THE VITAMIN G (B₂) CONTENT OF FROZEN STRAWBERRIES

by

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THE VITAMIN G ($B_2$) CONTENT OF FROZEN STRAWBERRIES

INTRODUCTION

The purpose of this study was to determine the vitamin G content of frozen strawberries that were frozen by the "quick-freezing" process. There has been considerable research in regard to the vitamin G content of some foodstuffs, but no reference was found in the literature to the vitamin G content of strawberries or of frozen strawberries. This study was undertaken because of the prevalence of this fruit during the spring and summer and because of the large amount consumed at that time.

Historical. The multiple nature of vitamin B has proved many times since Goldberger and Lillie (20) gave satisfactory evidence that the vitamin B complex involved at least two factors important for young rats. Chick and Roscoe (10) confirmed the existence of two separate dietary factors in the vitamin B complex, both necessary for young rats. Williams and Waterman (57) found a third factor associated only with the maintenance of the weight and the general condition of the adult pigeon. Reader (44) obtained further evidence of a third factor necessary for the growth of the rat. This factor she called vitamin $B_3$ and later
changed it to vitamin $B_4$, because the pigeon factor became known as vitamin $B_3$. Chick and Copping (7) indicated the probability of still another factor, "Y". Booher, Blodgett and Page (3) determined that the highly active vitamin G concentrate prepared in their laboratory and crystalline vitamin B, together did not constitute the whole of the so-called vitamin B complex.

Gurin and Eddy (23) questioned whether the vitamin B$_2$ (G)-free diet resulting in dermatitis owed its action to a lack of a factor which was identical with that preventive of human pellagra. They did not accept the vitamin B$_2$ (G) as then defined as identical with Goldberger's (20) pellagra-preventive substance.

Roscoe (49) found no support for the claim that separate dietary factors were necessary for the prevention and cure of dermatitis and for promoting growth. Roscoe (49) and Bourquin and Sherman (4) were agreed that the specific symptoms peculiar to vitamin G deficiency, as first described by Goldberger (20), and regarded by them as analogous with pellagra in man, appear only irregularly in rats.

György (24) found that rat pellagra could be produced almost without exception when crystalline vitamin B was added to a basal diet free of the vitamin B complex, and that neither lactoflavin nor a supplementary substance by itself possessed any growth promoting action. He found that Peter's "charcoal eluate" from yeast represented a
suitable source of this substance free from lactoflavin. György called this supplementary substance vitamin B₆ and suggested that it might be identical with the "Y" factor described by Chick and Copping (7). He further stated that vitamin B₆ and the flavin formed a combination having the biological action formerly attributed to vitamin B₂. Chick, Copping, and Edgar (8) concluded that this supplementary substance appeared to be identical with the "Y" factor as previously described (7).

Harris (26) concluded that flavin did not possess anti-pellagra activities, and both flavin and the anti-pellagra factor should be regarded as separate constituents of the heat-stable, growth-promoting vitamin B₂ complex.

**Vitamin G in Foods.** Salmon (50) tested the seeds or the velvet bean and the soy bean and found them to be about one-half as potent in vitamin G as the leaves. A daily intake of 1.85 grams of seeds produced about one-half the growth of 1.65 grams of leaves.

Hunt (31), after investigating the vitamin B complex as contained in wheat and corn, found these grains to be low in vitamin G.

Aykroyd and Roscoe (1) investigated the vitamin B₂ content of wheat and maize. Their technique consisted of feeding their basal diet, free of the vitamin B complex, plus 0.05 grams cod liver oil and 0.1 cc of Peter's anti-neuritic concentrate daily to rats. Of the wheat products, the bran
and embryo were found to be the best sources of vitamin B₂, normal growth (11-14 grams per week) being secured when from 15-30% of the ration consisted of the bran or embryo. The endosperm, either as top patent or household flour, was a poor source; 65% of the ration produced very little growth. Yellow corn was inferior to either of the samples of wheat tested, and white corn was equivalent to the wheat of the lowest value, of which 50% was required for normal growth. The endosperm or grits was very low in vitamin B₂, and corn germ meal contained about one-half as much vitamin B₂ as wheat germ meal. Other materials tested by the same workers were required in the following daily amounts for the standard growth during the four weeks test period: dried ox liver, 0.06-0.12 gm.; fresh whole milk, from 3 to 6 cc; dried yeast, 0.12-0.2 gm.; autoclaved yeast, 0.4 gm.; egg yolk, cooked and dried, 0.5-1.0 gm.; dried steak, 0.5-0.75 gm.

