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The Influence of Fertilizers on the Vitamin-B Content of Wheat

C. H. Hunt

OHIO
AGRICULTURAL EXPERIMENT STATION
Wooster, Ohio

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PREFACE

This work was undertaken to determine the relation, if any, between the mineral content of wheat and its vitamin-B content. The plots on which these wheats were grown had been receiving the same fertilizer and producing the same crops in five-year rotations for about 35 years and it was thought that this would afford an excellent opportunity to see whether the vitamin-B content of wheat is in any way associated with the kind of fertilizer applied to the soil on which the wheat is grown. Some of the plots have almost reached the stage where they cease to produce. This is probably not due to lack of necessary minerals, but rather to physical and chemical conditions brought on by the soil's becoming acid. No doubt there are farms which present similar conditions and since wheat and its by-products touch the interest of everyone the problem becomes one of practical importance.

The author wishes to thank those who have consciously or unconsciously aided him in this work. He particularly desires to express his thanks to Drs. J. F. Lyman and R. M. Bethke for their helpful suggestions; to Dr. G. Bohstedt for his suggestions and for aid that made the work possible. He also desires to express his thanks to the Department of Agronomy of this Station, who have so generously given of their time and efforts in securing the necessary material for the work.

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INTRODUCTION

There has appeared from time to time a vast amount of literature relating to the influence of fertilizers on the yield of crops. To determine this influence of fertilizers on crop yields has been the aim of many investigators and remarkable results have been obtained. The influence of fertilizers on the feeding value of crops is known to be of great importance; still comparatively few investigations have been made as to the detailed chemical composition or the feeding value, as measured by the biological method, of crops grown under various conditions.

It is well known that the composition of the plant is dependent upon the soil on which it is grown. Ames and associates (2)*, however, make mention of the fact that the character of the plant (wheat) is changed to a greater extent by seasonal and climatic influences than by soil and fertilizer, although the addition of fertilizers produced marked differences.

Although the question of the influence of soil (fertilizers) on the nutritive value of plants or crops has not been studied to any great extent, to the soil, indirectly, has been attributed the real cause of many abnormalities among livestock which have subsisted exclusively on feeds grown on certain soils or have grazed on certain pastures.

Patterson (43) working in Scotland reports the results of several years' study that seem to indicate that crops fertilized with basic slag are better for feeding sheep than crops fertilized with superphosphate. The slagged crop was lighter in yield but this was offset by the better feeding value. Somerville (51) in 1898 found that more hay was consumed per pound gain in live weight of sheep when the hay was grown without a fertilizer than when it was grown on a soil that received either superphosphate and potash or basic slag. Somerville and Middleton (52) in 1900 published the

*Reference is made by number to literature cited, page 40.

results of a four-year study, the conclusion of which was that fertilizers influence the feeding value of crops. Dymond, Hughes, and Jupe (7) investigated the influence of sulfates upon the yield and feeding value of crops. Their results show that the total sulfur and the total and albuminoid nitrogen were increased and they concluded that there was not enough sulfur in the soil for the production of crops of highest feeding value. Hall and Russel (22) in "A Study of the Cause of High Nutritive Value and Fertility of the Fattening Pastures of Romney Marshes and Other Marshes of S. E. England," reported that they found fields that grew grasses of varying feeding value. A chemical analysis did not reveal the differences in the crops. One noticeable difference, however, was found in the field that produced the most rapid fattening of cattle—the high rate at which nitrates were produced and the relatively large amount of total phosphoric acid. The soils of the fattening fields were reported to have had a better texture than those of the non-fattening field, allowing water to drain away in wet weather and holding moisture in dry weather.

McClendon and Henry (31) grew oats and barley on different soils and then fed these grains to rats. They found that growth was directly proportional to the phosphoric acid content of the grain. The number of animals in their experiment was not sufficient, and for that reason their results are open to criticism. Davis (6), reporting on "The phosphate depletion of the soils of Bihar (India); its effect on the quality and yield of crops and the contingent risk of malnutrition and endemic diseases in cattle and man", concluded that the soils had reached an impoverished state as regards phosphates, and that, as a result, the cattle were smaller and probably the milk produced was of less nutritive value than that from cattle of other districts where the conditions were more favorable for plant growth. Greaves and Carter (19), in a study of varying amounts of irrigation water on the quantity and quality of grains, report that the calcium, phosphorus, potassium, and magnesium content increased with the increase of water applied to the soil, but that there was a decrease of nitrogen. Although no feeding trials were conducted, they were of the opinion that, according to accepted feeding standards, some crops grown without sufficient water may have a low feeding value.

There are indications of poor feeding value other than that of slow growth or a "stand still" in weight. Probably one of the first indications of a deficiency in the quality of the feed from poor soils is an abnormal or perverted appetite in which case the animal may have an inclination to eat or lick rubbish of any sort. There are degrees of such an appetite, depending on the length of time the animal has been restricted to the deficient diet.

EFFECT OF FEEDS LOW IN MINERALS

Viljoen, Green, Theiler, and Der Toit (59, 58), after a number of years of study, found a direct connection between the diseases Osteophagia and Lamsiekte. Osteophagia, or "cravers for bones", is due to a deficiency of phosphorus in the soil. Grasses grown in districts where these diseases occur contain only as much phosphorus as ordinary wheat straw, entirely too little to supply the requirements of animals. In such districts animals develop an appetite for bones to the extent that herbivora eat carrion and hence become infected and contract lame sickness, or lamsiekte, due to ingestion of carcass material infected with a specific anaerobic saprophyte, now known as parabotulinis bovis. Cattle receiving feed with a normal phosphorus content do not have this craving for bones with adhering putrified flesh, but in areas where the phosphorus content of plants is low 90 percent of the cattle may show osteophagia.

Murphy (40) writes interestingly on the "Treatment of Pasture" as a preventive of rickets, cripples, and paralysis. He finds that the Victorian grasses, although of luxuriant growth, are low in important minerals, making it difficult for animals to thrive on them. Cattle on such grasses are troubled with rickets. Murphy concludes his remarks by saying that the pastures should be fertilized in order to feed and nourish the stock properly. Moreover, the absence of lime and potash renders the grass more liable to attacks of molds and fungi, which may be harmful to livestock. Henry (25), of New South Wales, reports the existence of a disease resembling lamsiekte and known as "impaction paralysis," which is attributed to feeds grown on poor soils low in calcium, phosphorus, and potassium.

Atkinson and Mohler (3) found that osteomalacia occurs on old worn-out soils devoid of lime. In the United States it is confined principally to local areas in the Southwest, known as the "Alkali Districts," and to the old dairy sections of New York. The disease generally follows a dry season, which, due to a diminished transpiration of water in plants, causes the plants to be low in minerals.

Huffman and Taylor (26) found depraved or perverted appetite among dairy cows and also among growing animals in the Thumb region of Michigan. They analysed the grasses and found that the calcium of the plants decreased and the phosphorus increased as the season advanced. They also found that the perverted appetite gradually abated as the phosphorus content of the pasture increased. Their results point to a phosphorus deficiency as the primary cause of the perverted appetite. Welch (63) found a disease called "Bone Chewing" among cattle in Montana, and he ascribed it to a deficiency of calcium phosphate in the soil and grasses. Eckles, Becker, and Palmer (8) made a study of perverted appetite among cows in Minnesota, and found that it was chiefly due to lack of phosphorus in the feeds which were grown on impoverished soils.

Certain sections of this country are endemic to goiter in the human being, hairlessness in pigs, big neck in cattle and sheep, and general weakness in other animals. This disease is probably due to a deficiency of iodine in the water, plants, and soil of these regions. Forbes (12), however, did not find that the iodine content of feeds from these regions was any lower, neither was it any higher, than that from the non-goiterous regions. McClendon and Williams (32) found a relation between the iodine content of drinking water and the distribution of goiter. If the diet lacks this element the thyroid gland enlarges in response to a stimulus to supply that which is lacking. This is one of the symptoms of the disease. It is well known that the thyroid gland produces a secretion, thyroxin, which regulates the metabolism of the body, and since iodine is essential for the production of thyroxin, the need of sufficient iodine in the food is evident.

Marine and Kimbal (37) made a study of the effect of feeding sodium iodide to a large group of girls in the public schools of Akron, Ohio, which is in a goiterous region. They found that when this study was begun 43.5 percent of the girls in the group had normal thyroids, while 56.5 percent had enlarged thyroid glands. The subjects were divided into two groups; one received the iodide prophylactic, and the other group did not, and after six months they were re-examined. It was found that the girls taking treatment and whose glands were normal at the beginning, still remained normal, while of those who did not take the treatment only 74 percent remained normal. Of those with a small goiter (enlarged gland) at the beginning and who took treatment, 33.5 percent were cured; while of those who did not take the treatment the disease continued in all but 1.2 percent. The results show clearly the efficacy of iodides as a phophylactic.

Investigations by Welch (62), Smith (49), Kalkus (28), and Hart and Steenbock (24) show that the disease among the lower animals can be prevented by feeding sodium iodide or potassium iodide, or administering tincture of iodine to the skin of the pregnant mother. As the loss among young livestock from this disease has been enormous, the feeding of iodine has become of economic importance.

Sometimes, in man at least, there is a hypersecretion of the thyroid gland and the feeding of iodine may do a distinct harm by too great stimulation of metabolism.

One naturally concludes then that the quality of pasture grass and other feeds varies greatly and it appears that this variation is due to soil and climatic factors. Under some conditions it seems that it is almost impossible to raise livestock because of the failure of the forage to support normal growth and well being of the animal.

VITAMINS

All attempts to produce growth of animals on purified rations have failed. Although these contain carbohydrates, fats, adequate proteins, and minerals in proper amount, and although they are palatable as judged by the amount consumed, and well digested, they lack a substance or substances which if not given to the young will stop growth and result in early death from various complications. If this substance is not administered to mature animals they become infertile (sooner or later), old age will set in prematurely, infections will occur, and death will soon follow. These substances, which we now call vitamins, have aroused wide interest and probably no greater impression has been made on the public mind by any other of the present day discoveries.

The investigations in the last 15 years have thoroughly demonstrated that vitamins play an important role in human and animal nutrition.

Up to the present time five vitamins have been recognized. They are designated by the first five letters of the alphabet, A, B, C, D, and E, with a possible division of B and E into two vitamins each. These vitamins are known by their physiological reactions, and not for what they are. So far they have remained unidentified as chemical entities, yet numerous attempts have been made to determine their identity. Several investigators have come forward with compounds which seem to have the properties of the vitamins, especially vitamin B, but in a few months or years the material changes, possibly to an isomeric form, or is oxidized and loses its properties. However, great progress has been made and future work may reveal the hidden secret.

VITAMIN A

Vitamin A is found chiefly in foods rich in fat, in animal organs such as the liver and kidneys, in vegetables, yellow corn, etc.; but is absent from most vegetable fats. It is necessary for growth. As soon as the store of this vitamin in the body is depleted the animal loses weight and the power to resist infection; the eyes become red and swollen, eventually followed by blindness. This disease of the eyes is known as xerophthalmia. Much information has accumulated with reference to the stability of vitamin A, and the opinion is that it is quite stable under most conditions except oxidation.

VITAMIN B

Vitamin B, like A, is necessary for growth. Although it is probably made up of two vitamins, one of which prevents polyneuritis in pigeons and the other produces growth in rats. Funk and Paton (16) found that if yeast be allowed to grow in the vitamin-B medium, a substance which they call vitamin D (not the anti-rachitic vitamin) is removed and vitamin B remains. These investigators believe that the vitamin-D fraction is essential for growth of yeast, although both fractions are necessary for the well being of animals. Results obtained more recently by several investigators (50, 18, 17, 45, 5) indicate rather conclusively that the original water soluble B vitamin is made up of two or more vitamins.

