chloride	200.00		
Crude protein (by assay)	24.9%		

* See appendix, table I.

It was added to the diets in a very dilute aqueous solution. Assay eggs were hard-boiled, ground after removal of the shell and mixed in the ration. Both yolks and whites were fed together in diet 3. Later it was reported by Yacowitz, Peeler, Miller, Carlson, Heuser and Norris (1950) that essentially all of the vitamin B_{12} is contained in the yolk, so only that part of the egg was fed in the assay with diet 4. Additions of eggs were made at the expense of corn in diet 3 and at the expense of cerelose in diet 4.

The New Hampshire chicks were fed diet 3 and were used to measure the vitamin B_{12} activity in eggs produced by group D pullets. These chicks were produced by dams maintained on wire floors, at the University poultry farm. The maternal ration was basal diet 1 supplemented with 1 microgram of vitamin B_{12}. The chicks were divided into groups on the basis of equalized weight. They were placed on test at one day of age for a period of 4 weeks. Data on mortality and growth responses were obtained. This assay was repeated three times. Rhode Island Red chicks were employed for the one study in which diet 4 was used. They were produced by dams fed a vitamin B_{12}-deficient breeding ration. Since these chicks were produced by pullets kept on litter which was only replaced every two or three weeks, they were judged to be less deficient in vitamin B_{12} than the New Hampshires (Kennard and Chamberlin, 1949). Therefore they were fed the vitamin B_{12}-deficient basal diet for a depletion period of 2 weeks. Then they were divided into groups on the basis of equalized weight and placed on test for the subsequent 3 weeks. Data on mortality, growth response, and feed efficiency were recorded. This method was used to measure the vitamin B_{12} activity of eggs produced by all five groups of pullets.

The egg samplesfor the microbiological analyses were prepared by the method outlined in the report by Yacowitz, Peeler, Miller, Carlson, Heuser and Norris (1950). Eggs were weighed, brought to room temperature, and steamed for ten minutes at 100° C. The albumen was discarded and the yolk weight was obtained. Each yolk was broken up in 100 cubic centimeters of acetate buffer at pH 4.5. Then this suspension was autoclaved for thirty minutes at 15 pounds pressure. Finally it was filtered and made up to volume. Attempts were made to use the cup assay for vitamin B_{12} as outlined by Foster, Lally and Woodruff (1949). That type of assay was found to be impractical for measuring the content in such a relatively low potency, crude material. A modification of the method of microbiological assay described by Peeler, Yacowitz and Norris (1950) with Lactobacillus leichmannii (ATCC 4797) was eventually employed. The tube assays were kindly conducted by Mr. Charles Denton, of the Bureau of Animal Industry, U. S. D. A., Beltsville, Maryland.

Effects on the chicks of the supplementation of basal diets 3 and 4 are summarized in tables 7 and 8. In every case the addition of either crystalline vitamin B_{12}, a low level of whole eggs or yolks promoted growth. Feed efficiency of the basal group of Rhode Island Red chicks was the lowest. In nearly every case addition of a higher level of the same type of egg improved growth and feed efficiency. Increasing the level of crystalline vitamin B_{12} fed improved growth and feed efficiency in every case. Mortality in the group fed the unsupplemented basal diet 3 was higher than in any other group fed diet 3. Among the groups fed diet 4, mortality seemed to bear no relationship to the level of supplemental vitamin B_{12}. The standard growth curves for the studies involving both chick diets are presented in chart 1. The values for

TABLE 7

Response of Chicks in Egg Assay
(Diet 3)

Group	Supplements Per Kilogram	Total No. of Chicks		Average Final Wt.* Grams
		At Start	At 4 Weeks	
1	None	32	18	166.9
2	2.5 mcg. of B_{12}	24	23	256.9
3	5.0 mcg. of B_{12}	24	19	248.4
4	1 D egg	24	18	230.2

* Average obtained by totaling the final weights of all chicks in three series and dividing by the number of individuals.

vitamin B_{12} activity, determined with these curves for the chick assays, are presented in table 9. The results obtained by the microbiological assays are also shown. All values are based on a yolk weight of 18 grams. The chick assay results obtained with the two different breeds and two entirely different types of basal diets show close agreement. The microbiological assay results agree well with each other, but credit the eggs with only 9.5 percent to 16.7 percent of the vitamin B_{12} activity found by chick assay. Both chick and microbiological assays produced results which indicated that within the levels fed to the pullets, there is a direct relationship between the vitamin B_{12} content of the diet and the vitamin B_{12} activity within the egg. The chick assays gave a value (0.604 micrograms) for group A eggs of one-half the activity found in group B eggs. Group D eggs, produced with four times as much supplemental vitamin B_{12} as the group B eggs, contained about 42 percent more activity, by chick assay. As determined by microbiological assay, the vitamin B_{12} activity of group E eggs was double that found in group B eggs. The value

TABLE 8

Response of Chicks in Egg Assay
(Diet 4)

Group	Supplements Per Kilogram	Total No. of Chicks		Av. Final* Wt. in Grams	Feed*** Efficiency
		At Start	At 4 Weeks		
1	None	21	21	246.8	0.250
2	1.25 mcg. B_{12}	15	11	306.2	0.280
3	2.50 mcg. B_{12}	14	12	357.4	0.317
4	5.00 mcg. B_{12}	14	11	392.2	0.355
5	1.5 A eggs	13	11	300.0	0.256
6	4.0 A eggs	14	13	313.6	0.324
7	2.0 B eggs	12	11	351.5	0.322
8	4.0 B eggs	15	11	386.9	0.360
9	1.5 C eggs	16	13	309.9	0.307
10	3.0 C eggs	15	9	366.4	0.270
11	0.5 D egg	13	12	294.3	0.290
12	1.0 D egg	14	13	277.6**	0.304
13	0.5 E egg	13	11	279.7	0.231
14	1.0 E egg	14	13	271.6**	0.246

* Weights on diet 4 represent the results of one series.