Aykroyd (2) showed that two samples of rice "raw" and "parboiled" were poor sources of vitamin B₂ and that millet was also a poor source.

Mussell (37) concluded that 30% of white corn substituted for an equal amount of cornstarch in the Sherman and Spohn (51) vitamin B and G-free diet did not supply sufficient vitamin G to promote growth or prevent the occurrence of the symptoms of pellagra.

Sherwood and Halverson (53) found peanuts to be much richer in the anti-neuritic (vitamin B) than in the
P-P (vitamin G) factor of the vitamin B complex.

Hoyle (30) tested a fresh sample of comb honey and a West Indian honey and found both samples deficient in vitamin G.

Hoagland and Snider (27) compared fat-free beef, pork, and lamb and found these foods to contain about the same quantity of vitamin G. From 15–25% of each of these furnished sufficient vitamin G for excellent growth. Beef spleen contained about as much vitamin G as beef muscle. Beef liver, pork liver, and beef kidney were found to be excellent sources of vitamin G. Apparently these substances contained about five to eight times as much vitamin G as beef, pork, or lamb muscle. They found 3% of beef liver, 3.05% of pork liver, or 2.77% of beef kidney furnished an ample source for excellent growth. Using 10% corn extract as a source of vitamin B, they (28) found samples of commercial moisture-free beef extract furnished sufficient vitamin G for excellent growth. One pound of moisture-free beef extract appeared to contain about the same amount of vitamin G as 3.4 pounds or dried beef or the same as 11 pounds of fresh lean beef muscle.

Day (15) used a modified Sherman and Spohn (51) diet by incorporating in it an alcoholic (80% by weight) extract of rice polishings. Using beef round steak as 1, he found the concentration of vitamin G in the fresh tissues of beef and veal to be as follows: beef heart, 3;
beef kidney, 8-10; and veal liver, 8-10.

Roscoe (47) found liver to have a potency practically equal to that of yeast; 0.1 gram of dry yeast daily produced a 50-60 gram gain in five to six weeks, while 0.12 gram of dried liver daily produced this gain. Roscoe (45) concluded that watercress, lettuce, spinach, and cabbage, when dry weights are considered, have a vitamin B₂ potency of about one-fourth that of dry brewer's yeast. She also found (49) that 0.5 gram of dried carrots, turnip, or potato would cure symptoms of dermatitis. No significant differences were noted between fresh spring carrots and those stored all winter.

Aykroyd and Roscoe (1) found milk to be about one-tenth as potent as yeast. Sherman (52) considers milk the most important source of vitamin G in the diet of the American and European peoples; 1.0 gram of milk contains 0.4 to 0.75 Bourquin units. A quart of milk furnishes as much as four or five eggs, or a pound and a quarter of lean beef, or two ounces of liver (52).

Quinn and Brabec (43) reported that 2.0 to 2.5 Bourquin units were contained in each gram of malted milk.

Chick, Copping, and Roscoe (9) found fresh egg white to be the one source of vitamin B₂ unaccompanied by vitamin B₁. Roscoe (47), in comparing various foodstuffs with yeast, found that 2.0 grams of egg yolk as compared with 0.5 gram of yeast (wet weights) had a potency of 14.
Jigg white was found to be less efficient than egg yolk.

Koscoe (46-47) carried on a series of experiments in which the potencies of various foods were tested in comparison to yeast. The foods were tested for their ability to produce 50-60 grams growth in five to six weeks. Using 0.1 gram of dry yeast as 100, the following potencies were determined: ox liver over 100; lean meat, egg white, watercress, dried peas, whole wheat, spinach, whole white corn, and green cabbage a potency of from 10 to 20; milk, lettuce, and carrot, from 5 to 10; and potato, apple, orange, tomato, and turnip, from 2.5 to 5.0. Onions were found to have a potency of less than 2.5.