Animals possess but little storage capacity for vitamin B, and, therefore, it must be in the food at all times. It is quite extensively distributed among natural foods, such as seed coats and germs of grains, vegetables, milk, fruit, and more or less in meat. The absence of this vitamin in the diet causes beriberi in man and polyneuritis in fowls. This disease is characterized by a nervous disorder and the loss of control of the muscles.

VITAMIN C

Scurvy has been known for a long time, but the cause of it was not linked up with the subject of nutrition until within the past decade and a half. Man and the guinea pig are about the only animals susceptible to this disease. Such is the opinion at the present time.

Vitamin C, or the antiscorbutic vitamin, can probably be synthesized in the body of other animals and as a result this vitamin does not necessarily have to be provided from an outside source. It is quite abundant in fruits and vegetables. Scurvy is a disease that attacks both old and young. It produces a faulty bone development and hemorrhage of the gums, ankles, and other tissues of the body. Vitamin C in an acid medium is quite stable to heat, but it is destroyed in an alkaline medium. It is also destroyed by oxidation.

VITAMIN D

Vitamin D, or the antirachitic vitamin, to distinguish it from the vitamin D which Funk separated out from yeast preparation, was discovered by McCollum and associates (34). They found that when cod-liver oil was oxidized it did not prevent xeropthalmia, but that it brought about a deposition of calcium in the bones and prevented rickets. Rickets is a disease of the bones and affects only the young. The symptoms are generally recognized by malformation of the bones of the legs, arms, and chest. Sunlight and ultraviolet light also have the power of preventing rickets.

VITAMIN E

Evans and Bishop (11) discovered a substance necessary for reproduction in the rat. They provisionally called this substance vitamin X, or the antisterility vitamin. They found this vitamin present in lettuce, wheat germ, wheat-germ oil, meat, dried alfalfa, rolled oats, etc. Sure (56) confirmed their findings and called the substance "vitamin E". This vitamin is considered to be quite stable under most normal and adverse conditions. Sure (57) also found that when wheat embryo, which is high in vitamin E, was autoclaved for 2 hours at 20 pounds pressure and then fed to rats, there was no noticeable effect on the fertility of the females, but it did produce a harmful effect on lactation. He presented this as evidence that wheat-germ oil contains two vitamins, one that promotes fertility and the other milk secretion.

FACTORS INFLUENCING VITAMIN-B CONTENT

It is established that the application of fertilizers to the soil affects the mineral content of the plants. The question then arises is there an increase in the vitamin-B content along with the increase in minerals? Voegtlin and Myers (60) reported that in a general way a high total phosphorus content is an indication that the particular corn or wheat product is relatively rich in vitamin B. Green

(20), however, did not find this parallelism between phosphorus and vitamins to hold, especially when different samples of grain were compared.

McCarrison (30) found that there are numerous factors influencing the vitamin-B content of plants, and he enumerated them in the following order: "difference in character of the soil, in soil composition, in soil deterioration, in its bacterial and protozoal content, in aeration, in weather, water, atmospheric temperature, etc.," but he concluded that natural fertilizers (farmyard manure) produce millet with a higher vitamin-B value than that produced with artificial fertilizers, although from his data artificial manures produced plants of a higher vitamin-B value than the control plots where no fertilizer was used.

VITAMIN B IN WHEAT AS FOUND BY OTHER INVESTIGATORS

Several investigators have made a study of the vitamin-B content of whole wheat, but there seems to be a diversity of opinion as to the quantity present. McCollum and associates (33) found that 15 percent of whole wheat in the diet is sufficient to supply the necessary amount of vitamin B for normal growth, reproduction, and nursing of the young, but not enough to enable the second generation to develop normally. They later concluded that when 33 percent of wheat in the diet was used as the sole source of vitamin B, it was doubtful whether sufficient vitamin B was present for the rearing of young. Osborne and Mendel (42) came to the same conclusion; viz., that 15 percent of whole wheat in the diet supplies sufficient vitamin B for normal growth. They mention that this is very near the minimum, for with 10 percent the rats ceased to grow, but recovered when 20 percent of the diet was whole wheat. Guest, Nelson, Parks, and Fulmer (21) found that 10 percent of wheat furnished enough vitamin B for normal growth of rats, and that when the amount of wheat in the diet was increased to 15 percent one female gave birth to six litters of young in 10½ months. The fact that their rats were on shavings for bedding probably explains their success in growth and reproduction on small amounts of wheat in the diet.

Probably these discrepancies were due to different varieties of wheat, whether spring or winter, different soil or climatic conditions under which the wheat was grown, or different conditions under which the experimental animals were kept. Bell and Mendel (4) found that spring wheat contains a larger amount of vitamin B than winter wheat, and that 15 percent of spring wheat or 40 percent of winter wheat was necessary to furnish enough vitamin B for normal growth.

Since there seems to be a quantitative difference in the vitamin-B content of different wheats another question is, what is the factor or factors influencing this difference? In an attempt to answer this and other questions, wheat from the five-year-rotation fertility plots of the Ohio Agricultural Experiment Station at Wooster was used.

These plots have been under experiment for 32 years, with the same treatment continued throughout, except that in 1906 and 1907 the liming was changed for one rotation from the west to the east side of the fields on which the wheat was grown in 1923 and 1924; hence the effect of the soils together with the fertilizers applied, on plant growth, development, vitamin-B content, etc., may be more pronounced and more easily detected than if the plants were grown on soils which had received a more diversified or less rigorous treatment. Since only one variety (Trumbull winter) of wheat was used, all of the factors previously mentioned as possibly having an influence, except soil condition or fertility, were eliminated for each particular year. The question can then be confined to this: Is there a difference in the vitamin-B value of wheat grown on these fertility plots? or, Does the wheat grown on soil receiving one kind of fertilizer have a different vitamin-B content from that grown on the same soil receiving another kind of fertilizer? The practical importance of such a study can be readily seen.

PLAN OF FERTILIZING IN THE 5-YEAR ROTATION

The plan of the 5-year rotation and the fertilizers used are shown in Table 1. Only three crops are shown in the table. Clover and timothy are grown in the rotation without fertilizers.

TABLE 1.—Plan of Fertilizing in the 5-year Rotation

Plots 1-10 acre—Fertilizing material in pounds per acre

Plot No.	On corn			On oats			On wheat		
	Acid phosphate	Muriate of potash	Nitrate of soda	Acid phosphate	Muriate of potash	Nitrate of soda	Acid phosphate	Muriate of potash	Nitrate of soda
2	80	....	....	80	....	....	160	....	....
3	....	80	....	....	80	....	....	100	....
4	....	....	....	....	....	....	....	....	....
5	....	....	160	....	....	160	....	....	160
9	....	80	160	....	80	160	....	100	160
11	80	80	160	80	80	160	160	100	160
17	160	80	80	160	80	80	160	100	80

CHEMICAL ANALYSES OF WHEAT

Samples of the wheat were finely ground and subjected to chemical analysis by standard methods. The data are given in Table 2.

TABLE 2.—Chemical Composition* of Wheat

Plot No.	Moisture %	Ash %	Nitrogen %	Protein N x 6.25 %	Calcium %	Magnesium %	Phosphorus %	Sulfur %
1923								
2, unlimed	13.11	1.85	2.19	13.69	.051		.40	
2, limed	12.98	1.95	2.28	14.25	.054		.43	
3, unlimed	13.01	1.79	2.50	15.62	.047		.35	
3, limed	12.83	1.78	2.52	15.75	.048		.40	
4, unlimed	12.97	1.56	2.53	15.81	.054		.32	
4, limed	12.91	1.90	2.57	16.06	.056		.40	
5, unlimed	13.18	1.66	2.77	17.31	.052		.32	
5, limed	13.38	1.78	2.70	16.87	.053		.39	
9, unlimed	13.17	1.50	1.98	12.37	.046		.29	
9, limed	12.62	1.94	1.97	12.31	.049		.39	
11, unlimed	12.11	1.72	2.27	14.18	.045		.36	
11, limed	12.94	1.76	2.21	13.81	.044		.39	
17, unlimed	12.55	1.77	1.99	12.44	.044		.39	
17, limed	13.38	1.88	2.08	13.00	.046		.42	
1924								
2, unlimed	14.15	1.65	2.26	14.12	.038	.158	.36	.174
2, limed	13.50	1.78	2.25	14.06	.052	.173	.43	.174
3, unlimed	14.02	1.66	2.07	12.94	.043	.153	.35	.177
3, limed	13.30	1.67	2.20	13.75	.046	.158	.36	.169
4, unlimed	14.20	1.60	2.13	13.31	.043	.135	.37	.179
4, limed	13.55	1.79	2.18	13.62	.055	.173	.39	.176
5, unlimed	12.64	1.63	2.23	13.94	.040	.154	.35	.161
5, limed	14.13	1.70	2.38	14.87	.048	.160	.40	.172
9, unlimed	12.85	1.42	2.34	14.62	.035	.127	.32	.170
9, limed	13.68	1.78	2.31	14.44	.049	.169	.37	.174
11, unlimed	13.98	1.56	2.17	13.56	.049	.141	.37	.196
11, limed	14.35	1.92	2.30	14.37	.044	.163	.46	.166
17, unlimed	13.46	1.51	2.11	13.19	.032	.162	.45	.168
17, limed	13.25	1.82	2.06	12.87	.040	.183	.47	.166
1925								
2, unlimed	12.55	1.62	2.05	12.81	.047	.162	.35	
2, limed	11.16	1.79	2.08	13.00	.050	.181	.39	
3, unlimed	12.43	1.54	2.13	13.31	.043	.147	.28	
3, limed	12.88	1.60	2.16	13.50	.046	.151	.32	
4, unlimed	12.62	1.44	2.12	13.25	.043	.142	.28	
4, limed	13.57	1.63	2.28	14.25	.052	.170	.32	
5, unlimed	11.55	1.39	2.20	12.75	.045	.133	.28	
5, limed	12.20	1.58	2.40	15.09	.049	.160	.33	
11, unlimed	11.77	1.46	2.16	13.50	.038	.148	.34	
11, limed	12.63	1.62	2.07	12.94	.041	.150	.38	
17, unlimed	11.52	1.66	2.12	13.26	.041	.151	.35	
17, limed	11.98	1.72	2.17	13.56	.041	.159	.41	

*Moisture-free basis.

Investigations by Ames (1) show that when phosphorus is applied to the soil deficient in this element, the amount of phosphorus in the grain is increased, and that the addition of lime to the soil also increases the amount of phosphorus assimilated by the plant. The chemical analyses confirm this conclusion and also show that liming not only increases the phosphorus content of the plant but the calcium, magnesium, and protein content as well. In some cases the difference in the calcium content of the wheat grown on the limed and unlimed sections was so small that it might be interpreted as due to experimental error; but, since the results for the three years are consistent, it would appear justifiable to conclude that liming caused a difference in the calcium content of the wheat. An analysis of the entire plant probably would have shown a greater difference.

Table 3 shows the ratio of protein to phosphorus and Table 4 of magnesium in ash to protein. Just how magnesium is related to protein is not known, but very likely the relationship comes through some photosynthetic process. This confirms the work of Sullivan and Near (55).

TABLE 3.—A Ratio of Protein to Phosphorus

Plot No.	Fertilizer treatment	1923	1924	1925
2, unlimed....	Acid phosphate..................	1:.0292	1:.0255	1:.0273
2, limed.......	Acid phosphate.....	.0301	.0305	.0300
3, unlimed....	Muriate of potash................	.0224	.0270	.0210
3, limed.......	Muriate of potash................	.0253	.0261	.0237
4, unlimed...	Check plot, no fertilizer...........	.0202	.0278	.0211
4, limed......	Check plot, no treatment..........	.0249	.0286	.0224
5, unlimed....	Nitrate of soda.......	.0184	.0251	.0203
5, limed......	Nitrate of soda...................	.0231	.0269	.0220
9, unlimed....	Nit. of soda and mur. of potash...	.0234	.0219	....
9, limed.......	Nit. of soda and mur. of potash...	.0316	.0256	....
11, unlimed....	Complete fertilizer................	.0253	.0272	.0252
11, limed.......	Complete fertilizer................	.0282	.0320	.0293
17, unlimed....	Complete fertilizer................	.0313	.0341	.0264
17, limed.......	Complete fertilizer......	.0323	.0365	.0302
Average..		1:.0261	1:.0282	1:.0249

As already stated, the fields on which the wheat for 1923 and 1924 was grown received lime on the east side instead of the west side in 1906 and 1907; and the effect of this one application is still evident, for the differences between the yield of wheat grown on the limed and unlimed parts in 1923 and 1924 were not so great as in 1925 on field C, which never received lime on the east side. This is shown in Table 5.