** Results not used in calculating the vitamin B_{12} activity of the egg.

*** Feed efficiency = $\frac{\text{Total gain during test period}}{\text{Total feed consumption during same period}}$.

Chart 1. Standard Growth Curves for Egg Assays with Chicks

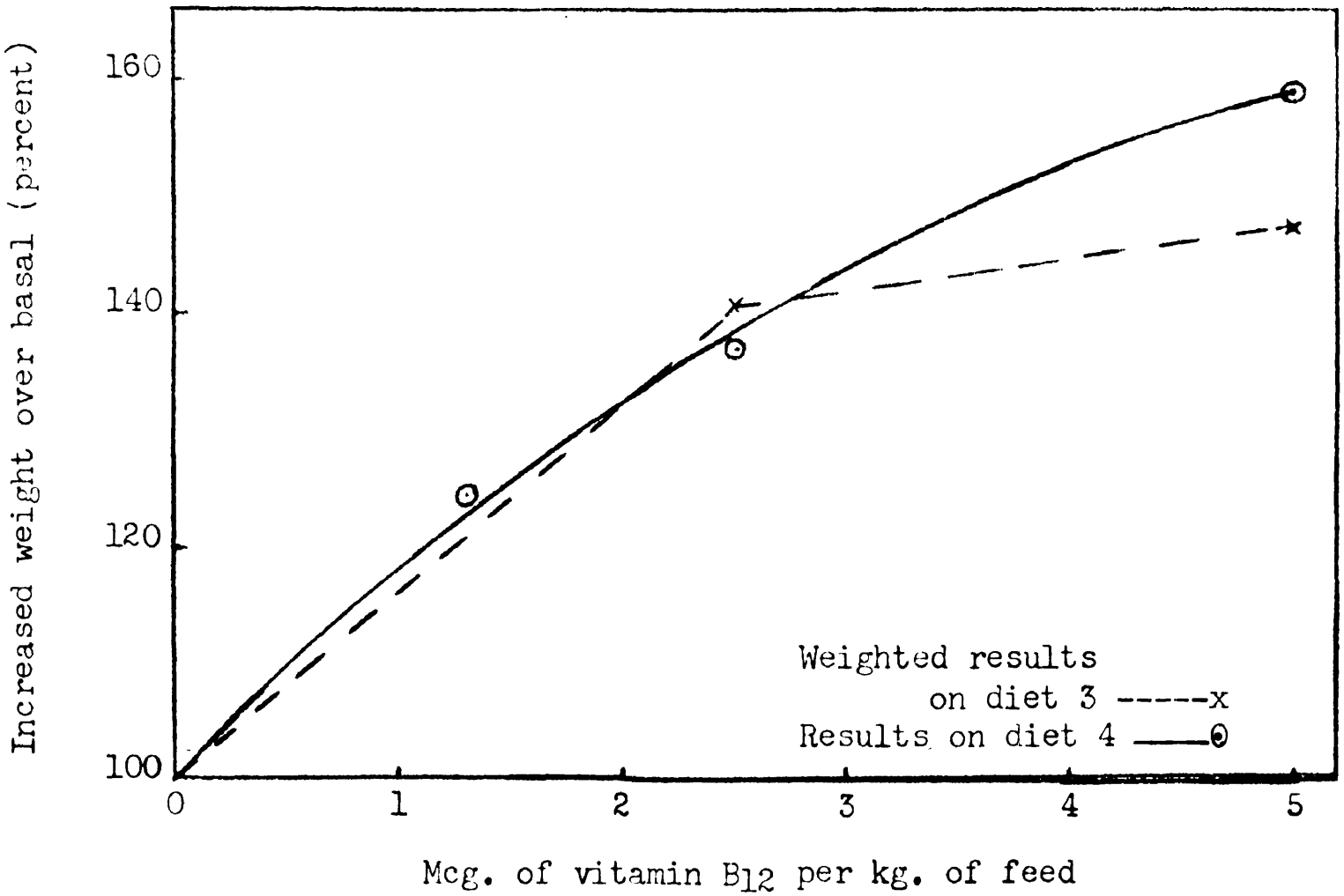


TABLE 9

Vitamin B_{12} Activity per Egg as Influenced By Diet

Type of Assay	Pullet Group A		Pullet Group B		Pullet Group C		Pullet Group D		Pullet Group E	
	Values in Micrograms									
	Single	Average	Single	Average	Single	Average	Single	Average	Single	Average
Chick assay, diet 3							2.12 2.42 3.10	2.23*		
Chick assay, diet 4	0.813 0.395	0.604	1.450 1.100	1.275	0.987 1.183	1.080	2.120	2.120	1.560	1.560
Microbiological assay			0.094 0.109 0.171 0.108	0.121					0.231 0.277 0.279	0.262

* Weighted average of 3 series.

obtained for group C eggs is probably lower than it should be. The cause of this inconsistency in the results is unknown. The results indicated that storage increased at a diminishing rate in the egg with increasing level of the vitamin in the pullet diet.

The percentage transmission of vitamin B_{12} from the pullet diet to the egg may be calculated, using the results of the chick assay, if one assumes that all the vitamin B_{12}, if any, contained in basal diet 1 is completely unavailable. As shown in table 10, the percentage transmission from the pullet diet dropped as higher levels were fed. The results indicate that probably between 30 and 46 percent of the vitamin B_{12} fed to the pullet was transmitted to the egg. These figures are based on the arbitrary assumption that every pullet consumed 127 grams of feed daily.

3. Results of pullet experiment 2. Results of experiment 1 showed that the requirement for optimum hatchability was less than 4 micrograms of supplemental vitamin B_{12} per kilogram under the experimental conditions. Therefore the procedure was altered with the intention of determining more closely this requirement. The length of time required for depletion of hens previously fed known levels of supplemental vitamin B_{12} was also studied. The specific dietary changes which were made are shown in table 2. All the groups were continued on the same basal diets, and group B was continued on the same level of vitamin B_{12}. Group A received a supplement of 3 micrograms of vitamin B_{12} per kilogram, in the form of the Rubramin concentrate produced by Squibb and Sons. Group E received an additional supplement of approximately 27.5 micrograms of vitamin B_{12} in the form of Merck and Co. experimental APF concentrate #3.

TABLE 10

Experiment 1: Percentage Transmission of Vitamin B_{12} from Pullet Diet to Egg

Supplemental B_{12} in Pullet Diet (mcg.)	Feed per Egg (kg.)	Supplemental Fed B_{12} per Egg (mcg.)	B_{12} in Egg (mcg.)	Increase in B_{12} Content of Egg with Supplementation (mcg.)	Transmission of B_{12} from Diet (%)
0	.303	0.000	0.604	0.000	00.0
4	.347	1.388	1.275	0.671	48.3
8	.363	2.904	1.080	0.476	16.4
16	.325	5.200	2.175*	1.571	30.2

*Average of results of both types of chick assay.

Mortality

As shown in table 11, percentage mortality was highest in group A, next highest in group B and was approximately constant at a lower level for the other groups. There was no indication that the levels of vitamin B_{12} which were fed affected mortality.

TABLE 11

Experiment 2: Mortality and Egg Production

Group	% Mortality	Egg Production Total	Hen-day Total	% Production on Hen-day Basis	Decline in % Production from Experiment 1
A	30 (10)*	256	929	27.6	14.3
B	16.7(12)	337	1214	27.8	8.8
C	9.1(11)	190	1122	17.3	18.1
D	8.3(12)	341	1272	26.8	12.3
E	8.3(12)	351	1225	28.7	16.1

* Number in parentheses indicates number of surviving birds at start of experiment 2.

Egg Production

Egg production declined universally and seemed to be unrelated to the level of supplemental vitamin B_{12} in the diet during experiment 2. These data are also presented in table 11.

Fertility

The data on fertility are presented in table 12. Fertility declined for the two groups from which the supplemental vitamin B_{12} was withdrawn. The decline was greater in the group which had been receiving the highest level of supplemental vitamin B_{12} previously. Fertility was maintained or slightly improved in the other groups. The percentage fertility of E group, fed a very high level of vitamin B_{12}, was much lower than the fertility in group B, fed a low level of vitamin B_{12}. Therefore it was concluded that the vitamin B_{12} content of the diet was not the cause of fluctuating fertility.

TABLE 12

Experiment 2: Fertility and Hatchability

Group	No. of Eggs Set	% Fertility	Change in % Fertility from Experiment 1	% Hatchability of Fertile Eggs	Change in % Hatchability from Experiment 1
A	159	50.3	(+) 0.1	65.0	(+) 2.8
B	243	80.7	(+) 4.7	73.0	(-)10.7
C	135	74.1	(-)13.9	68.0	(-)19.6
D	259	49.8	(-)17.9	83.2	(+) 2.7
E	216	66.7	(+) 0.5	75.7	(-)11.3

Hatchability

Overall hatchability of fertile eggs improved slightly in groups A and D as shown in table 12. But it declined noticeably for the other groups including the positive control group E. Therefore no additional

information on the hatchability requirement could be derived from experiment 2.

Depletion Rate

It is difficult to ascertain the rate of depletion. Table 13 presents the hatchability data for experiment 2 in two periods to facilitate study of the depletion rate. The first period terminated 47 days after the start of experiment 2. The next period terminated 56 days later. Limited numbers of eggs made it impossible to further subdivide the test. Hatchability appeared to drop in C group immediately after removal of the 8 micrograms of vitamin B_{12} from the diet. It also appeared that hatchability was unaffected in D group for the first 47 days after the removal of the 16 micrograms of vitamin B_{12}. The hatchability data are of very limited value because hatchability showed wide fluctuations.

4. Summary of pullet experiments. Pullets were fed a practical-type basal ration composed of feedstuffs very low in vitamin B_{12} content. During experiment 1 this basal ration was supplemented with graded levels of vitamin B_{12} or natural ingredients considered to be potent sources. In experiment 2 the higher levels of supplemental vitamin B_{12} were withdrawn and a low level added to the group previously fed the basal diet. During experiment 1 body weight and hemoglobin values increased but bore no measurable relationship to the vitamin B_{12} content of the diets. Mortality was highest in the unsupplemented group in experiment 1, but continued to be highest in that group after a low level of supplemental vitamin B_{12} was added to the diet for experiment 2.

TABLE 13

Experiment 2: Maternal Depletion Rate as shown by Percentage Hatchability of Fertile Eggs

Period Represented	A Eggs		B Eggs		C Eggs		D Eggs		E Eggs	
	No. Fertile	Hatch-ability %	No. Fertile	Hatch-ability %	No. Fertile	Hatch-ability %	No. Fertile	Hatch-ability %	No. Fertile	Hatch-ability %
Experiment 1	119	62.6	184	83.7	161	87.6	174	80.5	208	87.0
April 21 - May 25	51	72.5	109	72.3	53	67.9	71	93.0	74	83.8
May 26 - July 16	29	51.7	87	73.7	47	68.1	48	68.8	70	72.9

In all cases the numbers of individuals involved were small. There was no significant evidence that the levels of vitamin B_{12} involved affected mortality in mature stock. Egg production declined for all groups during both experiments, but decreased less in the supplemented groups during experiment 1. During experiment 2, the decline in egg production seemed to be unrelated to the level of supplementation. Fertility was lowest in the group fed the unsupplemented basal diet during experiment 1, but extreme variability occurred between the groups fed additional vitamin B_{12}. During experiment 2, groups C and D, from which all supplemental vitamin B_{12} had been withdrawn, showed sharp declines in fertility, while the other groups, fed supplemental vitamin B_{12}, either maintained fertility or increased it. Fluctuations in percentage fertility between groups made it seem unlikely, however, that the vitamin B_{12} content was a controlling factor. Hatchability was maintained on 4 micrograms of supplemental vitamin B_{12} per kilogram of feed and on all higher levels, but it was depressed on the unsupplemented basal diet 1, during the 154 day duration of experiment 1. Fluctuations in hatchability only partially reconcilable with the diets occurred during experiment 2. Results indicated that when the 8 micrograms of supplemental vitamin B_{12} were removed from the diet of C group, hatchability dropped immediately. Apparently the hatchability in group D was not depressed for at least the first 47 days after removal of the supplement of 16 micrograms of vitamin B_{12} from its diet. Since hatchability dropped sharply for group E during the second period, it is believed that the seasonal factor was a primary determinant toward the last of the test.