Kühn defined the unit of vitamin B2 as that amount which produced in thirty days a gain in weight of 40 grams, and found the following foods to contain that amount: beef liver and kidney, 0.2 to 0.4 gram; beef heart, 0.5 gram; chicken muscle (leg), 1.5 gram; chicken (breast), 3 grams; beef muscle 2 to 3 grams; cow's milk, 10 to 20 grams; egg white, 2.5 to 5.0 grams; green leaves of cabbage, 5.0 grams; and bananas 5.0 grams.

Munsell (36), using 30% of white corn to replace an equivalent weight of cornstarch in the Sherman and Spohn diet, tested watermelon for the vitamin C content and found small, but detectable amounts, approximately 0.12 Bourquin units per gram.

Daniel and Munsell (13), using white corn extract
as a source of vitamin B, tested Sultanina and Malaga grapes and two brands of commercial grape juice for their vitamin G content. Those animals receiving daily portions of 5.0 grams of Sultanina grapes made an average gain of 1.7 grams per week over a test period of eight weeks, thus showing a small but significant amount of vitamin G. The animals receiving the Malaga grapes and the grape juice did not make sufficient growth to indicate the presence of vitamin G.

Eddy (17) reported that daily feedings of 6.0 grams of banana furnished sufficient vitamin G for nearly normal growth and that the banana was relatively richer in vitamin G than in vitamin B. Zucker (59) found that only traces of vitamin G were present in cranberries.

Day and Darby (14) assayed fresh apples, avocados, oranges, and Bartlett pears for their vitamin G content, using essentially the Bourquin and Sherman method. Oranges and apples were found to be poor sources of vitamin G, each containing about 0.2 unit per gram of fresh edible fruit; avocados were slightly better as they contained 0.67 unit per gram, and Bartlett pears were even higher as they furnished 1.0 Bourquin unit per gram.

Douglass et al (16), working in this laboratory and using a modified Munsell diet, concluded that 0.5 gram of dried Bosc pear (4.64 grams of fresh) contained 1.0 Bourquin unit of vitamin G.
Morgan, Hunt, and Squier (35), using the technique of Bourquin and Sherman (4), determined the vitamin G content of prunes. They found that California dried prunes contained 2.66 Bourquin units per gram.

Morgan, Field, Kemmel, and Nichols (34), using the Bourquin and Sherman (4) basal diet with 0.05 gram of a concentrate from rice polishings daily as a source of vitamin B, tested the vitamin G content of four varieties of figs. They found the dried product to contain practically the same amount of vitamin G in all cases. The tentative conclusion was drawn that 1.0 to 1.5 Bourquin units were contained in 3.0 grams of figs, or 0.33 to 0.5 units per gram of fruit. They (33) likewise examined Sultanina (Thompson Seedless) grapes and raisins for the vitamin G content, but without satisfactory conclusions. Fresh grapes in 2.0 gram daily feedings produced an appreciable increase in growth as did 1.0 gram of raisins. There was about 0.5 Bourquin unit in 1.0 gram of raisins whether sulfured or not.

Poe and Gambill (41) tested home canned tomato juice using the Bourquin and Sherman (4) basal diet. The average content was 0.21 Bourquin unit per cc or about 95 Bourquin units per pound of tomatoes. Hanning (25) studied the vitamin G content of canned strained foods (Gerber Products) and found the following vitamin G units per ounce of food: spinach, 7.5 to 11.0 units; peas, 7.5
to 8.6 units; tomatoes, 4.0 to 5.0 units; string beans, 6.0 units; beets, 3.3 units; and carrots, 2.5 units.
The plan of this study was to use the rat growth method, feeding a modified Munsell basal diet (37) plus graded amounts of frozen strawberries for a six weeks experimental period. The frozen strawberries were fed with the purpose of determining the amount of this food necessary to produce in a rat, on a diet otherwise devoid or nearly devoid of vitamin G, a 3.0 gram gain per week over a period of four to six weeks. This is the measure for a unit of vitamin G which has been established by Bourquin and Sherman (4).