A similar effect on the composition of the grain was not noted, but in 1923 and 1924 the limed end of Plot 11, receiving complete

TABLE 4.—Ratio of Magnesium in Ash to Protein

Plot No.	Fertilizer treatment	1924	1925
2, unlimed	Acid phosphate	1:1.47	1:1.28
2, limed	Acid phosphate	1.44	1.29
3, unlimed	Muriate of potash	1.40	1.40
3, limed	Muriate of potash	1.46	1.42
4, unlimed	Check plot, no fertilizer	1.58	1.38
4, limed	Check plot, no fertilizer	1.40	1.37
5, unlimed	Nitrate of soda	1.48	1.43
5, limed	Nitrate of soda	1.58	1.48
9, unlimed	Nitrate of soda and muriate of potash	1.64	....
9, limed	Nitrate of soda and muriate of potash	1.54	....
11, unlimed	Complete fertilizer	1.49	1.34
11, limed	Complete fertilizer	1.67	1.40
17, unlimed	Complete fertilizer	1.24	1.45
17, limed	Complete fertilizer	1.27	1.47
Average		1:1.47	1:1.39

fertilizer, produced wheat of a lower calcium content than the unlimed end, while in 1925 the reverse was true. This peculiarity of Plot 11 may have been due to the change in the application of lime as noted above, but the idea seems rather unreasonable.

TABLE 5.—Yield of Wheat in Bushels per Acre

Plot No.	1923	1924	1925	1926
2, unlimed	21.50	26.67	12.17	10.33
2, limed	26.06	27.83	26.42	28.42
3, unlimed	12.17	13.67	8.67	1.92
3, limed	19.75	15.75	23.67	17.25
4, unlimed	12.25	9.75	7.42	1.42
4, limed	14.08	12.67	21.25	12.67
5, unlimed	11.50	11.67	14.50	8.92
5, limed	14.83	13.92	24.83	27.42
9, unlimed	13.42	10.17	16.17	5.58
9, limed	16.83	15.58	26.67	17.50
11, unlimed	19.42	36.33	26.58	24.67
11, limed	36.33	37.25	36.42	43.75
17, unlimed	25.17	35.17	20.83	25.17
17, limed	28.50	36.92	32.00	33.75

CHEMISTRY OF VITAMIN B

Before discussing the various methods used in testing for the presence of vitamin B it seems desirable to say something about the chemistry of vitamin B.

Funk (14) was the first who attempted to isolate and identify vitamin B. After fractionating the precipitate formed with phosphotungstic acid from autolysed yeast, he obtained, with the silver nitrate-baryta reagent, a crystalline substance which melted at 233° C. He gave it the formula $C_{17}H_{20}O_7N_2$ and placed it in the class of pyrimidine bases. Edie etal. (10) obtained an active fraction from yeast which they also regarded as a pyrimidine base. Williams and Seidell (67) prepared adenin from yeast and found that it had antineuritic properties. On standing, it lost this property and they attributed this change to isomerization. They treated the inactive preparation with alcoholic sodium hydroxide and found that it became active again. They absorbed the antineuritic substance from autolyzed yeast by means of fuller's earth, and extracted the earth with 5 percent sodium hydroxide and found that the extract was active. When this extract was evaporated and crystallized they found that the crystals were inactive, but upon boiling with glacial acetic, dilute hydrochloric, or sulfuric acid the substance acquired antineuritic properties. These crystals were identified as adenin. Others (23, 36) have attempted to verify this, but with negative results. Seidell (46) prepared a picrate of vitamin B and found it very potent in curing pigeons of polyneuritis.

Funk (14) prepared a crystalline substance which possessed antineuritic properties from rice polishings. He identified this as nicotinic acid. Eddy, Kerr, and Williams (9) isolated from yeast a crystalline substance that they believed to be "bios." It had a melting point of 223° C. and contained 43.29 percent carbon, 8.31 percent hydrogen, and about 25 percent nitrogen. Numerous attempts have thus been made to isolate vitamin B, but the general opinion is that all have failed and the evidence in support of any particular formula for it is inconclusive. There is some evidence, however, that vitamin B is probably a derivative of pyrimidine.

Vitamin B is soluble in water, dilute alcohol, or weak acid solutions, but it is insoluble in strong alcohol or ether. It is stable at 100° C. and in cold or hot dilute acids, but it is rapidly destroyed in hot alkaline solution. One of the fractions of vitamin B is destroyed when autoclaved for 2½ hours at 15 pounds steam pressure.

METHODS OF STUDY

Several methods have been proposed for the quantitative measure of vitamin B. Among these methods, and one that was used first, was an attempt to find the smallest amount of a food that would cure a pigeon of polyneuritis that had been induced by a diet of polished rice. This method has been severely criticised, for, as McCollum (36) says, "substances of a stimulating nature might whip up motor nerve cells which had lost their functional capacity, and temporarily restore the bird to an apparently normal condition". Some of the substances that are able to induce "cures" are thyroxin, distilled water, hydroxy pyridin, thymus nucleic acid, and adenin. Another method, which has been almost universally adopted, is that of feeding rats a diet made up of an adequate purified protein, dextrin, a salt mixture, and a fat containing vitamin A. Either the food or preparation under study is added to this diet and the growth and well being noticed; or the animal is kept on the diet, which is devoid of vitamin B, until growth ceases, when the substance to be tested is added to the diet and the animal's behavior noticed. If the animal recovers and resumes growth, vitamin B is present. This will be referred to hereafter as the "curative" method.

Jendrassik (27) proposed a qualitative test for vitamin B. He made extracts of substances (foods), some of which were known to contain vitamin B and others in which it was entirely absent. He also used solvents that are known to extract the vitamin and others that are known not to extract it. He found that those extracts that contained vitamin B gave a blue color with ferric ferricyanide. The method has been criticised from the fact that many phenols and their derivatives reduce ferric ferricyanide giving this same color.

Williams (66) proposed yeast as a test organism for vitamin B. He found that when a yeast culture was added to a medium of inorganic salts and cane sugar very little growth was obtained, but when extracts of natural foods were added the yeast grew very rapidly. Several modifications of this method have been proposed.

Souza and McCollum (53) and Whipple (64) found that extracts of foods, the vitamin-B content of which was supposed to have been destroyed, were as effective in inducing growth of yeast as were the original extracts containing vitamin B. Fulmer, Nelson, and Sherwood (13) found that when extracts of wheat embryo and alfalfa were treated with alkali (a treatment which destroys vitamin B) their property of causing the growth of yeast was not destroyed. Neither was the growth stimulus improved by adding vitamin B.

Wildiers (65) found that yeast required some unknown substance for growth which is found in beer wort and also in yeast cells themselves. He called this substance "bios." Funk and Dubin (15) separated from autolyzed yeast, by means of fuller's earth, a substance which stimulated the growth of yeast and which they found to be different from vitamin B. They provisionally called this substance vitamin D. From the references cited it seems likely that the original vitamin B as first studied consists of two fractions, one that stimulates the growth of yeast and the other that similarly affects animals.

All of the methods that have been proposed for the quantitative study of vitamin B may possess certain merits, but the most reliable methods are those in which either rats or pigeons are used as the experimental animals and the food in question is fed with an otherwise complete diet.

The methods adopted in this study are, first, the prophylactic method in which the substance under study is added to the diet and the growth and well-being of the animal is noticed, and second, the curative method as previously mentioned. The prophylactic method was used in the three-year study and will be described first.

THE EXPERIMENTS

The wheats from the different plots (Table 1) were finely ground and fed as the sole source of vitamin B. They were thoroughly incorporated in the diets in proportions as follows

DIETS

Wheat	35	40	45	50	60	65	70
Casein	19	18	17	16	15	15	15
Starch	29	25	22	18	11	6	1
Salt mixture*	5	5	4	4	3	3	3
Crisco	10	10	10	10	9	9	9
Cod-liver oil	2	2	2	2	2	2	2

Since a preliminary trial had shown that at least 40 percent of wheat in the diet is necessary to furnish enough vitamin B to produce growth to any degree, a minimum of 35 percent was adopted. For the years 1923 and 1924 the amounts varied from 35 to 60 percent, while in 1925 they varied from 45 to 70 percent.

Four rats, two males and two females, were used in the biological analysis of each diet. For each level at which the wheat was fed there were 8 rats, 4 for the wheat from the limed portion and 4 for that from the unlimed portion of a plot. The rats were kept in cylindrical wire cages, 10 by 15 inches, equipped with ¼-inch mesh screen bottoms to prevent them from eating their own feces. Steenbock and associates (54) and our own experiences have shown that the amount of vitamin B necessary for normal growth is considerably greater if feces cannot be consumed. The rats in most cases weighed 45 to 55 grams at the beginning. The diet and

*McCollum and associates, J. Biol. Chem. 32, 191, (1917).

history of all of the rats were identical, except that in 1923 the feed of the stock colony contained 2 percent of cod-liver oil. The young rats were weaned at 24 days of age and then placed on test. If the weight of a rat was not normal (40 grams or more) it was discarded. The feeds, given in the proportions as stated, were made up in 1000-gram portions and kept in covered one-half gallon mason jars. Feed and distilled water were kept before the animals at all times. The rats were weighed weekly.

The feeding trials or biological analyses for the first three years were carried through a period of about six months. The original intention was to run all of the trials for the same length of time, but some plots of wheat did not yield as much as others and as a result the rats fed from these plots had to be discontinued sooner than they otherwise would have been. In order to conserve the wheat for the higher levels, some of the trials on the lower levels were not run. All of the lots, however, were kept going for a sufficient length of time to allow the rats to reach sexual maturity and, in case of females, time enough for conception, gestation, and nursing of the young to the weaning age.

Primarily the object of the experiment was to study the vitamin-B content of wheat. Whether these diets were suitable for a study of the reproductive factor, or vitamin E remains to be determined.

If crisco contain vitamin E, as shown by Kennedy and Palmer (29), then all of the diets were supplied with a sufficient quantity of this factor. Since the question of vitamin E in wheat was of secondary importance, although vitamin B is associated with E in reproduction, as indicated later, it was thought best not to change the diet from that determined upon at the beginning of the experiment. During the four years of this study the ideas regarding diets have changed somewhat, but in order that one may study the influence of climate as well as fertilizers over such a period the basal diet must of necessity remain the same.

In the series of trials of 1923 the mother rats were allowed to nurse all of the young that were born; while in 1924 and 1925 all litters were reduced to six, and in Table 6 the percentages of young weaned are based upon these facts. The trials of 1923 were begun on October 1, about eight weeks after threshing the wheat; those on wheat grown in 1924 were begun September 9, 1925, more than a year after threshing. The wheats of 1924 were kept in paper sacks at a low temperature (3.5° C.) until needed. The trials on wheat grown in 1925 were begun January 21, 1926, about five months after threshing. The trials of 1926 were begun September 12, about 6 weeks after threshing. All of the wheats were kept at a low temperature while the experiments were in progress.

DISCUSSION OF RESULTS

The record of all females, the number of weeks on test, the increase in weight, and the reproductive history for the first three series of experiments are shown in Table 6. The composite growth curves of all animals are shown in Charts 1 and 2.