Eggs produced by each group during experiment 1 were assayed for vitamin B_{12} activity. According to chick assays, eggs produced by

group A pullets contained activity equivalent to approximately 0.604 microgram of vitamin B_{12} per average egg. Eggs produced by group D pullets contained activity equivalent to about 2.175 micrograms. Microbiological assays yielded values only 9.5 percent to 16.7 percent as high as those obtained by chick assays. According to arbitrary calculations based on the results of the chick assays, 30 to 48 percent of the vitamin B_{12} fed to the pullet was transmitted to the egg.

B. Studies with Chicks.

1. General experimental procedure. Progeny produced during experiment 1 were studied as a part of experiment 1, and those produced during experiment 2 were studied as a part of the latter test. During both periodsall chicks were pedigreed according to maternal group and wing banded. Initial weights were recorded. Then chicks from each pullet group were distributed evenly into all chick pens according to equalized weight. At one day of age they were placed on test in heated metal batteries with raised screen floors. Feed and water were supplied ad libitum. Whenever the number of chicks and available space permitted, pens were replicated to reduce the importance of the positional factor. Tests were terminated when the chicks were four weeks old unless otherwise stated. During several series, the degree of feathering was scored. The system used is described in table 14. Basal diet 5, used in these tests, was identical with basal diet 3, shown in table 6, except that the dried whey was omitted. It was adequately fortified with choline and methionine to meet all normal requirements. The supplements to basal diet 5 are listed in table 15 for experiments 1 and 2.

TABLE 14

Grading System for Degree of Feathering

Adjective Score	Numerical Score
No feathers	0
Very poor	25
Poor	50
Fair	75
Good	90
Excellent	100

Chief criterion of the test was the final average weights, although mortality and feathering data were also obtained.

While the hatchability data were not considered until the hatchability began to drop in pullet group A, all chicks produced from eggs laid after November 20, 1949, are included in the progeny studies in experiment 1. In order to simplify the presentation, chicks produced by group A pullets, group B pullets, etc., will be described simply as A chicks, B chicks, etc. The final setting date for chicks for studies in experiment 1 was March 16, 1950. Eggs laid by the pullets between April 21st and July 20th were also incubated. Hatches of eggs laid between April 21st and July 6th were large enough to use for chick studies. Consequently the chick studies in experiment 2 covered a period of 89 days after the change in maternal diets.

2. Chick trials during experiment 1. A range of supplemental vitamin B_{12} levels was fed in an effort to bracket all chick requirements for that vitamin. The actual levels are shown on table 15.

TABLE 15

Levels of Supplemental Vitamin B_{12} Fed in Progeny Studies

Chick Pen	Micrograms of B_{12} per Kilogram of Feed	
	Experiment 1	Experiment 2
1	0	0
2	8	12
3	4	15
4	27	18

Initial Weight and Mortality

The initial weight of the chick was unaffected by maternal diet, according to the data included in table 16. Table 17 shows the effect of maternal diet on average liveability of chicks in each chick group. When the dam received no supplemental vitamin B_{12} the chick required somewhere between 9 and 27 micrograms of supplemental vitamin B_{12} for reasonably good liveability to 4 weeks. If the dam received 4 micrograms of supplemental vitamin B_{12} the chick required no supplemental vitamin B_{12} for reasonably good liveability to 4 weeks.

Final Weight

The influence of the maternal diet on the requirement of chicks for vitamin B_{12} for growth is shown on chart 2. Each column represents the final average weight for all chicks produced on a given maternal diet during experiment 1, and fed a given level of supplemental vitamin B_{12}.

TABLE 16

Experiment 1: Initial Weight of Chicks as Influenced by Maternal Diet

Maternal Group	No. of Chicks	Av. Hatching Weight (gm.)
A	82	41.4
B	168	41.9
C	155	41.5
D	161	40.5
E	182	40.7

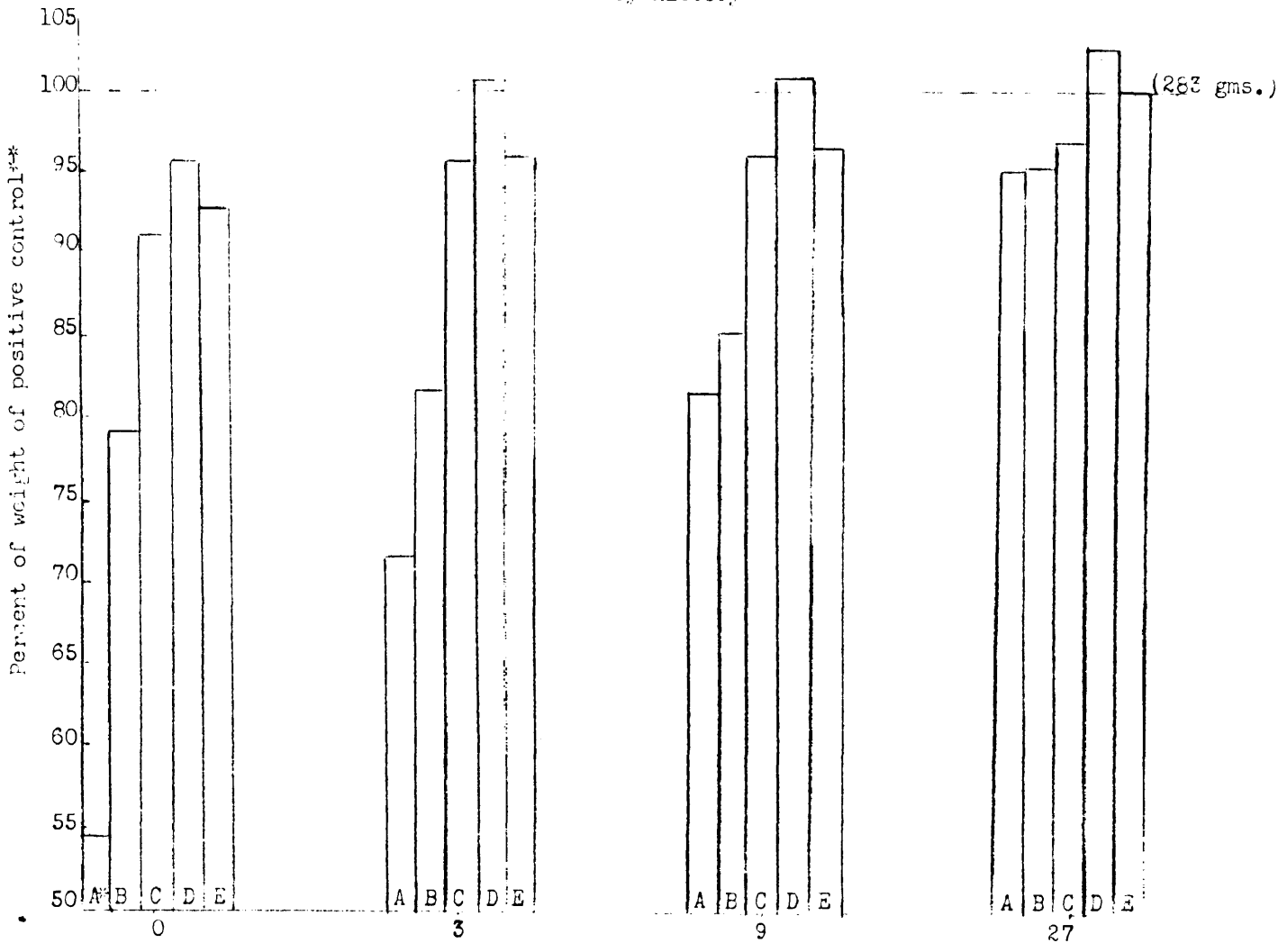
TABLE 17

Experiment 1: Percentage Liveability of Chicks as Influenced by Maternal Diet

Maternal Group	% Liveability to 4 Weeks Chick Pen			
	1	2	3	4
A	75.2	69.0	85.7	91.2
B	89.4	96.1	92.5	95.6
C	92.5	96.8	93.8	90.6
D	98.4	96.9	95.3	100.0
E	93.1	90.7	98.6	91.4

Chart 2

Experiment 1: Vitamin B_{12} Requirement of Chicks for Growth as Affected by Maternal Dietary History