Animals. The albino rats used in this experiment were raised in the stock colony of the Foods and Nutrition Department and in the colony of the Animal Nutrition Department at Oregon State College. The stock animals had been fed for a number of generations on a slightly modified form of Steenbock's Stock Diet (54). This diet has been found adequate for satisfactory growth and reproduction. The constituents of this diet are as follows: ground yellow corn, 32%; linseed oil meal, 8%; technical casein, 2.5%; ground alfalfa, 1%; sodium chloride, 0.25%; calcium carbonate, 0.25%; wheat germ, 5%; yeast, 1%; whole milk powder, 50%; and butter fat, 5%.

The animals were weaned when twenty-eight days old and placed in metal cages with raised screen bottoms to prevent coprophagy. Fresh distilled water was given daily.
The animals were weighed weekly during the first part of the depletion period and every other day during the six weeks experimental period.

**Basal Diets.** The Munsell vitamin G deficient diet (37) was used as a basal ration. It was fed *ad libitum* and records of the food intake were kept. The diet contained the following constituents: leached casein, 18%; dextrinized corn starch, 38%; ground whole white corn, 30%; butterfat, 8%; cod liver oil, 2%; and Osborne and Mendel Salt mixture (38), 4%. The caloric value of this diet was 408.6 calories per 100 grams. The Munsell diet was selected as a basal diet in preference to other vitamin G-free diets, because the white corn which was used as a source of vitamin B was less expensive than the vitamin B concentrates, and because the laboratory does not have the facilities for extensive purification.

Diet B was a modified Sherman and Spohn (51) vitamin B and G-free diet used to determine whether the casein and dextrinized starch were entirely free of vitamin G. This diet contained the following constituents: leached casein, 18%; dextrinized corn starch, 66%; butterfat, 8%; cod liver oil, 2%; Osborne and Mendel salt mixture, 4%; and agar, 2%. One hundred grams of this diet furnished 388.6 calories.

**Purification of Diets.**

**Casein:** The method used for purifying was that of
Steenbock, Sell, and Nelson (55) and has been used in other laboratories. This method consisted of placing 466.6 grams of technical casein in a cheese cloth bag, tying loosely, and immersing in 5,000 cc of tap water containing 5 cc of glacial acetic acid. The casein was worked with the hands and allowed to stand in the acidified water. This water was changed twice daily for five and one-half days. For the last five washings distilled water was used instead of tap water. At the end of this time the casein was rinsed in distilled water several times, dried for three or four days, put through a sieve, and ground.

**Dextrinized Starch:** The method used in the laboratory for dextrinizing starch was that described by Palmer and Kennedy (40) in which 800 cc of distilled acidified water (1% citric acid) were mixed with two pounds of Argo cornstarch. This was autoclaved for one and one-half hours at 15 to 18 pounds pressure, dried, and ground to a fine powder.

**Butterfat:** Fresh creamery butter was melted at a temperature below 60 degrees centigrade, decanted, and filtered through absorbent cotton in order that the salts and the water might be removed (40).

**White Corn:** The white corn used was the Champ White Pearl and was ground either in the laboratory or in the Science building.

**Strawberries:** The strawberries used in this inves-
tigation were of the Narcissa variety and were grown on the South Farm of the Oregon State College. The berries were hulled, washed, and drained, then packed in half-pint Mason jars, the lids placed on the jars, and the berries "quick-frozen" by the brine method. This process was done by the Horticultural Department. The frozen fruit was stored at a temperature of 5 degrees Fahrenheit. Eight jars, a week's supply, were placed in the freezing compartment of a General Electric Refrigerator. The temperature in the freezing compartment was 10 degrees Fahrenheit. The daily feedings were weighed while the berries were frozen and the ice particles in the bottom of the jar were discarded. One hundred grams of strawberries furnished 41 calories (5).

**Dried Yeast:** The analysis of the dried yeast as given by the company was as follows: moisture, 7.44%; protein, 48.5%; glycogen, 28%; cellulose, 6.38%; and ash, 8.09%. One hundred grams of yeast furnished approximately 300 calories (5).