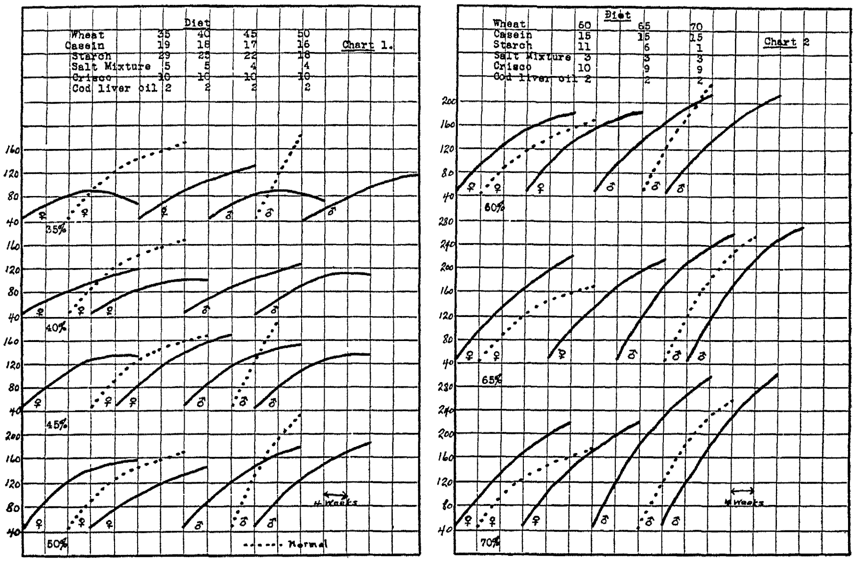

Fig. 1.—Charts 1 and 2 are composite growth curves of rats on different amounts of wheat in the diet

RESULTS OF 1923

The results as recorded by the composite growth curves indicate that wheat as the sole source of vitamin B should make up at least 45 percent of the diet to produce growth, at a slow rate, for any length of time, and at least 60 percent for normal growth. No difference was observed in the rate of growth produced by the various wheats used.

TABLE 6, PART 1.—Wheat From Plot 2, ACID PHOSPHATE, Unlimed and Limed, Fed in Different Proportions in the Diet of Rats

Percent of wheat in diet	35		40		45		50		60		Total
1923, unlimed											
Rat, No.	1338	1340	1343	1345	1346	1348*	1350	1353	1355	1357	....
Weeks on test, No.	16	16	18	18	21	21	21	25	26	24	....
Gain in weight, gm.	117	57	94	56	113	138	86	115	150	132	....
Times pregnant, No.	0	0	0	0	1	2	0	2	3	2	8
Litters born, No.	0	0	0	0	1	2	0	2	3	2	8
Young born, No.	0	0	0	0	7	12	0	17	28	15	67
Young weaned, No.	0	0	0	0	0	10	0	2	8	2	12
Young weaned, pct.	0	0	0	0	0	100	0	12	29	13	....
1923, limed											
Rat, No.	1358*	1361	1362	1364	1366	1368	1370	1373	1375	1376	....
Weeks on test, No.	25	18	20	19	19	23	22	24	19	25	....
Gain in weight, gm.	146	93	119	114	89	152	146	150	126	162	....
Times pregnant, No.	2	0	0	0	0	2	2	2	0	1	7
Litters born, No.	2	0	0	0	0	2	1	2	0	1	6
Young born, No.	7	0	0	0	0	5	8	19	0	9	41
Young weaned, No.	5	0	0	0	0	0	0	0	0	3	3
Young weaned, pct.	70	0	0	0	0	0	0	0	0	33	....
1924, unlimed											
Rat, No.	1178	1181‡	1184	1185‡	1188‡	1189	1191	1192	1195	1196†	....
Weeks on test, No.	20	19	21	19	20	21	24	24	27	13	....
Gain in weight, gm.	52	21	46	14	80	72	103	154	152	44	....
Times pregnant, No.	0	0	0	0	0	0	0	0	2	0	2
Litters born, No.	0	0	0	0	0	0	0	0	2	0	2
Young born, No.	0	0	0	0	0	0	0	0	9	0	9
Young weaned, No.	0	0	0	0	0	0	0	0	0	0	0
Young weaned, pct.	0	0	0	0	0	0	0	0	0	0	....
1924, limed											
Rat, No.	1323	1324	1327	1330	1332	1333	1335	1336	1339	1340	....
Weeks on test, No.	18	18	24	24	28	28	23	23	26	26	....
Gain in weight, gm.	60	57	130	100	114	100	94	102	120	118	....
Times pregnant, No.	0	0	0	1	0	0	0	0	0	0	1
Litters born, No.	0	0	0	1	0	0	0	0	0	0	1
Young born, No.	0	0	0	6	0	0	0	0	0	0	6
Young weaned, No.	0	0	0	0	0	0	0	0	0	0	0
Young weaned, pct.	0	0	0	0	0	0	0	0	0	0	....
Percent of wheat in diet	**45**		**50**		**60**		**65**		**70**		**Total**
1925, unlimed											
Rat, No.	1928	1930	1932	1935§	1937	1938	1942	1943	1946	1947	....
Weeks on test, No.	21	21	22	21	23	23	23	23	23	23	....
Gain in weight, gm.	102	115	70	12	108	178	181	188	106	160	....
Times pregnant, No.	0	0	0	0	0	2	2	0	0	1	5
Litters born, No.	0	0	0	0	0	2	2	0	0	1	5
Young born, No.	0	0	0	0	0	7	15	0	0	2	24
Young weaned, No.	0	0	0	0	0	0	9	0	0	0	9
Young weaned, pct.	0	0	0	0	0	0	75	0	0	0	....
1925, limed											
Rat, No.	1948	1951	1952	1953	1956	1959	1960	1961	1963	1966	....
Weeks on test, No.	21	21	24	24	23	23	23	23	25	24	....
Gain in weight, gm.	120	76	154	115	186	163	144	176	156	155	....
Times pregnant, No.	0	0	1	0	3	1	1	0	3	3	12
Litters born, No.	0	0	1	0	3	1	1	0	3	3	12
Young born, No.	0	0	7	0	14	7	7	0	17	19	71
Young weaned, No.	0	0	1	0	6	0	5	0	6	10	28
Young weaned, pct.	0	0	14	0	43	0	71	0	36	52	....

*Yeast added to diet. Results not included in total. †Killed accidentally. ‡Died. §Killed by mates.

TABLE 6, PART 2.—Wheat From Plot 3, MURIATE OF POTASH, Unlimed and Limed, Fed in Different Proportions in the Diet of Rats

Percent of wheat in diet	35		40		45		50		60		Total
1923, unlimed											
Rat, No.	1379	1380	1383	1384	1386	1389	1390	1392	1394	1396	...
Weeks on test, No.	20	20	19	21	21	21	23	19	26	19	...
Gain in weight, gm.	114	118	102	110	124	123	115	106	130	140	...
Times pregnant, No.	0	0	0	0	0	1	0	0	1	2	4
Litters born, No.	0	0	0	0	0	1	0	0	1	?	2
Young born, No.	0	0	0	0	0	7	0	0	7	?	14
Young weaned, No.	0	0	0	0	0	0	0	0	0	0	0
Young weaned, pct.	0	0	0	0	0	0	0	0	0	0	...
1923, limed											
Rat, No.	1399	1400	1402	1403	1406	1408	1411	1413	1415	1417	...
Weeks on test, No.	13	13	23	22	20	19	19	21	24	19	...
Gain in weight, gm.	70	90	106	125	123	108	96	108	138	104	...
Times pregnant, No.	0	0	2	1	0	0	0	0	0	0	3
Litters born, No.	0	0	2	1	0	0	0	0	0	0	3
Young born, No.	0	0	14	7	0	0	0	0	0	0	21
Young weaned, No.	0	0	0	4	0	0	0	0	0	0	4
Young weaned, pct.	0	0	0	57	0	0	0	0	0	0	...
1924, unlimed											
Rat, No.	1219	1220	1223	1224	1227	1228	1231	1233	1235	1237	...
Weeks on test, No.	19	19	21	21	22	22	25	25	26	26	...
Gain in weight, gm.	52	60	92	16	96	47	144	70	114	142	...
Times pregnant, No.	0	0	0	0	0	0	2	0	0	0	2
Litters born, No.	0	0	0	0	0	0	2	0	0	0	2
Young born, No.	0	0	0	0	0	0	5	0	0	0	5
Young weaned, No.	0	0	0	0	0	0	0	0	0	0	0
Young weaned, pct.	0	0	0	0	0	0	0	0	0	0	...
1924, limed											
Rat, No.	1361*	1364	1365	1366	1371	1372	1373	1376	1378	1379	...
Weeks on test, No.	14	17	19	19	21	21	25	25	26	25	...
Gain in weight, gm.	4	108	24	90	88	110	116	102	150	80	...
Times pregnant, No.	0	0	0	0	0	0	0	0	2	0	2
Litters born, No.	0	0	0	0	0	0	0	0	2	0	2
Young born, No.	0	0	0	0	0	0	0	0	17	0	17
Young weaned, No.	0	0	0	0	0	0	0	0	9	0	9
Young weaned, pct.	0	0	0	0	0	0	0	0	75	0	...
Percent of wheat in diet	**45**		**50**		**60**		**65**		**70**		**Total**
1925, unlimed											
Rat, No.	...	...	2013	2016	2019	2020	...	...	2025	2027	...
Weeks on test, No.	...	...	20	20	21	21	...	...	22	22	...
Gain in weight, gm.	...	...	148	148	178	172	...	...	150	164	...
Times pregnant, No.	...	...	1	1	2	0	...	...	2	1	7
Litters born, No.	...	...	1	1	2	0	...	...	2	1	7
Young born, No.	...	...	5	3	15	0	...	...	15†	8	46
Young weaned, No.	...	...	0	0	12	0	...	...	6	0	18
Young weaned, pct.	...	...	0	0	100	0	...	...	50	0	...
1925, limed											
Rat, No.	2029	2031	2033	2036	2038	2040	2041	2044	2045	2047	...
Weeks on test, No.	20	23	22	22	23	25	23	23	23	23	...
Gain in weight, gm.	38	150	149	153	148	180	183	139	188	184	...
Times pregnant, No.	0	1	0	0	1	3	2	2	2	3	14
Litters born, No.	0	1	0	0	1	3	2	2	2	3	14
Young born, No.	0	3	0	0	3	31	12	12	18	17	96
Young weaned, No.	0	0	0	0	0	18	9	12	11	1	51
Young weaned, pct.	0	0	0	0	0	100	100	100	91	8	...

*Killed by mates. †3 dead.