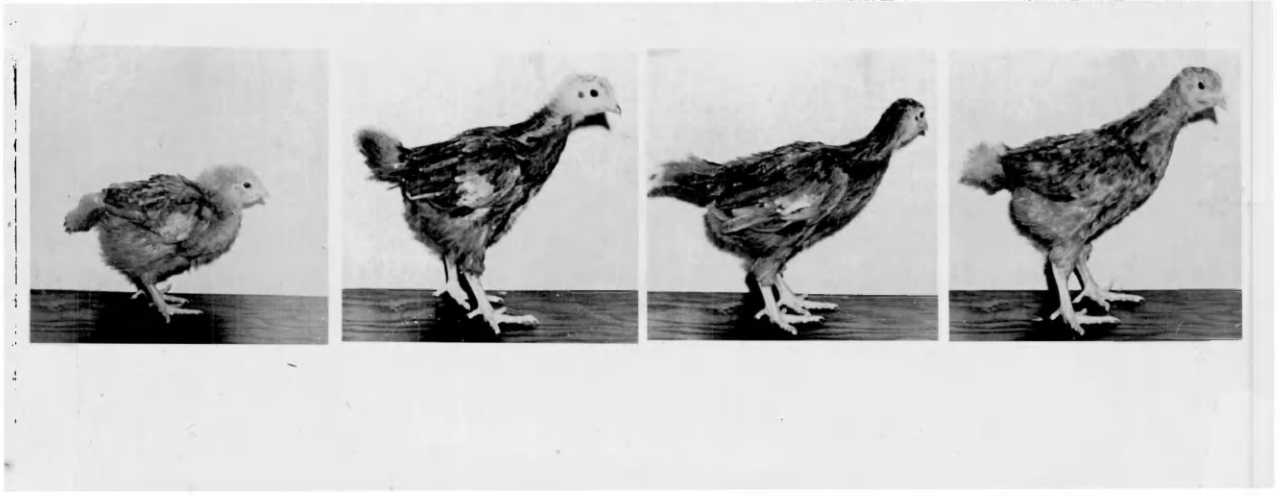
* Capital letters in columns refer to the maternal group which produced the chicks. The maternal diets are listed in table 2.

** Weighted average of final weights expressed as percent of that produced by progeny of group E pullets on basal chick diet 5, supplemented with 27 mcg. of B_{12} per kg.

The average final weight is expressed in terms of percent of that attained by E chicks fed 27 micrograms of supplemental vitamin B_{12} per kilogram of feed. The type of chick represented by a column is described by the capital letter at the base. Lowest final weight was produced by A chicks, although the average weight of both A and B chicks was approximately equal when the chick ration contained 27 micrograms of supplemental vitamin B_{12} per kilogram of feed. Best growth was produced by D chicks on every chick diet. The supposition that the vitamin B_{12} content of basal diet 2 was intermediate between the level furnished group C and group D pullets is borne out by the average final weight attained by the E chicks. When the chicks were produced on diet 1 supplemented with 4 micrograms of vitamin B_{12} they grew the most satisfactorily on the highest level of supplementation, 27 micrograms in the chick diet. When the chicks were produced by pullets fed 8 micrograms of supplemental vitamin B_{12}, they required only 5 micrograms of supplemental vitamin B_{12} in the chick diet to grow as well as if fed 27 micrograms. D chicks appeared to require 3 micrograms or less of supplemental vitamin B_{12} in the chick diet for satisfactory growth to 4 weeks, although there was a tendency towards slightly higher final weight with 27 micrograms in the diet. Figure 1 illustrates pictorially the effect of the maternal diet on chick growth. All the chicks in the photographs were reared in the same chick group on the unsupplemented basal diet 5. The effect of supplementation of the maternal diet with vitamin B_{12} is evident.

FIGURE 1

Experiment 1: Effect of Vitamin B_{12} in Maternal Diet on Chick Growth*



Maternal Group	A	B	C	D
Av. Wt. (gm.)**	154	215	282	289

* All chicks were reared in the same group on basal diet 5.

** Average weight at 4 weeks of age.

Feathering

Progeny produced during the period from February 17, 1950 to March 16, 1950 (2 hatches) were graded for degree of feathering. The number of individuals involved was small. The degree of feathering of A chicks, shown in table 18, was clearly less satisfactory when the progeny were fed the chick basal diet than that of any other type of chicks on the same ration. The A chicks feathered satisfactorily on 3 micrograms of vitamin B_{12} in the chick diet. When the dams were fed 4 micrograms of vitamin B_{12} the chicks required no vitamin B_{12} to 4 weeks of age for good feathering.

TABLE 18

Experiment 1: Degree of Feathering as Influenced by Diet*

Chick Pen	Maternal Group A	Maternal Group B	Maternal Group C	Maternal Group D	Maternal Group E
1	48.6(7)**	70.0 (7)	75.4(12)	75.0(13)	72.9(12)
2	75.0(1)	61.8(10)	79.6(12)	74.6(14)	76.2(13)
3	73.8(4)	71.0(10)	77.9(12)	78.8(13)	77.5(12)
4	56.3(4)	69.5(11)	79.1(11)	80.0(13)	77.8(12)

* Observations made on chicks hatched from eggs produced between February 17th and March 16th. Method of scoring shown in table 14.

** Number in parentheses indicates total number of chicks represented.

(Occasional examples of poor feather quality also appeared in the form of broken shafts or easily fractured tips, but this type was not confined to any specific group of chicks. The author has noted the same type of feathering difficulty with Rhode Island Red chicks produced on a good breeder mash and fed a Connecticut broiler ration or other good starting ration. For this reason he believes the cause is not related to the problem under study.)

In table 19 the final weights at 6 weeks are compared with the fourth week weights. The numbers of chickens involved in some cases were very small, but the same relationships between the groups prevailed at both ages. This result indicated that the requirements determined for the first 4 weeks are adequate for 6 weeks. It does not prove, however that the requirement remains constant between the fourth and sixth week. It may diminish.

The relative effectiveness of vitamin B_{12} furnished by the chick diet and by the egg is shown in table 20. The calculations are based on the admittedly arbitrary assumption that all chicks consumed 454 grams of feed during the first 4 weeks of life. Vitamin B_{12} furnished by the chick diet was at best only 25 percent as efficiently used as vitamin B_{12} furnished by the egg and the relative efficiency of utilization of that furnished in the diet dropped as higher levels were supplied.

Tabel 21 presents the relative quantitative efficiency of supplying the vitamin B_{12} for the chick through the maternal diet and through the chick diet. The data show that the vitamin B_{12} requirement of the chick for growth, on a quantitative basis, can be more efficiently met to 4 weeks of age by supplementation in the maternal diet. Supplementation in the chick diet was at best only 51 percent as efficient.

TABLE 19

Experiment 1: Chick Weight at 6 Weeks Compared to 4 Weeks Expressed as Percentage of Average Weight of E Chicks in Pen 4*

Chick Pen	A		B		C		D		E	
	4th Week	6th Week	4th Week	6th Week	4th Week	6th Week	4th Week	6th Week	4th Week	6th Week
1	44.3 (4)**	46.8 (4)	41.3 (2)	38.9 (2)	62.6 (7)	64.8 (7)	83.7 (8)	81.9 (8)	74.5 (6)	75.7 (6)
2	58.3 (1)	59.8 (1)	69.8 (4)	68.0 (4)	98.0 (7)	94.8 (7)	87.7 (9)	85.6 (9)	85.1 (7)	83.2 (7)
3	56.7 (2)	59.8 (2)	66.7 (5)	64.4 (5)	88.8 (7)	89.1 (7)	91.4 (8)	94.9 (8)	76.8 (7)	79.1 (7)
4	65.5 (2)	65.4 (2)	89.6 (4)	93.9 (4)	97.9 (7)	96.6 (7)	100.8 (8)	99.3 (8)	100.0 or 322.7 gm. (7)	100.0 or 602.4 gm. (7)

* Data obtained on chicks hatched from eggs produced between February 17th and March 2nd.

** Number in parentheses indicates total number of individuals represented.

TABLE 20

Experiment 1: Relative Effectiveness of Vitamin B_{12} Furnished to Chicks by Diet and via Egg

	Comparison of Chicks					
	A and B		A and C		A and D	
Level of supplemental B_{12} (mcg.) in chick diet	9	3	27	3	27	0
Estimated av. feed cons. (kg.) per chick per 4 weeks	.454	.454	.454	.454	.454	.454
Mcg. B_{12} required for equal growth:						
a. when furnished in chick diet	2.724		10.896		12.258	
b. when furnished via egg*		0.671		0.476		1.571
Relative effectiveness of B_{12} fed in chick diet	24.6%		4.4%		12.8%	

* Values obtained from table 10.

TABLE 21

Experiment 1: Relative Effectiveness of Vitamin B_{12} Fed in the Maternal Diet and in the Chick Diet for Chick Growth

	Comparison of Chicks					
	A and B		A and C		A and D	
Level of supplemental B_{12} (mcg.) in chick diet	9	3	27	3	27	0
Estimated av. feed cons. (kg.) per chick per 4 weeks	.454	.454	.454	.454	.454	.454
Mcg. B_{12} Required for Equal Growth:						
a. when furnished in chick diet	2.724		10.896		12.258	
b. when furnished in maternal diet*		1.388		2.904		5.200
Relative effectiveness of B12 fed in chick diet	51.0		26.7		42.4	

* Values obtained from table 10.

3. Chick studies during experiment 2. The levels of supplemental vitamin B_{12} fed the chick were changed for experiment 2 as shown in table 15. Slightly under maximum growth was believed to have been obtained with E chicks when 9 micrograms of vitamin B_{12} were added in the chick diet. For that reason the levels of supplemental vitamin B_{12} were fixed at 12, 15 and 18 micrograms per kilogram.