TABLE 6, PART 3.—Wheat From Plot 4, Check Unfertilized, Unlimed and Limed, Fed in Different Proportions in the Diet of Rats

Percent of wheat in diet	35		40		45		50		60		Total
1923, unlimed											
Rat, No.	1419*	1421	1423	1425	1427	1429	1432	1433	1435†	1436	....
Weeks on test, No.	23	19	24	22	26	26	26	28	10	26	....
Gain in weight, gm.	104	95	108	67	117	116	140	132	28	156	....
Times pregnant, No.	1	0	0	0	0	0	2	2	0	2	6
Litters born, No.	1	0	0	0	0	0	2	2	0	2	6
Young born, No.	4	0	0	0	0	0	13	15	0	18	46
Young weaned, No.	0	0	0	0	0	0	0	2	0	3	5
Young weaned, pct.	0	0	0	0	0	0	0	13	0	16	....
1923, limed											
Rat, No.	1439	1440	1443	1444	1447	1449	1450	1453	1775	1776	....
Weeks on test, No.	23	23	24	24	25	25	24	24	20	20	....
Gain in weight, gm.	92	104	90	113	113	124	151	172	178	142	....
Times pregnant, No.	0	0	0	0	1	0	2	2	1	2	8
Litters born, No.	0	0	0	0	?	0	2	2	1	2	7
Young born, No.	0	0	0	0	?	0	10	16	4	6	36
Young weaned, No.	0	0	0	0	0	0	0	1	4	0	5
Young weaned, pct.	0	0	0	0	0	0	0	6	100	0	....
1924, unlimed											
Rat, No.	1199	1200‡	1202	1205	1208	1209	1211	1213	1215	1216	....
Weeks on test, No.	20	12	21	21	21	21	22	22	25	25	....
Gain in weight, gm.	43	25	72	90	88	80	70	94	112	116	....
Times pregnant, No.	0	0	0	0	0	0	0	0	1	0	1
Litters born, No.	0	0	0	0	0	0	0	0	1	0	1
Young born, No.	0	0	0	0	0	0	0	0	3	0	3
Young weaned, No.	0	0	0	0	0	0	0	0	0	0	0
Young weaned, pct.	0	0	0	0	0	0	0	0	0	0	....
1924, limed											
Rat, No.	1381	1383	1387	1388	1390	1391	1395	1396	1397	1399	....
Weeks on test, No.	17	17	17	17	25	25	19	19	25	25	....
Gain in weight, gm.	19	28	34	64	114	138	58	32	160	154	....
Times pregnant, No.	0	0	0	0	0	0	0	0	2	2	4
Litters born, No.	0	0	0	0	0	0	0	0	2	2	4
Young born, No.	0	0	0	0	0	0	0	0	7	19	26
Young weaned, No.	0	0	0	0	0	0	0	0	0	11	11
Young weaned, pct.	0	0	0	0	0	0	0	0	0	91	....
Percent of wheat in diet	**45**		**50**		**60**		**65**		**70**		**Total**
1925, unlimed											
Rat, No.	2117	2118	2121	2122	2124	2126	......	......	2129	2130	....
Weeks on test, No.	22	24	22	22	22	22	......	......	22	22	....
Gain in weight, gm.	179	147	95	119	119	158	......	......	174	182	....
Times pregnant, No.	2	3	1	0	0	0	......	......	1	2	9
Litters born, No.	2	3	1	0	0	0	......	......	1	2	9
Young born, No.	16	20	3	0	0	0	......	......	6	20	65
Young weaned, No.	1	12	0	0	0	0	......	......	5	10	28
Young weaned, pct.	8	87	0	0	0	0	......	......	83	83	....
1925, limed											
Rat, No.	2133	2134	2136	2137	2139	2142	2277	2278	2143	2145	....
Weeks on test, No.	20	20	20	20	24	20	21	21	22	22	....
Gain in weight, gm.	118	72	140	142	140	124	37	172	170	188	....
Times pregnant, No.	0	0	0	1	2	2	0	2	2	2	11
Litters born, No.	0	0	0	1	2	2	0	2	2	2	11
Young born, No.	0	0	0	8	11	15	0	20	17	11	82
Young weaned, No.	0	0	0	6	3	8	0	3	12	5	37
Young weaned, pct.	0	0	0	100	33	80	0	25	100	50	....

*Yeast added to diet. Results not included in total. †Died. ‡Killed by mates.

TABLE 6, PART 4.—Wheat From Plot 5, Nitrate of Soda, Unlimed and Limed, Fed in Different Proportions in the Diet of Rats

Percent of wheat in diet	35		40		45		50		60		Total
1923, unlimed											
Rat, No.	1455	1457	1464	1465	1467	1468*	1472	1473	1782	1784	....
Weeks on test, No.	22	18	26	26	26	10	26	26	18	18	....
Gain in weight, gm.	124	82	174	188	172	110	97	134	108	159	....
Times pregnant, No.	0	0	3	3	1	1	0	0	0	0	8
Litters born, No.	0	0	2	3	1	1	0	0	0	0	7
Young born, No.	0	0	11	14	4	8	0	0	0	0	37
Young weaned, No.	0	0	1	0	0	0	0	0	0	0	1
Young weaned, pct.	0	0	9	0	0	0	0	0	0	0	....
1923, limed											
Rat, No.	1475	1476	1479	1481	1483	1485	1487	1489	1631	1633	....
Weeks on test, No.	21	21	22	17	23	23	24	24	21	21	....
Gain in weight, gm.	43	48	98	62	100	107	136	125	158	102	....
Times pregnant, No.	0	0	0	0	0	0	0	1	0	0	1
Litters born, No.	0	0	0	0	0	0	0	1	0	0	1
Young born, No.	0	0	0	0	0	0	0	5	0	0	5
Young weaned, No.	0	0	0	0	0	0	0	0	0	0	0
Young weaned, pct.	0	0	0	0	0	0	0	0	0	0	....
1924, unlimed											
Rat, No.	1239	1240	1243	1244	1248	1250	1251	1252*	1255	1256	....
Weeks on test, No.	19	19	20	20	26	26	26	19	26	26	....
Gain in weight, gm.	68	60	62	52	100	154	124	58	154	154	....
Times pregnant, No.	0	0	0	0	0	1	0	0	1	1	3
Litters born, No.	0	0	0	0	0	1	0	0	1	1	3
Young born, No.	0	0	0	0	0	6	0	0	10	7	23
Young weaned, No.	0	0	0	0	0	0	0	0	6	6	12
Young weaned, pct.	0	0	0	0	0	0	0	0	100	100	....
1924, limed											
Rat, No.	1418	1420	1421	1423	1425	1427	1429	1432	1433	1434	....
Weeks on test, No.	14	14	13	13	20	20	25	25	26	26	....
Gain in weight, gm.	61	7	48	80	94	102	136	150	103	136	....
Times pregnant, No.	0	0	0	0	0	0	2	1	0	0	3
Litters born, No.	0	0	0	0	0	0	2	1	0	0	3
Young born, No.	0	0	0	0	0	0	18	7†	0	0	25
Young weaned, No.	0	0	0	0	0	0	6	3	0	0	9
Young weaned, pct.	0	0	0	0	0	0	50	50	0	0	....
1925, unlimed											
Percent of wheat in diet	45		50		60		65		70		Total
Rat, No.	2085	2086	2087	2088	2093	2094	2021	2023	2097	2098	....
Weeks on test, No.	20	20	19	19	20	20	20	20	20	20	....
Gain in weight, gm.	148	164	106	126	214	178	118	170	153	160	....
Times pregnant, No.	0	0	0	0	2	1	0	1	0	2	6
Litters born, No.	0	0	0	0	2	1	0	1	0	2	6
Young born, No.	0	0	0	0	19	1	0	6	0	14	40
Young weaned, No.	0	0	0	0	0	0	0	3	0	5	8
Young weaned, pct.	0	0	0	0	0	0	0	50	0	55	....
1925, limed											
Rat, No.	2101	2102	2103	2105	2109	2110	......	......	2113	2114	....
Weeks on test, No.	19	19	22	22	24	23	......	......	23	23	....
Gain in weight, gm.	88	124	54	96	175	154	......	......	172	210	....
Times pregnant, No.	0	0	0	0	3	3	......	......	3	2	11
Litters born, No.	0	0	0	0	3	3	......	......	3	2	11
Young born, No.	0	0	0	0	19‡	18§	......	......	18(1)	19	74
Young weaned, No.	0	0	0	0	0	0	......	......	6	11	17
Young weaned, pct.	0	0	0	0	0	0	......	......	43	91	....

*Died. †3 dead. ‡4 dead. §6 dead. (1)8 dead.

TABLE 6, PART 5.—Wheat From Plot 9, Nitrate of Soda and Muriate of Potash, Unlimed and Limed, Fed in Different Proportions in the Diet of Rats

Percent of wheat in diet	35		40		45		50		60		Total
					1923, unlimed						
Rat, No.	1490	1492*	1494	1496	1499	1501	1503	1505	1778	1780	....
Weeks on test, No.	16	24	19	19	21	21	23	23	19	19	....
Gain in weight, gm.	16	114	58	35	88	40	103	88	170	124	....
Times pregnant, No.	0	1	0	0	0	0	0	0	0	0	0
Litters born, No.	0	1	0	0	0	0	0	0	0	0	0
Young born, No.	0	5	0	0	0	0	0	0	0	0	0
Young weaned, No.	0	0	0	0	0	0	0	0	0	0	0
Young weaned, pct.	0	0	0	0	0	0	0	0	0	0	....
					1923, limed						
Rat, No.	1507	1509	1511	1513†	1515	1517	1519	1521	1627	1629	....
Weeks on test, No.	20	20	20	18	23	23	24	24	17	17	....
Gain in weight, gm.	67	66	89	50	104	118	96	127	102	98	....
Times pregnant, No.	0	0	0	0	0	0	0	0	0	0	0
Litters born, No.	0	0	0	0	0	0	0	0	0	0	0
Young born, No.	0	0	0	0	0	0	0	0	0	0	0
Young weaned, No.	0	0	0	0	0	0	0	0	0	0	0
Young weaned, pct.	0	0	0	0	0	0	0	0	0	0	....
					1924, unlimed						
Rat, No.	1259	1260	1264	1265	1267	1268	1272	1273	1276	1277	....
Weeks on test, No.	19	19	18	18	20	20	21	21	25	25	....
Gain in weight, gm.	60	59	75	56	70	86	99	120	118	158	....
Times pregnant, No.	0	0	0	0	0	0	0	0	1	1	2
Litters born, No.	0	0	0	0	0	0	0	0	1	1	2
Young born, No.	0	0	0	0	0	0	0	0	7	10	17
Young weaned, No.	0	0	0	0	0	0	0	0	6	6	12
Young weaned, pct.	0	0	0	0	0	0	0	0	100	100	....
					1924, limed						
Rat, No.	1441	1444	1445	1446	1449‡	1450	1453	1454	1457	1458	....
Weeks on test, No.	15	15	19	19	16	19	26	26	27	25	....
Gain on weight, gm.	22	81	76	75	36	92	137	124	157	124	....
Times pregnant, No.	0	0	0	0	0	0	0	0	1	0	1
Litters born, No.	0	0	0	0	0	0	0	0	1	0	1
Young born, No.	0	0	0	0	0	0	0	0	8	0	8
Young weaned, No.	0	0	0	0	0	0	0	0	5	0	5
Young weaned, pct.	0	0	0	0	0	0	0	0	83	0	....

*Yeast added to diet. Results not included in total. †Died. ‡Killed by mates.