Initial Weights and Mortality

Initial weights of chicks produced during experiment 2, presented in table 22, again were not measurably affected by the diet of the dams. The data on mortality of chicks in experiment 2 are presented in table 23. The percentage liveability to 4 weeks of A chicks was improved over experiment 1 by the addition of 5 micrograms of vitamin B_{12}

TABLE 22

Experiment 2: Effect of Maternal Diet on Initial Weight of Chicks

Maternal Group	Number of Chicks	Average Weight (gm.)
A	43	43.5
B	121	43.9
C	48	43.6
D	75	42.8
E	93	44.1

TABLE 23

Experiment 2: Percentage of Chicks Alive at 4 Weeks

Chick Group	A Chicks		B Chicks		C Chicks		D Chicks		E Chicks	
	No. Started	% Surviving	No. Started	% Surviving	No. Started	% Surviving	No. Started	% Surviving	No. Started	% Surviving
1	13	92.3	37	91.9	14	92.9	21	90.5	28	82.1
2	13	76.9	32	93.8	11	100.0	21	81.0	28	78.6
3	8	75.0	31	90.3	10	100.0	17	94.1	16	100.0
4	13	92.3	29	86.2	15	80.0	21	90.5	25	92.0

per kilogram to the maternal diet. Removal of the vitamin B_{12} supplement from maternal groups C and D did not impair the viability of the progeny. These results indicated that the dams' stores of vitamin B_{12} were sufficient to protect the chicks from excessive mortality for the duration of the experiment.

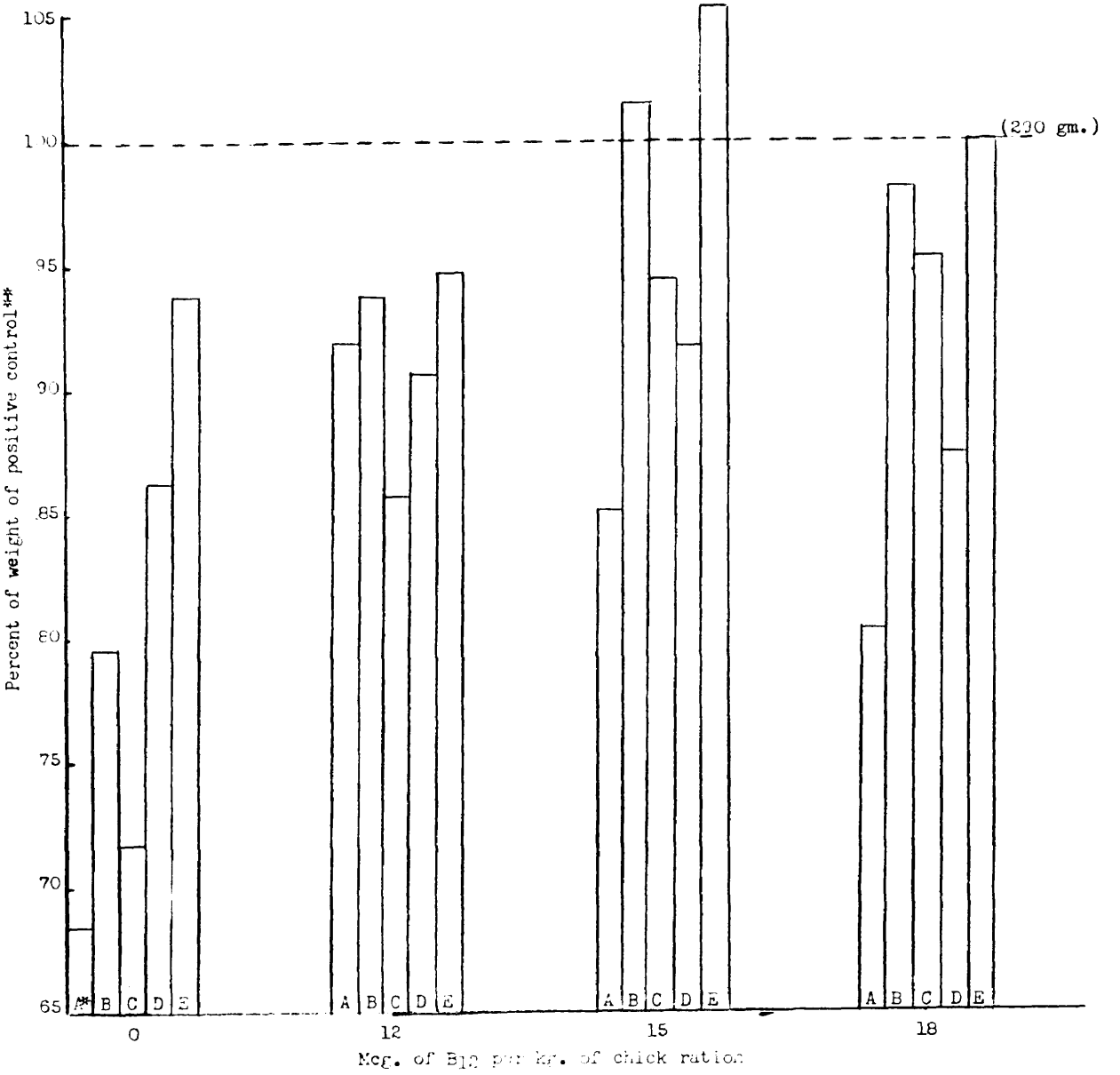
Final Weight

Chart 3 summarizes the average final weights attained by the chicks, expressed as percentage of that attained by the positive control group. That group consisted of the E chicks fed the highest level of supplemental vitamin B_{12}. The average weight of the positive control chicks was practically identical with the average weight of the E chicks fed 27 micrograms of supplemental vitamin B_{12} in experiment 1. B chicks, produced by pullets fed 4 micrograms of vitamin B_{12}, grew approximately as well as the control group when fed 15 micrograms of vitamin B_{12}. The average weight for the E chicks fed this level of vitamin was higher than for the controls. This result indicated that no advantage could be gained by feeding higher levels to E chicks.

Considering the results on all chick diets, the average weights of both C and D chicks, whose dams were presumably being depleted of their vitamin B_{12} stores, were lower than the average weight of the B chicks. The final weight of the A chicks was generally lower than the final weight of any other type of chick. The results suffered from the variability to be expected with small numbers of individuals, but generally indicated that 15 micrograms of supplemental vitamin B_{12} added to such a chick diet would satisfy the requirement of chicks produced on no more than 4 micrograms of vitamin B_{12}.

Chart 7

Experiment 2: Vitamin B_{12} Requirement of Chicks for Growth as Affected by Maternal Dietary History



* Capital letters in columns refer to maternal group which produced the chicks. Maternal diets fed during experiment 2 are listed in table 2.

** Weighted average of final weights at 4 weeks, expressed as percent of that produced by progeny of group E pullets, on basal chick diet B, supplemented with 27 mcg. of B_{12} per kg.

Depletion Rate

Table 24 presents, by division into 2 periods, the final average weight of the chicks fed only the unsupplemented basal diet 5. Period 1 includes the first 47 days of experiment 2. The second period includes results obtained on the subsequent 42 days. As shown in the table, numbers of individuals representing most of the groups were very small. It is noticeable, however, that the growth of A chicks increased immediately after supplementation of the maternal basal diet 1 with 3 micrograms of vitamin B_{12}. The B chicks produced on the same maternal diet used for experiment 1, maintained the same general rate of growth throughout experiment 2. Growth of D chicks remained relatively constant during experiment 2, indicating that the dams had sufficient stores of vitamin B_{12} remaining from experiment 1 to supply the chicks with enough vitamin B_{12} to maintain the same rate of growth for at least 89 days on the chick basal diet. The average weight of D chicks fed supplemental vitamin B_{12} (chart 3) indicated however that some depletion had occurred in pullet group D. Growth of E chicks, presented in table 24, increased following supplementation of their maternal basal diet with 27.5 micrograms of additional vitamin B_{12}. It is evident from these data that addition of a small amount of supplemental vitamin B_{12} to the diet of depleted pullets produces an immediate growth stimulation in resultant progeny. It is also evident that supplementation of pullet basal diet 1 with 8 micrograms of vitamin B_{12} did not permit storage of a satisfactory reserve supply. Supplement of the pullet basal diet 1 with 16 micrograms of vitamin B_{12} did permit storage of a considerable reserve.

TABLE 24

Experiment 2: A Study of the Maternal Depletion Rate as Shown by Final Weights of Chicks Fed Unsupplemented Basal Diet 5.