TABLE 6, PART 6.—Wheat From Plot 11, Complete Fertilizer, Unlimed and Limed, Fed in Different Proportions in the Diet of Rats

Percent of wheat in diet	35		40		45		50		60		Total
1923, unlimed											
Rat, No.	1523	1525	1527	1529	1531	1533	1535	1537	1539	1541	....
Weeks on test, No.	19	19	22	22	22	22	24	24	27	27	....
Gain in weight, gm.	68	74	64	71	75	99	155	85	180	80	....
Times pregnant, No.	0	0	0	0	0	0	1	0	1	0	2
Litters born, No.	0	0	0	0	0	0	1	0	1	0	2
Young born, No.	0	0	0	0	0	0	6	0	10	0	16
Young weaned, No.	0	0	0	0	0	0	3	0	0	0	3
Young weaned, pct.	0	0	0	0	0	0	50	0	0	0	....
1923, limed											
Rat, No.	....	....	....	....	....	....	1459	....	1461	....	....
Weeks on test, No.	....	....	....	....	....	....	22	....	26	....	....
Gain in weight, gm.	....	....	....	....	....	....	132	....	140	....	....
Times pregnant, No.	....	....	....	....	....	....	2	....	3	....	5
Litters born, No.	....	....	....	....	....	....	2	....	3	....	5
Young born, No.	....	....	....	....	....	....	9	....	20	....	29
Young weaned, No.	....	....	....	....	....	....	7	....	6	....	13
Young weaned, pct.	....	....	....	....	....	....	77	....	30	....	....
1924, unlimed											
Rat, No.	1284	1285	1287	1289	1291	1294	1297	1298	1299	1300	....
Weeks on test, No.	18	18	18	18	22	22	23	23	27	27	....
Gain in weight, gm.	66	32	36	53	22	104	90	92	166	152	....
Times pregnant, No.	0	0	0	0	0	0	0	0	1	1	2
Litters born, No.	0	0	0	0	0	0	0	0	1	1	2
Young born, No.	0	0	0	0	0	0	0	0	6	9	15
Young weaned, No.	0	0	0	0	0	0	0	0	0	0	0
Young weaned, pct.	0	0	0	0	0	0	0	0	0	0	....
1924, limed											
Rat, No.	1461	1463	1465	1467	1469	1472	1473	1474	1477	1478†	....
Weeks on test, No.	15	15	15	15	16	16	16*	22	25	23	....
Gain in weight, gm.	58	48	38	35	73	108	43	118	104	68	....
Times pregnant, No.	0	0	0	0	0	0	0	0	0	0	0
Litters born, No.	0	0	0	0	0	0	0	0	0	0	0
Young born, No.	0	0	0	0	0	0	0	0	0	0	0
Young weaned, No.	0	0	0	0	0	0	0	0	0	0	0
Young weaned, pct.	0	0	0	0	0	0	0	0	0	0	....
Percent of wheat in diet	**45**		**50**		**60**		**65**		**70**		**Total**
1925, unlimed											
Rat, No.	2147	2148	2152	2153	2155	2156	2266	2268	2161	2162	....
Weeks on test, No.	24	22	22	18†	16†	22	21	21	22	22	....
Gain in weight, gm.	139	76	104	36	94	140	139	209	176	154	....
Times pregnant, No.	2	1	0	0	0	1	1	2	2	3	12
Litters born, No.	2	1	0	0	0	1	1	2	2	3	12
Young born, No.	15	6	0	0	0	?	8	22	18	17‡	86
Young weaned, No.	7	5	0	0	0	0	0	8	0	0	20
Young weaned, pct.	58	83	0	0	0	0	0	66	0	0	....
1925, limed											
Rat, No.	2163	2164	2167	2168	2173	2174	2270	2272	2177	2178	....
Weeks on test, No	22	22	22	22	23	22	21	21	23	23	....
Gain in weight, gm.	120	107	126	137	168	132	160	116	198	152	....
Times pregnant, No.	0	0	0	1	2	1	2	2	3	2	13
Litters born, No.	0	0	0	1	1	1	2	2	3	2	12
Young born, No.	0	0	0	6	5	9	12	10	25	13	80
Young weaned, No.	0	0	0	6	3	0	4	0	18	5	36
Young weaned, pct.	0	0	0	100	60	0	40	0	100	21	....

*Died. †Killed by mates. ‡7 dead.

TABLE 6, PART 7.—Wheat From Plot 17, Complete Fertilizer, Unlimed and Limed, Fed in Different Proportions in the Diet of Rats

Percent of wheat in diet	35		40		45		50		60		Total
1923, unlimed											
Rat, No.	1543	1545	1546	1548	1551	1553	1555	1557	1559	1561	......
Weeks on test, No.	20	20	20	20	21	21	25	25	25	25	......
Gain in weight, gm.	—2	62	120	60	100	91	150	118	178	166	......
Times pregnant, No.	0	0	0	0	1	0	2	1	3	1	8
Litters born, No.	0	0	0	0	1	0	2	1	1	1	6
Young born, No.	0	0	0	0	3	0	11	9	2	4	29
Young weaned, No.	0	0	0	0	0	0	7	8	0	4	19
Young weaned, pct.	0	0	0	0	0	0	63	89	0	100	......
1923, limed											
Rat, No.	......	......	1563	1565	1567	1569	1571	1573	1575	1577	......
Weeks on test, No.	......	......	19	19	20	20	24	24	24	24	......
Gain in weight, gm.	......	......	74	100	108	84	94	168	168	122	......
Times pregnant, No.	......	......	0	0	0	0	0	3	1	0	4
Litters born, No.	......	......	0	0	0	0	0	3	1	0	4
Young born, No.	......	......	0	0	0	0	0	10	3	0	13
Young weaned, No.	......	......	0	0	0	0	0	5	2	0	7
Young weaned, pct.	......	......	0	0	0	0	0	50	66	0	......
1924, unlimed											
Rat, No.	1303	1305	1308	1309	1311	1312	1316	1317	1319	1322	......
Weeks on test, No.	17	17	17	17	21	21	26	26	26	26	......
Gain in weight, gm.	8	34	67	83	73	86	126	150	84	109	......
Times pregnant, No.	0	0	0	0	0	0	0	1	0	0	1
Litters born, No.	0	0	0	0	0	0	0	1	0	0	1
Young born, No.	0	0	0	0	0	0	0	6	0	0	6
Young weaned, No.	0	0	0	0	0	0	0	6	0	0	6
Young weaned, pct.	0	0	0	0	0	0	0	100	0	0	......
1924, limed											
Rat, No.	1481	1482	1487	1488	1489	1490	1493	1496	1497	1499	......
Weeks on test, No.	15	15	16	16	25	18*	20	20	25	25	......
Gain in weight, gm.	34	82	58	38	131	54	31	102	120	196	......
Times pregnant, No.	0	0	0	0	1	0	0	0	0	2	3
Litters born, No.	0	0	0	0	1	0	0	0	0	2	3
Young born, No.	0	0	0	0	5†	0	0	0	0	22	27
Young weaned, No.	0	0	0	0	0	0	0	0	0	12	12
Young weaned, pct.	0	0	0	0	0	0	0	0	0	100	......
Percent of wheat in diet	**45**		**50**		**60**		**65**		**70**		**Total**
1925, unlimed											
Rat, No.	2181	2182	2184	2186	2187	2188	2273	2276	2193	2194	......
Weeks on test, No.	22	22	21	21	20	20	21	21	21	21	......
Gain in weight, gm.	101	102	108	136	163	151	153	206	188	156	......
Times pregnant, No.	0	0	0	0	0	0	2	2	2	2	8
Litters born, No.	0	0	0	0	0	0	2	2	2	2	8
Young born, No.	0	0	0	0	0	0	4	17	12	23	56
Young weaned, No.	0	0	0	0	0	0	0	12	5	11	28
Young weaned, pct.	0	0	0	0	0	0	0	100	62	91	......
1925, limed											
Rat, No.	2206	2207	2210	2213	2214	2216	2218	2220	2222	2225	......
Weeks on test, No.	21	21	22*	21	21	21	22	20	21	22	......
Gain in weight, gm.	75	97	145	93	149	108	146	66	186	169	......
Times pregnant, No.	0	0	0	0	0	0	2	0	2	3	7
Litters born, No.	0	0	0	0	0	0	2	0	2	3	7
Young born, No.	0	0	0	0	0	0	13	0	17	23	53
Young weaned, No.	0	0	0	0	0	0	0	0	7	5	12
Young weaned, pct.	0	0	0	0	0	0	0	0	58	28	......

*Killed accidentally. †All dead.

The reproductions as recorded in Table 6 were probably due to the presence of two vitamins, B and E. The number of pregnancies and the number of young born are believed to be the combined results of vitamins B and E, since all other factors were provided for; while the number, or percentage, of young weaned was due to vitamin B and possibly other factors, at present unidentified. This latter assumption is supported by Sure (56), who found that vitamin B plays an important role in lactation; while vitamins B and E are necessary for successful reproduction. He found that the vitamin-B requirement for lactation is much higher than for growth. Nelson, Jones, etal. (41) also present data which seem to indicate that the vitamin-B requirement for normal reproduction and rearing of young is much higher than for growth. Miller (39) on the other hand contends that the amount of vitamin B necessary for gestation and lactation does not need to be greater than for normal growth. Mattill (38) has shown that apparently vitamin E is concerned only with degeneration of the testes, while vitamin-B starvation lowers the quality of sex expression, due to the lowering of the plane of metabolism, and as a result there is no reproduction. Sure (57) in a more recent paper says that vitamin E not only is a specific antisterility vitamin but also possesses lactation properties. The results of his investigation suggest that vitamin E is composed of two fat soluble vitamins, one affecting fertility and the other lactation.

With these evidences in mind, the results seem to indicate, judging from the smallest amount of wheat that produced pregnancy and weaning of young, that those wheats grown on Plot 2, receiving acid phosphate; Plot 5, nitrate of soda; and Plots 11 and 17, receiving a complete fertilizer, contain the highest amount of vitamin B. The reproduction on 40 percent of wheat from Plot 3, limed and fertilized with muriate of potash, can probably be accounted for by the fact that the rats were on a screen bottom the mesh of which was too fine to allow the feces to drop through. After this screen was replaced with one of a coarser mesh there were no more pregnancies. Wheat from Plot 4, which did not receive any fertilizer, was next best in the matter of vitamin-B content. No reproduction was obtained from rats fed wheat from Plot 9, receiving muriate of potash and nitrate of soda. The records for grow and reproduction from rats receiving wheat from the unlimed part of Plot 11, receiving complete fertilizer, are scanty, as only a small amount of wheat was available for the test.

None of the wheat, even when as much as 60 percent was used in the diet, furnished sufficient vitamin B for the successful rearing of the young. The young were generally normal in weight at birth, but the percentage weaned generally was small and the weight at 24 days of age below normal (40 grams) (See Table 7). The number of young in the litter was, as a rule, below that of our stock colony average. With one or two exceptions, which have been noted, none of the rats reproduced and nursed the young to maturity on less than 50 percent of wheat in the diet.

TABLE 7.—Reproductions and Growth of Rats on Wheat Grown on Differently Fertilized Plots

Plot and treatment	Total pregnancies No.	Total litters born No.	Total young born No.	Total weaned No.	Average young per litter No.	Av. time of birth first litter	Av. wt. of young at birth Gm.	Av. wt. at weaning 24 days Gm.
Section B, 1923								
2 Acid phosphate,								
unlimed	8	8	67	12	8.3	14	5.2	32
limed	7	6	41	3	6.8	17	4.7	30
3 Muriate potash,								
unlimed	4	2	14	0	3.5	18	4.7	0
limed	3	3	21	4	7.0	11	5.0	31
4 Unfertilized,								
unlimed	6	6	46	5	7.6	17	5.0	33
limed	8	5	30	5	3.8	15	4.5	18
5 Nitrate soda,								
unlimed	8	7	37	1	4.6	11	5.0	30
limed	1	1	5	0	5.0	17	5.0	0
9 Nit. soda and mur. pot.								
unlimed	0	0	0	0	0	0	0	0
limed	0	0	0	0	0	0	0	0
11 Complete fertilizer,								
unlimed	2	2	16	3	8.0	18	4.8	43
limed	5	5	29	15	6.0	11	4.5	27
17 Complete fertilizer,								
unlimed	8	6	29	10	3.6	11	4.6	34
limed...	4	4	13	7	3.2	18	5.0	46
Section A, 1924								
2 Acid phosphate,								
unlimed ...	2	2	9	0	4.5	20	5.0	....
limed......	1	1	6	0	6.0	24	5.0	....
3 Muriate potash,								
unlimed ...	2	2	5	0	4.0	20	5.5	....
limed.....	2	2	17	9	8.5	15	5.0	32
4 Unfertilized,								
unlimed ...	1	1	3	0	3.0	24	5.0	....
limed......	4	4	26	11	6.5	17	4.6	40
5 Nitrate soda,								
unlimed ...	3	3	23	12	8.0	20	4.8	37
limed......	3	3	25	9	8.0	20	4.6	33
9 Nit. soda and mur. pot.								
unlimed ...	2	2	19	12	8.5	20	4.6	32
limed......	1	1	8	5	8.0	24	5.0	36
11 Complete fertilizer,								
unlimed ...	2	2	15	0	7.5	22	5.0	....
limed......	0	0	0	0	0	....	0	....
17 Complete fertilizer,								
unlimed ...	1	1	6	6	6.0	26	5.3	26
limed	3	3	27	12	9.0	15	4.9	39
Normal wheat grown 1925..	....	....	....	....	6	10	5.0	34

TABLE 7.—Reproductions and Growth of Rats on Wheat Grown on Differently Fertilized Plots—Continued

Plot and treatment	Total pregnancies No.	Total litters born No.	Total young born No.	Total weaned No.	Average young per litter No.	Av. time of birth first litter	Av. wt. of young at birth Gm.	Av. wt. at weaning 24 days Gm.
Section C, 1925								
2 Acid phosphate,								
unlimed ...	5	5	24	9	5.0	13	4.5	36
limed	12	12	71	28	6.0	12	5.0	31
3 Muriate potash,								
unlimed ...	7	7	46	18	6.6	11	5.0	28
limed	14	14	96	51	7.0	10	5.0	35
4 Unfertilized,								
unlimed ...	9	9	65	28	7.0	10	5.0	38
limed	11	11	82	37	7.4	10	5.0	37
5 Nitrate soda,								
unlimed ...	6	6	40	8	6.7	9	5.0	36
limed	11	11	74	17	6.6	12	5.0	39
9 Nit. soda and mur. pot.								
unlimed ...	....	....	....	....	....	....	....	....
limed	....	....	....	....	....	....	....	....
11 Complete fertilizer,								
unlimed ...	12	12	86	20	7.3	10	5.0	29
limed	13	12	80	36	6.3	12	5.5	41
10 Complete fertilizer,								
unlimed ...	8	8	56	28	7.0	9	4.5	39
limed	7	7	53	12	7.6	11	4.2	37

RESULTS OF 1924

The results obtained from wheat grown in 1924 are not so easily interpreted. The limed part of Plot 17, receiving a complete fertilizer, and of Plot 5, receiving nitrate of soda, probably produced wheats of the highest vitamin-B content. Wheat from the unlimed half of Plots 4, unfertilized, and 9, muriate of potash and nitrate of soda, contained vitamin B in almost as high a degree. The fact that the wheat grown in 1924 was in cold storage for about 13 months before using may account for part of the disagreements in the results from those of the other two years. The time of the first reproduction, Table 7, is rather late, much later than in comparable cases with wheat grown in 1923 and 1925. At that time it was thought that storage at the low temperature might have caused a destruction of vitamins B and E, but later evidence indicates that the wheat for that particular year contained a relatively small amount of these vitamins. This was indicated by the fact that several more weeks than usual were necessary for the animal to store enough of these vitamins to produce the conditions necessary for pregnancy. Also the growth curves of rats which received wheat grown in 1924 show that a smaller amount of vitamin B was present than in 1923. This was rather striking when small percentages of wheat were used, but when larger amounts were used this difference was not so apparent (Charts 3 to 5).

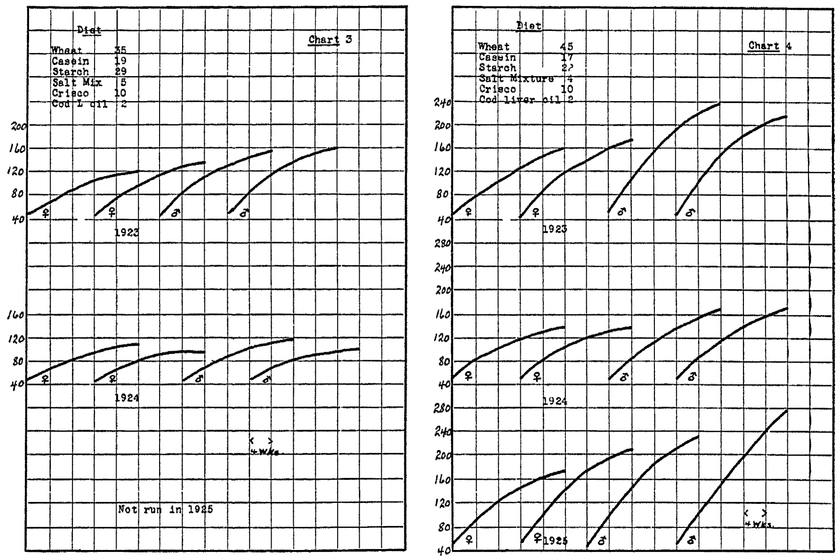

Fig. 2.—Chart 3 illustrates the rate of growth of rats induced by the same amount of wheat grown in different years

Chart 4 shows the rate of growth of rats induced by the same amount of wheat but grown in different years

The rainfall for the time the grain was filling, May and June, 1924, was 10.5 inches. This was greater than in 1923 or 1925 with 6.1 and 4.6 inches, respectively. It is not my wish to infer that vitamin B is associated with rainfall, but since no one has investigated this, the matter is presented for record. The amount of sunshine during the time of filling of the kernel was 23 days, as compared with 26 days in 1923 and 32 days in 1925. Again the writer does not care to say that vitamin B and sunshine are associated, nor that sunshine only during the days when the compounds are being synthesized in the kernel has its effect, for, throughout the entire dormant and growing periods, there may be processes influenced by weather that are associated with this vitamin.

As in 1923, with only two or three exceptions, there were no cases of reproduction on less than 50 percent of whole wheat in the diet.

There did not appear to be any parallelism between the amount of calcium and phosphorus or ash in the wheat and vitamin B.

No definite conclusions can be drawn from the data of 1924 as to the effect of fertilizers on the vitamin-B content of wheat, although Plot 17, nitrate of soda, acid phosphate, and muriate of potash; Plot 5, nitrate of soda; and Plot 9, muriate of potash and nitrate of soda, evidently produced wheat that contained a higher vitamin-B content than wheat from any of the other plots.

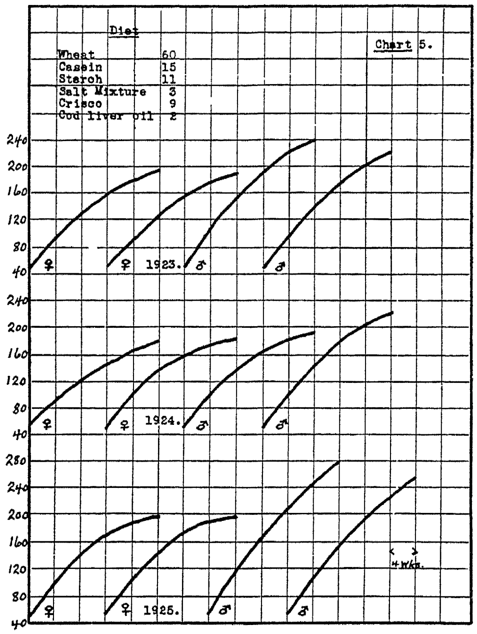

Fig. 3.—Chart 5 illustrates the rate of growth of rats as induced by the same amount of wheat but grown in different years.

RESULTS OF 1925

As in the other two years there did not appear to be any correlation between the rate of growth of rats as affected by the different fertilizers applied to the soil.

If the total number of pregnancies, the number of young born, and the number or percentage of young weaned are an indication of the amount of vitamins B and E that are present, then one may conclude that the limed section of the plots produced wheat with higher vitamin content than the unlimed section. However, Plots 11 and 17, complete fertilizers, gave as good or slightly better results on the unlimed section. Wheats grown on Plots 4 and 11, unlimed, caused reproduction on the 45 percent level, and the mother rats were able to nurse part of their young to the weaning age. This would indicate that these plots produced wheat of higher vitamin-B content than that produced by the other plots. Plots 2 and 11, limed, were next best—the wheat from these plots caused reproduction and nursing of some of the young to the weaning age on the 50 percent level. Probably the lowest amount of wheat that will produce growth, reproduction, and nursing of the young to the weaning age, is as good a criterion on which to draw a conclusion as the data from the total results of the reproductive history; since Plots 2 and 11 partially confirm the conclusion from the results of 1923, that acid phosphate produces wheat of the highest vitamin-B content. Just why there were reproduction and partial nursing of the young on 45 percent of wheat in the diet and almost failure on some of the higher percentages, is not known. Possibly certain definite ratios between the inorganic constituents are upset as the wheat is increased, and as the higher levels are approached, with no increase or decrease in the mineral mixture added, the proper ratio is again approached. Unfortunately the wheat from Plot 9 was not saved in 1925.

The results for the three years do not show a definite difference in the vitamin-B content of the wheats from the several plots; yet one or more of the plots receiving acid phosphate (2, 11, and 17) were always among those that showed the highest vitamin-B content. At least 60 or 65 percent of wheat was necessary to produce good growth and attain partial success in rearing the young. Only a small number of the young were normal in weight at 24 days of age, Table 6, part 2.

RESULTS OF 1926

The curative method of determining vitamin-B was used in the fourth year's study. This method was used by Sherman and Spohn (48) and found to be a very satisfactory quantitative method. Young rats, 24 days of age, with the same previous nutritional history as in the other years, and weighing 50 to 70 grams, were placed on a vitamin-B free diet as follows:

VITAMIN-B FREE DIET

Casein	19
Starch	63
Salt mixture	4
Crisco	10
Agar agar	2
Cod-liver oil	2

At the end of three weeks all were losing weight or were in equilibrium.* Six rats were placed in each cage.

After three weeks the rats were transferred to individual cages and they were so distributed that an equal number of males and females were used with each wheat. The wheat was fed separately, one gram each day for five weeks. The rats were weighed weekly and a total quantitative record of the food intake was kept.

*Since this work was started Sherman and MacArthur (47) found that it is not necessary to subject the animal to a "depletion period" before the quantitative determinations are made.

TABLE 8.—Gain in Weight, Food Intake, and Reproductive Record of Rats Receiving Wheat From Differently Treated Plots

Plot No.	Fertilizer treatment	Amount of wheat fed daily gm.	No. of rats	Average initial weight gm.	Average gain in weight 5 weeks gm.*	Average gain in weight mean gm.*	Average food (basal) intake gm.	Average gain in weight per 100 gms. food consumed gm.	Reproductive record 1923—25
2	Acid phosphate, unlimed	1	8	62	23.0±2.99	21.5 ±1.72	147	15.6	3
2	Acid phosphate, limed	1	12	65	20.0±2.06		164	12.2	4
3	Muriate of potash, unlimed	1	8	63	15.0±2.21	16.4 ±1.75	134	11.2	5
3	Muriate of potash, limed	1	12	62	17.7±2.44		147	12.0	2
4	Check plot, unlimed	1	8	61	24.0±1.99	20.5 ±1.66	173	13.9	3
4	Check plot, limed	1	12	61	17.0±2.45		138	12.4	1
5	Nitrate of soda, unlimed	1	8	64	21.8±2.74	19.9 ±1.71	144	15.1	2
5	Nitrate of soda, limed	1	12	65	18.0±2.17		147	12.2	5
9	Muriate potash, and nitrate soda, unlimed	1	8	61	14.5±1.72	11.05±1.40	142	10.2	4
9	Muriate potash, and nitrate soda, limed	1	8	63	7.6±1.88		159	4.8	5
11	Complete fertilizer, unlimed	1	12	69	22.2±1.72	20.9 ±1.53	158	14.1	2
11	Complete fertilizer, limed	1	12	61	19.5±1.76		138	14.1	2
17	Complete fertilizer, unlimed	1	8	66	17.5±2.07	17.4 ±1.32	149	11.7	1
17	Complete fertilizer, limed	1	11	67	17.3±1.74		143	12.1	2
Idaho	Spring wheat	1	12	55	22.4±1.02	....	175	12.9	....
Ohio	Spring wheat	1	8	65	19.4±2.61	....	168	11.5	....
Marquis	Spring wheat	1	8	65	18.9±1.91	....	157	12.0	....

*Includes probable error.