Period Represented	A Chicks		B Chicks		C Chicks		D Chicks		E Chicks	
	No. Chicks	Weight (gm.)	No. Chicks	Weight (gm.)	No. Chicks	Weight (gm.)	No. Chicks	Weight (gm.)	No. Chicks	Weight (gm.)
Final average weight for experiment 1	31	153.8	59	223.4	62	256.9	62	269.6	67	262.1
April 21 - May 25	8	190.1	21	213.8	9	201.2	14	245.0	15	251.5
May 26 - July 6	4	214.8	13	257.8	4	221.8	5	262.4	8	309.3

3. Summary of results with progeny. During both experiments, initial weight of the chicks was unaffected by maternal diet. During experiment 1 it was shown that absence of supplemental vitamin B_{12} in diet 1 for the dams produced chicks of poor liveability. The excessive mortality could be prevented by the addition of between 9 and 27 micrograms of supplemental vitamin B_{12} per kilogram of chick diet. During experiment 2 there was apparently enough vitamin B_{12} transmitted to the chick to prevent excessive mortality in all groups. Results of experiment 1 indicated that slightly more than 8 micrograms of supplemental vitamin B_{12} per kilogram of pullet feed would permit progeny to grow at an optimum or nearly optimum rate, if the chick diet contained 3 micrograms of supplemental vitamin B_{12}. Results of experiment 2 indicated that the chicks produced on 4 micrograms of supplemental vitamin B_{12} would grow at an excellent rate on 15 micrograms of supplemental vitamin B_{12}.

The growth response of both C and D chicks was depressed somewhat by removal of the supplemental vitamin B_{12} from the maternal diet. Division into 2 periods of final weight data, obtained for chicks fed the unsupplemented basal diet 5, showed that C chicks immediately dropped in growth rate. D chicks maintained their rate of growth fairly well. These results indicated that 8 micrograms of supplemental vitamin B_{12} in the pullet diet would permit little if any storage in the dam's body. The addition of 3 micrograms of vitamin B_{12} to the maternal diet of A chicks did not restore the growth rate to normal in the 89 day period represented, when the chicks received as much as 18 micrograms of vitamin B_{12} per kilogram, but it did produce an immediate growth stimulation.

Limited data on growth to 6 weeks indicate that the same levels of supplemental vitamin B_{12} required for the first 4 weeks are adequate for the first 6 weeks of chick life. Degree of feathering was poorest in A chicks fed the unsupplemented basal diet. The degree of feathering was easily increased by addition of vitamin B_{12} to either the chick or the maternal diet.

The growth results indicate that vitamin B_{12} furnished through the maternal diet is more efficiently used than when it is supplied through the chick diet.

DISCUSSION

A. Studies with Laying Pullets

The vitamin B_{12} content of the basal diet was apparently adequate for maintenance of body weight. This result agrees with the report by Whitson, Titus and Bird (1948) that body weight was not affected by feeding a similar ration to layers.

There was some published evidence that vitamin B_{12} influenced hemoglobin values in chickens. The report by Nichol, Harper and Elvehjem (1949) had indicated that vitamin B_{12} improved the rate of hemoglobin regeneration in folic acid-deficient chicks, although only in the presence of adequate levels of folic acid. However, in the study reported herein, hemoglobin values of pullets were not reduced by the low dietary level of vitamin B_{12}. Mushett and Ott (1949) reported similar results with chicks. These results prove that if vitamin B_{12} is required for maintenance of hemoglobin, the quantitative requirement must be extremely low.

Although mortality was highest in the group which was fed no supplemental vitamin B_{12} in experiment 1 and fed a very low level of vitamin B_{12} in experiment 2, the limited numbers involved make it impossible to determine if the effect was due to a vitamin B_{12} deficiency in the pullets. There have been no reports of mortality in mature birds as a result of such a deficiency. It is doubtful that body weight would be maintained when the deficiency for maintenance alone were so great that it caused increased mortality.

While egg production seemed to be benefited in experiment 1 by supplemental vitamin B_{12} in the diet, the results in experiment 2 were

inconclusive. Skinner and Quisenberry (1950) reported that absence of an APF supplement or animal protein from a high efficiency laying mash resulted in lowered production. Hill, McConachie, Gartley and Branion (1950) stated that birds fed a cereal grain-soybean meal type ration unsupplemented with vitamin B_{12} came into production later than others which were furnished the vitamin, and produced fewer eggs. It is not clear whether the birds laid fewer eggs than the others on a total quantitative basis due to slowness in attaining sexual maturity or the egg production was actually less intense after they came into lay. Olcese, Couch and Lyman (1950) have recently reported that the addition of vitamin B_{12} to a sucrose-soybean protein diet improved egg production of hens. When starch replaced the sucrose in the absence of vitamin B_{12}, egg production and hatchability improved, possibly indicating increased intestinal synthesis of the vitamin. On the other hand, Whitson, Titus and Bird (1946), Forrest, Bisly and March (1950) and others, working with all-vegetable protein practical diets deficient in vitamin B_{12} have found no reduction in egg production. In other work carried out by the writer, an excellent rate of production was found to be maintained from September to the following June (when the test was terminated for unrelated reasons) on a similar ration. More than 125 Rhode Island Red pullets were used. They were maintained on litter, however, and therefore probably received slightly more vitamin B_{12}. Arscott (1950) reported no significant differences in the egg production between pens of pullets maintained on wire with and without supplemental vitamin B_{12}. The basal ration employed was basal diet 1. Fifty New Hampshire pullets were used per pen. Perhaps vitamin B_{12} is required for egg production, but in no larger quantities than are usually contained in cereal grain-soybean meal rations without

supplementation with vitamin B_{12}. At any rate the evidence produced in experiment 1 favoring the hypothesis that egg production can be benefited by supplementation of such a ration with vitamin B_{12} is by no means conclusive because of the limited number of individuals and the small differences in production.

There was considerable variability in fertility among the groups at different times, but there was no evidence that fertility was influenced by vitamin B_{12}. During experiment 1, 4 micrograms of vitamin B_{12} were found to satisfy the requirement for good hatchability when a normal level of protein was fed and no stress factors were introduced aside from the elimination of coprophagy. Recently several studies on hatchability have been reported which have a direct bearing on the work being discussed. Petersen, Wiese, Lampman and Dahlstrom (1950) studied the effect of vitamin B_{12} on hatchability by injecting graded levels of the vitamin into white leghorn pullets kept on wire and previously fed a corn-soybean meal depletion diet of unstated protein level for 4 months. Their data indicated that approximately 1.0 micrograms of vitamin B_{12} injected per hen per week would restore hatchability to 89.6 percent within 3 weeks as compared to 65.0 percent hatchability for the birds injected with 0.5 micrograms of B_{12} per week. If one assumes that each hen consumed approximately 800 grams of feed per week, the higher injection rate furnished the vitamin to the bird at a rate of 1.25 micrograms per kilogram of feed. Growth of progeny was highest when the hen was injected with the highest level of vitamin B_{12} used, 40 micrograms per hen per week, or approximately 5.0 micrograms per kilogram of feed. The progeny were fed a vitamin B_{12} deficient all-plant protein diet for 4 weeks. Stokstad, Jukes, Pierce, Page and Franklin (1949) found the oral

potency of vitamin B_{12} to be 50 percent that of the injected potency. In 1950 Menge reported work in agreement essentially with this figure. On the basis of these conversion factors, the levels of vitamin B_{12} which Petersen et al found satisfied the requirement for hatchability and the requirement for growth were 2.5 micrograms and 10 micrograms, respectively, per kilogram when orally administered.

Carver and McGinnis (1950) reported that hatchability was greatly reduced when hens in batteries were fed an unsupplemented cereal grain-soybean meal type of diet of unspecified protein level. When 1.0 microgram of crystalline vitamin B_{12} was added per kilogram of diet, hatchability markedly improved. Data were insufficient to indicate a quantitative requirement for hatchability. In December, 1949, Lillie, Olsen, and Bird, reported that hatchability of eggs from depleted hens was restored by injecting 0.5 microgram of B_{12} into each egg. Growth of resulting chicks also increased markedly for at least 6 weeks. In studies involving administration of vitamin B_{12} to the dam the level of protein fed the hen must be considered as an important factor. Yacowitz, Peeler, Miller, Carlson, Heuser and Norris (1950) reported that when birds were fed an all-vegetable protein type of diet containing no supplemental vitamin B_{12}, hens on a level of 25 percent protein produced eggs of 26 percent hatchability after 12 weeks of depletion. If the protein level in the hen diet was 18 percent, however, hatchability was 53 percent after 15 weeks. They estimated that the requirement for hatchability on a 25 percent protein diet was 2 micrograms of supplemental vitamin B_{12} per kilogram of feed.

While 4 micrograms of vitamin B_{12} per kilogram of feed supported satisfactory hatchability on the 15 percent protein diet 1, the results

with 3 micrograms per kilogram in experiment 2 were not clear. True, the overall hatchability of that group did improve very slightly after the vitamin B_{12} was added, but hatchability in E group declined considerably and overall hatchability in D group, from which all supplemental vitamin B_{12} had been removed, increased slightly. Group B, fed 4 micrograms of vitamin B_{12} declined considerably in overall hatchability. In spite of the fact that hatchability in A group remained lower than that in any other group, the spread between the positive control group E, group A and group B was so narrow as to indicate that all of the fluctuations might have been the result of other factors. It is quite possible, therefore, that 3 micrograms of vitamin B_{12} supplemented to such a ration may maintain good hatchability. Indeed concurrent work conducted at this station by Arscott (1950) strongly indicates that on the same basal diet, birds maintained under flock conditions on wire require slightly more than 2 micrograms of vitamin B_{12} per kilogram of diet for optimum hatchability. This figure is in close agreement with the adjusted figure of 2.5 micrograms cited for Petersen et al.