The data are summarized in Table 6, part 3. Results as given show the gain in weight and the mean of the gain in weight and the probable error for each of the wheats under study. Since vitamin-B stimulates the appetite, the increase in weight of the animal was attributed to a high economical consumption of the basal diet, which in turn was influenced by the amount of vitamin-B in the wheat. In this connection the table also gives the average increase in weight per 100 grams of food (basal diet) consumed. When the probable error of the means was calculated by the method of Wallace and Snedecor (61) and the odds determined from the table of Pearl and Miner (44) it was found that there was no significant difference between the wheat grown on the limed and unlimed ends of the plots. The mean of the limed and unlimed end of each plot was compared with that of the other plots, using the same statistical method. The odds show that the wheat from Plot 9, muriate of potash and nitrate of soda, was lowest in vitamin B. The differences between the other plots were not significant, although, when the increase in weight per 100 grams of food (basal) consumed is considered, the wheat from Plot 3, muriate of potash, was lower in vitamin B than the wheat from the remaining plots.

No significant difference was found in the vitamin-B content of spring wheat and the winter wheat of any of the plots except Plot 9.

In Table 8 the figures in the "Reproductive record" column are arbitrary and represent the order in which reproduction was most successful in the three preceding years. The order is based upon the smallest amount of wheat in the diet in which there was reproduction and nursing of the young to the weaning age, the number of pregnancies and the number of young weaned. This agrees very well with the data obtained by the curative method and shows that Plot 9, muriate of potash and nitrate of soda, produced wheat of the lowest vitamin-B and -E content. The fact that Plot 4, a check plot, produced wheat having as high a vitamin-B content as any plot would indicate that, in the absence of artificial plant food, nature provides the most of what she has, and when the plant food is at a minimum she sacrifices quantity for quality.

GENERAL DISCUSSION

When this work was begun four years ago, the impression was obtained from the literature that the vitamin-B content of wheat is high. As the work progressed the data more and more showed the reverse to be true. At the start of the project there was no evidence that the water soluble vitamin, then known as vitamin B, might be a mixture of two or more vitamins. Papers by other investigators (50, 17, 18, 45, 5) have shown rather conclusively that the "original vitamin B" is made up of two vitamins, one that will prevent polyneuritis in pigeons or rats and the other, called by Goldberger the P-P factor, a heat stable factor that will produce growth and prevent pellagra. Our data show that wheat is very low in the growth promoting or P-P, factor.

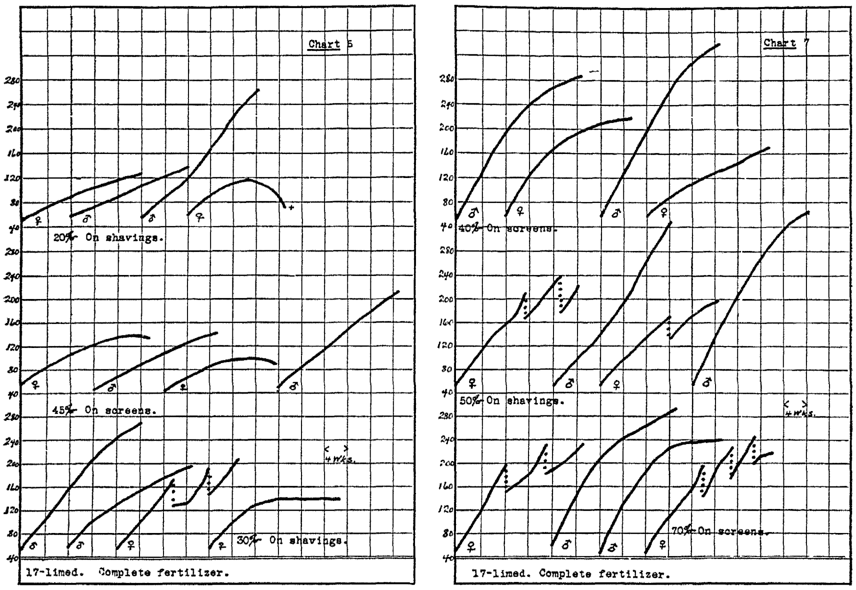

Fig. 4.—Charts 6 and 7 are growth curves showing the effect of shavings and excreta on rate of growth and reproduction of rats on different amounts of wheat in the diet

One of the most noticeable results of the diets as used—and this was especially true of the rats that received 35 and 40 percent of wheat—was the perverted appetite; that is, a desire to eat or pull out the hair of their mates. Another form in which this appetite manifested itself was that of coprophagy. With many this craving became so great that the animal would find the droppings before they were entirely voided. There were also some cases of cannibalism. When rats on a diet containing as low as 20 percent of wheat were placed in a cage without a screen bottom, and were given shavings as bedding, this perverted appetite was not noticed. When it was already in evidence and dried yeast was added to the diet, it disappeared. From the curves shown in Charts 6 and 7 for rats with and without shavings, it is quite evident that the shavings and feces consumed were equivalent to 25 percent of wheat; or, in other words, the rats with shavings as bedding and 20 percent of wheat grew as rapidly as those on screen bottoms with 45 percent of wheat in their diet. It appears then that the feces contained the growth promoting vitamin. It appears from Chart 6 that access to the shavings and feces did not improve reproduction, for the rats on shavings as bedding and receiving a diet containing 50 percent of wheat grew almost as rapidly as the rats on screens and receiving a diet containing 70 percent of wheat; yet they showed no greater reproduction or number of pregnancies than those kept on screens and receiving similar amounts of wheat.

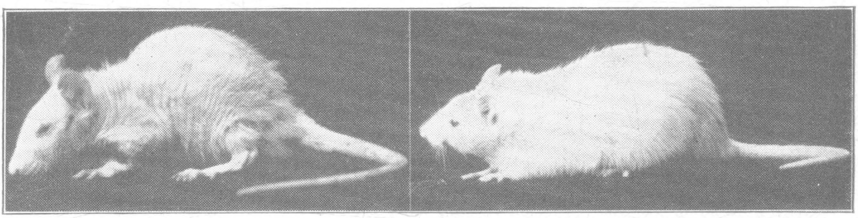

Fig. 5.—The rat at the left shows symptoms indicative of pellagra. At the right, the same rat after administering yeast for two weeks

Many of the rats on the low percentage of wheat also developed sore eyes and later lesions appeared around the mouth, feet, and different parts of the body. In many cases the feet became edematous. The eye disease resembled xeropthalmia. Cod-liver oil given seperately, however, failed to clear up the symptoms. When dry yeast was given in addition to the regular diet the eye disease disappeared; the lesions healed; the feet became normal; and a new coat of fur grew. Some of the females conceived, gave birth to young, and nursed them to maturity. Since all of these symptoms disappeared with the feeding of yeast, it seems that wheat on the lowest percentages as fed is deficient in vitamin B, the growth promoting, or Goldberger's P-P, factor. Whether the eye disease of the rats on the diets low in wheat is the same as that observed by McCollum (35) remains to be determined; but it is very likely the same as that described by Goldberger (17), Salmon (45), and Chick and Roscoe (5). The rat at the left in Figure 5 shows the symptoms of this disease.

It is evident that climatic and other factors must be considered in interpreting the results. The biological analysis of wheat grown in 1923 seemed to indicate that the acid phosphate applied to the soil influenced the vitamin-B content of the wheat. Similar analysis of the wheat grown in 1924 did not show the same effect on reproduction. Why the two soils produced thus was probably due to the climate—a difference in the rainfall, sunshine, and cloudiness of the two years—or to the physical or biological factors in the soil, which may be dependent upon or independent of the fertilizers used.

The third year's (1925) results were somewhat indefinite. Judged from the reproductive history, there were indications, that the plots receiving acid phosphate, a complete fertilizer, or no fertilizer produced wheat of the highest vitamin-B content. There was no relation between the rate of growth and the kind of fertilizer used in producing the different wheats.

The fourth year's (1926) work confirmed to some extent the conclusion of the first and third year, namely, that acid phosphate alone or in a complete fertilizer, nitrate of soda, and muriate of potash alone (not mixed with nitrate of soda) produce wheat of the highest vitamin-B content. Muriate of potash and nitrate of soda together produced wheat of the lowest vitamin-B content.

Possibly the soil on which the wheat for these experiments was grown was incapable of producing a wheat with a high vitamin-B content. To determine this point a wheat of the same variety and grown under normal conditions in 1925 was obtained and fed in the same proportions as previously mentioned. This wheat was fed along with the 1924 crop as a check on the effect of storage. The reproductive history of these rats is shown in Table 9.

TABLE 9.—Total Reproduction Records for all Wheats

Percent wheat in diet	No. of female rats used	No. litters born	No. of young born	No. of young weaned	Percent of young weaned
35*	52	0	0	0	0
40*	54	9	52	5	7.6
45†	76	20	117	35	16 4
50†	79	35	208	63	37.6
60†	79	61	408	126	41.1
65‡	18	23	158	65	53 3
70‡	24	48	349	139	49.0

*Total results for two years †Total results for three years. ‡Total results for one year.

The results again show that wheat as grown under the described conditions is low in vitamin B. Table 9 shows that as the amount of whole wheat in the diet is increased so is the amount of vitamins B and E increased, as would be expected. This is indicated by the increase in the number of litters and young that were born. The size of the litters also increased as the amount of wheat in the diet was increased. The percentage of young weaned is surprisingly low.

Plot 4, unfertilized, throughout the entire three years produced wheat with a rather constant vitamin-B content. The fact that this plot produced a low yield of wheat may be significant.

There did not appear any parallelism, for any of the years, between the percentage of calcium and phosphorus in wheat and its vitamin-B content.

SUMMARY AND CONCLUSIONS

This investigation was an attempt to determine the relation, if any, between the mineral and vitamin-B content of wheat, as they are influenced by fertilizers. Other factors which might show a relation to vitamin B were noted.

The experimental part consist of a study of the vitamin-B content of wheat grown for four consecutive years on soils of known and constant treatment covering a period of thirty-five years.

The finely ground wheat furnished the only source of vitamin B, and was thoroughly incorporated into an otherwise complete diet, which was then fed to rats; or, when the curative method was used, it was fed separately. The criteria used as to whether a wheat was high or low in vitamin B, were the effect on growth, and, in case of females, the degree of fertility as evidenced by the number of pregnancies and the success attained in nursing their young.

The results of the first year's study, judged from the rate of growth, show no relation between the kind of fertilizer used and the vitamin-B content of wheat, while if the results are judged from the reproductive history data it appears that acid phosphate, either alone or mixed with other fertilizer elements, produced wheat with a higher vitamin-B content than either nitrate of soda or muriate of potash or both Wheat grown without any fertilizer was next highest in point of vitamin-B content.

The second year's results show no relation between the kind of fertilizer applied to the soil and the vitamin-B content of the wheat, although nitrate of soda and muriate of potash alone or mixed caused the best reproduction and nursing of the young.

The data from the third year show no relation between the kind of fertilizer used and the vitamin-B content of wheat, as judged by the growth curves, but if the reproductive history be used as a criterion then the results seem to indicate that plots receiving acid phosphate and a complete fertilizer, and a plot receiving no fertilizer produced wheat of the highest vitamin-B content.

The results for the first three years show no relation between the vitamin-B content of wheat, judged by the rate of growth of animals, and the kind of fertilizer applied to the soil on which the wheat was grown. From the reproduction viewpoint there are some indications that a high vitamin-B content of wheat is associated with a fertilizer containing acid phosphate.

The fourth year's study, using the curative method of study, which is no doubt more sensitive than the prophylactic method, gave data that coincide with the reproductive records of the three previous years. There are indications that acid phosphate alone or a complete fertilizer, which contains acid phosphate, muriate of potash, and nitrate of soda, produces wheat with the highest vitamin-B content. Muriate of potash and nitrate of soda together produced wheat of the lowest vitamin-B content. This was true two years out of three.

There were indications that the climate had a significant influence on the vitamin-B content of wheat and may cause the results to vary from year to year.

There was no evidence that spring wheat contained a larger amount of vitamin-B than winter wheat.

Sixty-five percent of wheat was necessary to furnish the vitamin-B requirement for normal growth.

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