The study on the rate of depletion of pullets following removal of supplemental vitamin B_{12} was handicapped by limited egg production. The results did show an immediate drop in hatchability when the 8 micrograms of supplemental vitamin B_{12} were removed from the pullet diet. The rapid decline is evidence that those pullets were unable to store a reserve of vitamin B_{12} sufficient to carry them over even a temporary shortage of the vitamin. It indicates that 8 micrograms of supplemental vitamin B_{12} in such a diet is inadequate if storage is desired as a safety factor.

The results of the analyses of the eggs for vitamin B_{12} content demonstrate the current difficulty in assaying any crude feedstuffs for

this vitamin. The results with the different chick assays show good correlation. The microbiological assay results are also reasonably consistent. But 90.5 percent of the vitamin B_{12} activity found in B eggs and 83.3 percent of the vitamin B_{12} activity found in E eggs by chick assay were microbiologically unavailable. In 1947 Rubin and Bird reported the occurrence of the cow manure factor in eggs. They assayed the egg for potency with chicks, feeding what later proved to be a vitamin B_{12} concentrate as a positive control. Since that time Lillie and Bird (1949) have determined the vitamin B_{12} content of that concentrate. By applying the now known vitamin B_{12} potency one finds that Rubin and Bird found approximately 1.23 micrograms of vitamin B_{12} activity per egg when it was produced on a vitamin B_{12}-deficient ration similar to basal diet 1. They determined normal eggs to contain roughly 2.89 micrograms of vitamin B_{12} activity, apiece. Since their laying birds were maintained on litter and had access to their droppings, it is logical that the vitamin B_{12} activity of their eggs should be slightly higher for both the normal eggs and for the deficient ones. The correlation between the results of their study and the ones reported herein is remarkable. The preliminary value for vitamin B_{12} content of eggs produced by undepleted chickens as determined by the rat assay, reported by Lewis, Register, Thompson and Elvehjem (1949) conflicts with the chick results. Their result showed good correlation with the microbiological results reported herein. They found 252 millimicrograms of vitamin B_{12} per egg, whereas the latter results indicated 262 millimicrograms of vitamin B_{12} per egg.

Results of the microbiological assays reported in this thesis verify the results reported by Yacowitz, Peeler, Miller, Carlson, Heuser and Norris (1950). They found 158 millimicrograms of vitamin B_{12} activity

per "normal" egg. Variations in experimental procedure reconciled the small differencies between the microbiological results. The question of which results are nearest the absolute value, those obtained by microbiological, rat or chick assays, plagues research workers today. Each type of assay probably measures the actual vitamin B_{12} content of some materials more accurately than any other assay. Stokstad, Hoffmann and Jukes (1949) reported discrepancies between microbiological and chick assays for vitamin B_{12}. Results of rat and microbiological assays frequently disagree widely. Certainly as far as chick nutrition is concerned, values determined with chick assays are the most reliable. The variable results between species probably are caused by (1) differential ability of the species to utilize bound forms, (2) differential stimulatory or sparing effect of various compounds, or (3) a combination of the two. Among factors other than vitamin B_{12} which might conceivably affect assay results with the chicks is the whey factor. It was considered likely that the whey factor would be present in the eggs which were assayed. In addition it was felt that the whey factor might exert a stimulatory effect on growth which could be interpreted as being caused by the presence of vitamin B_{12}. For that reason 3 percent of dried whey was added to diet 3. It was not added to diet 4. Since the results of the two types of chick assays showed very close agreement, the whey factor is believed to have no effect on the results of chick assays for vitamin B_{12} when chicks of such dietary background are used.

Results of both microbiological and chick assays demonstrate a definite correlation between the level fed the hen and the level found in the egg. Study of table 8 reveals that the chick assays found increased storage in the egg at a diminishing rate with higher levels of vitamin B_{12}

in the pullet diet. The efficiency of transmission of vitamin B_{12} from the pullet ration to the egg decreased with higher levels of supplementation as shown in table 10. It is probable that the vitamin B_{12} content of group C eggs as determined by the chick assay is too low. If more chick assays had been conducted, it is believed that a higher vitamin B_{12} content would have been determined for C eggs. The growth of the chicks hatching from such eggs indicate that the vitamin B_{12} content in them is intermediate between the level in group B and group D eggs.

B. Studies on the Progeny

The data on liveability indicate that a deficiency of vitamin B_{12} in the maternal diet leads to excessive mortality among the chicks which can only be counteracted by the use of high levels of vitamin B_{12} in the chick diet. A recent report by Bird (1950) substantiates this result. He suggests that the mortality is caused by the physical inability of the chick to ingest enough vitamin B_{12} during the first few days of life for survival. Intramuscular injection of the vitamin would prevent the excessive mortality.

Interesting evidence for the influence of maternal diet on the requirement of the chick for vitamin B_{12} for normal growth has been obtained. The final weight of A chicks, produced on a maternal diet including no supplemental vitamin B_{12} and fed a chick diet containing 27 micrograms of supplemental vitamin B_{12}, was 5 percent less than the final weight of chicks produced on 16 micrograms in the maternal diet and fed only 3 micrograms in the chick diet. When A pullets subsequently received 3 micrograms of vitamin B_{12}, growth of the progeny was unsatisfactory on 18 micrograms of vitamin B_{12}, the highest level used in

experiment 2. By contrast B chicks produced by dams fed on 4 micrograms of vitamin B_{12} throughout both experiments, apparently required only 15 micrograms of vitamin B_{12} in the diet for good growth. The quantitative requirement of chicks for vitamin B_{12} is therefore seen to be influenced tremendously by the maternal diet. Although the figures presented in tables 20 and 21 are based on assumed values for pullet and chick feed consumption, they clearly show that vitamin B_{12} supplied through the maternal diet is much more efficiently used than if supplied in the chick diet. Obviously the level furnished in the chick diet would increase in importance as the birds became older.

There is further evidence of an admittedly inconclusive nature for a limiting effect on chick growth of low levels of vitamin B_{12} in the maternal diet, regardless of level of supplementation in the chick diet. During experiment 1, the growth of B, C, and E chicks tended to plateau at a slightly lower level than the growth of D chicks as shown in chart 2. During experiment 2 the growth of A chicks also tended to plateau at a low level. It seems unlikely to the writer that such a limiting effect actually exists irrespective of chick diet. Rather does it seem probable that higher levels of vitamin B_{12} than were fed in the chick diet were required for maximum growth of such progeny to 4 weeks. The data obtained by Bird (1950) on excessive mortality in depleted chicks support this belief. As previously mentioned, he found that excessive mortality was prevented by injection of vitamin B_{12}. The results with D and E chicks in experiment 1 do present the possibility that higher levels of vitamin B_{12} than are usually found in breeding rations may produce chicks that will grow at an initially faster rate on customary broiler rations. It is

evident that the level of vitamin B_{12} fed in the breeding pen is as important to the broilerman as the level fed in the broiler house.

The results of these experiments also prove that some supplemental vitamin B_{12} is needed in chick diets composed primarily of cereal grains and soybean protein. When a good breeding ration was used, the requirement of E chicks for supplemental vitamin B_{12} was no less than 3 micrograms, possibly as much as 15 micrograms, for optimum growth. Since these chicks were reared in batteries they did not have the advantage of access to nutritional factors in droppings and litter, which most broiler chicks would have. (Microbiological analyses of hen feces and of deep litter itself by Halbrook, Sutton, and Winter (1950) indicated that they may act as limited sources of the vitamin.) Nevertheless such an advantage is of dubious importance during the critical first 4 weeks of life. At this time the ability of the chick to ingest large quantities of bulky material is particularly limited, and the amount of vitamin B_{12} available in the litter would be at the lowest point unless old litter were used to start new chickens. Therefore, the availability of vitamin B_{12} in the maternal and chick mashes would probably be nearly as important for chicks reared under commercial conditions. Robblee, Nichol, Cravens, Elvehjem and Halpin (1948) described work which indicated that even if the maternal diet contained supposedly adequate levels of vitamin B_{12}, the progeny still required supplemental vitamin B_{12} to 4 weeks of age. Stevens, Biely and March (1949) reported that even on a commercial chick starter containing mixed grains, soybean meal, fish meal and dried whey, the feed efficiency was markedly improved by the addition of the supplemental vitamin B_{12}, although growth in their study was unaffected. Their basal ration in this case was certainly as

high in vitamin B_{12} from natural sources as could be expected. Unfortunately the exact composition of it is not given and the choline and methionine levels are not stated. A deficiency of these two compounds is quite possible in such a ration. A low level of these two compounds in the chick diet has been shown by Briggs, Hill and Giles (1950) and others to increase the requirement for vitamin B_{12}. It should be pointed out also that the growth relationships expressed for the first 4 weeks, probably also hold to 6 weeks as shown on table 15. This does not mean that the chick requirement is as high at 6 weeks as it is at 4 weeks, but it does mean that all growth requirements are satisfied with these levels. Lillie, Marsden, Groschke and Bird (1949) found that the apparent requirement of the growing chicken for vitamin B_{12} dropped during the later stages of growth.

The degree of feathering is clearly influenced by vitamin B_{12}. Either 4 micrograms of vitamin B_{12} in the breeding ration or 8 micrograms in the chick diet supported good feathering. Therefore the requirement is so low that the effect of vitamin B_{12} on degree of feathering is of little practical importance. A possible relationship between feathering and vitamin B_{12} is easily discerned. Feathers contain a high level of cystine, which may be produced from methionine. The work of Briggs, Hill and Giles (1950), Gillis and Norris (1949), Schaefer, Salmon and Strength (1949) and others have indicated that there is a mutually sparing action between the vitamin and methionine. One may assume that presence of a high level of vitamin B_{12} would increase the amount of methionine available for secondary physiological functions, while a vitamin B_{12} deficiency would correspondingly decrease the supply of methionine available for such functions. The results of the depletion

study show that more than 8 micrograms of supplemental vitamin B_{12} is required in the maternal diet to permit adequate storage, as a safety factor.

The figures cited for the vitamin B_{12} requirements have not credited the basal diets with content of any vitamin B_{12}. The reason is that there is no reliable vitamin B_{12} assay available for cereal grains, soybean meal and dried brewers' yeast. These feedstuffs are believed to contain unidentified growth factors (Menge, 1950) which may cause microbiological assays to yield inflated values. If the results of a microbiological assay (published by Peeler, Yacowitz and Norris, 1950) are used, basal diet 1 may be considered to contain approximately 8.6 ~~10~~ micrograms of vitamin B_{12} per kilogram. Basal diet 2 may be estimated to contain 23 micrograms of vitamin B_{12} per kilogram. Approximately 15 micrograms of the total content of basal diet 2 has been determined with chick assays, and therefore is believed definitely to be present. The existence of the other 8 micrograms is questionable. It is felt that the calculated content of vitamin B_{12} is much too high where values obtained by microbiological assays of these feedstuffs are employed. For that reason they have not been used in interpreting these results.

The requirements determined for the progeny are based on results obtained with diet 5 which contained slightly more protein than is normally fed. Since a high level of protein in the diet increases the need for vitamin B_{12} for growth, the actual requirements of the progeny for vitamin B_{12} may be slightly lower than those determined. On the other hand normal levels of pantothenic acid (a vitamin which has been shown by Yacowitz, Norris and Heuser, 1950, to spare vitamin B_{12} for chicks), methionine and choline were fed in the pullet diets

and in chick diet 5. Therefore, it is believed that any undue effect of these compounds on the vitamin B_{12} requirements has been eliminated.

It should be stated that possibly the pullets which were selected for experiments 1 and 2 were relatively refractory to a vitamin B_{12} deficiency. Bird, Rubin, and Groschke (1947) have shown that there is a genetic relationship to the vitamin B_{12} requirement of the chicken which permits some birds to function satisfactorily on levels quite deficient for others. The test birds were selected on the basis of an average hatchability of 80.8 percent. Only 1 bird in each pullet group had a record of less than 66 percent overall hatchability, when experiment 1 was started. Perhaps it would have been wiser to permit birds with records of lower hatchability during the preliminary period to be used in the 2 experiments. The results of experiment 2 suffer from variability as a result of limited numbers, and possibly from seasonal effects.

SUMMARY

The vitamin B_{12} requirements of New Hampshire pullets and their progeny were studied, using a cereal grain-soybean meal type of diet. In the pullet studies average weights, hemoglobin values and mortality bore no measurable relationship to the level of vitamin B_{12} in the diet. During experiment 1, egg production declined in direct relationship to decreasing level of vitamin B_{12} supplementation. The results of experiment 2 demonstrated no clear relationship of the dietary level of vitamin B_{12} to egg production. Reasons discussed previously lead the writer to conclude that any effect of vitamin B_{12} on egg production cannot be measured with such test rations. No relationship was proven between fertility and the level of vitamin B_{12} fed to the pullets. Hatchability declined sharply in experiment 1 when no supplemental vitamin B_{12} was furnished in pullet diet 1. The results of experiment 1 showed that the requirement for hatchability was adequately met by 4 micrograms of supplemental vitamin B_{12} per kilogram under the conditions of the test. In experiment 2, fluctuations in hatchability occurred between groups in such a manner that any relationship of hatchability to a lower level of vitamin B_{12} was obscured. Rate of depletion studies with hatchability indicated, however, that even 8 micrograms of supplemental vitamin B_{12} might not permit enough storage in the pullets' bodies for safety.

The vitamin B_{12} activity of eggs was measured by both microbiological and chick assays. It was shown to bear a direct relationship to the level fed the pullet. When pullets were fed a practical breeding mash, the vitamin B_{12} activity of the average egg was 1.560 micrograms by chick assay. When they were fed a cereal grain-soybean protein type

of diet unsupplemented with vitamin B_{12} the content was 0.604 micrograms of vitamin B_{12} activity by chick assay. The microbiological assay results showed only 9.5 to 16.7 percent as much vitamin B_{12} activity in the eggs as did the chick assays. The results of the chick assays indicated that between 30 and 48 percent of the vitamin B_{12} fed to the pullets was transmitted to the egg. The transmission was more efficient at low levels of supplementation in the pullet diet.

Initial weight of progeny was unaffected by the variations in the diet. Mortality was considerably higher in progeny of the pullet group fed the unsupplemented basal diet 1 during the first experiment. The excessive mortality among progeny could be avoided by the addition of 3 micrograms of vitamin B_{12} to pullet basal diet 1 or the addition of between 9 and 27 micrograms of vitamin B_{12} to chick basal diet 5. The effect on liveability of lower levels of supplemental vitamin B_{12} in the maternal diet is unknown.

When there was no supplemental vitamin B_{12} in both the maternal diet 1 and the chick diet 5, degree of feathering was poor. When 4 micrograms of supplemental vitamin B_{12} were fed in the maternal diet 1, or 3 micrograms were added to the chick diet 5, degree of feathering was satisfactory. The effect of lower levels of supplemental vitamin B_{12} has not been studied.

Growth of progeny was directly affected by both the maternal and chick dietary level of vitamin B_{12}. Final weight of the chick plateaued at a nearly optimum point when a kilogram of the maternal diet contained 8 micrograms of supplemental vitamin B_{12} and a kilogram of the chick diet contained 3 micrograms of supplemental vitamin B_{12}. These levels of vitamin B_{12} are believed to mark the lower limits for best

growth on such a ration. When 16 micrograms of supplemental vitamin B_{12} were fed to the pullets instead of 8, the final weight of the progeny plateaued at a slightly higher level. The point of plateauing involved the same level of supplemental vitamin in the chick diet for both groups. Correlation of the results of the egg assays by chicks and the growth of progeny showed that vitamin B_{12} furnished by the egg was far more effectively utilized than vitamin B_{12} furnished in the chick diet. Less vitamin B_{12} was required, quantitatively, for good growth of chicks when most of it was furnished via the maternal diet than if furnished through the chick diet. These results indicate that the level of vitamin B_{12} fed to the dam is of vital importance for the production of rapidly growing chicks. Growth in one series to 6 weeks indicated that the levels determined for satisfactory growth to 4 weeks of age may also be used for the first 6 weeks of life.

Sixteen micrograms of supplemental vitamin B_{12} per kilogram permitted the pullets to build up a reserve. Eight micrograms of supplemental vitamin B_{12} did maintain hatchability and did maintain growth of progeny fed 3 micrograms of the vitamin. The immediate drop in hatchability of eggs and drop in growth following omission of the vitamin, however, showed that such a level did not permit build-up of an adequate reserve in the pullet for emergencies.

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APPENDIX

TABLE I

Composition of Salt Mix and Vitamin Mix for Basal Diet 4

Dietary Ingredient	% of Diet
$CaCO_3$	1.5000
K_2HPO_4	0.9000
$Ca_3(PO_4)_2$	1.3000
NaCl	0.6800
Na_2HPO_4	0.7300
$MgSO_4.7\ H_2O$	0.5000
$Fe(C_6H_5O_7)_2.6\ H_2O$	0.1400
KI	0.0040
$MnSO_4.4\ H_2O$	0.0410
$ZnCl_2$	0.0020
H_3BO_4	0.0009
$CoSO_4.7\ H_2O$	0.0001
$CuSO_4$	0.0013
H_3PO_4	0.0058
(milligrams/100 grams diet)	
Thiamine HCL	1.00
Riboflavin	1.00
Calcium pantothenate (dextro)	2.00
Niacin (nicotinic acid)	5.00
Pyridoxine HCL	0.60
Choline chloride	200.00
Biotin	0.02
Folic acid	0.30
i-inositol	100.00
p-Amino benzoic acid	0.20
Menadione	0.50
alpha-Tocopherol acetate	0.50
Vitamin A*	1200.00**
alpha-Tocopherol acetate (mg.)*	0.50

* Administered by dropper per chick per week in addition to that included in the diet.

** International units per 100 grams of diet.