**Autoclaved Yeast:** Autoclaved yeast was added to the basal ration for one group of animals to serve as a positive control. Hunt (31) obtained desired results when autoclaving for 4 hours at 15 pounds pressure. Hogan and Hunter (29) used 2 hours at 120 degrees centigrade. Williams, Waterman and Gurin (58) concluded that much of the original vitamin G survived autoclaving for 6 hours at 120 degrees centigrade if the reaction were that natural to yeast
(pH 4.5), and more if it were acid (pH 1.0 to 2.0), and further, that some of the vitamin B would survive if the reaction were acid. If the material heated were alkaline (pH 8.0 to 14.0), both of the vitamins were destroyed.

Chick and Roscoe (11) found that yeast, after exposure to moist heat at 120 degrees centigrade for 5 hours, contained 50% of the vitamin B₂ originally present, but no significant amount of vitamin B₁. In 1930 Chick and Roscoe (12) concluded that at pH 5.0 no loss in the potency of vitamin B₂ could be detected when yeast was heated for two hours at 90 to 100 degrees centigrade; about 50% of the potency was lost when yeast was heated for four to five hours at 123 degrees centigrade. When the reaction was alkaline (pH 8.3), the loss was about 50% on autoclaving for two hours at 98 to 100 degrees centigrade, and on autoclaving for four to five hours at 122 to 125 degrees centigrade the loss was from 75 to 100%. Chase and Sherman (6) autoclaved dried baker's yeast for two, four, and six hours at 15 pounds pressure. They found the results for their yeast to be practically the same regardless of the length of time autoclaved, but they emphasized that each kind of yeast must be adequately tested for freedom from vitamin B after autoclaving. Daniel and Munsell (13) autoclaved yeast for four hours at 20 pounds pressure. Elvehjem, Kline, Keenan, and Hart concluded that the accepted method for autoclaving yeast was at 15 pounds pressure and 120 degrees centigrade.
for five hours, and since vitamin B was more easily destroyed when autoclaved in moist heat than in dry heat, the presence of moisture became an important factor in autoclaving. Evans, Lepkovsky, and Murphy (19) autoclaved yeast for six hours at 18 to 20 pounds pressure. Gorcia, Peterson, and Steenbock (22) concluded that yeast autoclaved for five hours at 15 pounds pressure was free from vitamin B and adequate in vitamin G when fed at a 4% level.

From this evidence it was concluded that autoclaving yeast for six hours at 15 to 18 pounds pressure at the original hydrogen ion concentration (pH 5.96) was sufficient for destroying vitamin B and for retaining sufficient vitamin G when fed at a level of 0.5 gram daily. The final pH of the autoclaved yeast was pH 4.6.
The animals were divided into eight groups with three to six animals in each series. Two groups of negative controls were established, not only because it was desired to observe symptoms of G avitaminosis, but also to test the deficiency of the Basal diet A in vitamin G.

**Series I:** Basal diet A, modified Munsell vitamin G deficient diet.

**Series II:** Basal diet B, modified Sherman and Spohn diet.

This diet was included for comparison with basal diet A and, with the addition of yeast, to furnish a positive control.

To prove that the basal diet was adequate with the exception of a source of vitamin G, two groups of positive controls were established (Series III and IV).

**Series III:** Modified Munsell diet (Basal Diet A) plus 0.5 grams autoclaved yeast fed as a daily supplement, seven days a week. This series was included to demonstrate that the addition of this amount of autoclaved yeast daily will produce nearly normal growth, when fed as a daily supplement to the basal diet.

**Series IV:** Modified Sherman and Spohn diet (Basal diet B) plus 0.5 gram of whole dried yeast fed as a daily supplement furnished a positive control which might be compared to that of Series III.
Series V: Basal diet A, plus three grams frozen strawberries as a daily supplement, seven days a week.

Series VI: Basal diet A, plus six grams frozen strawberries as a daily supplement, seven days a week.

Series VII: Basal diet A, plus nine grams frozen strawberries as a daily supplement, seven days a week.

Series VIII: Basal diet A, plus twelve grams frozen strawberries as a daily supplement, seven days a week.
Series I. Four animals were placed in this series using the modified Munsell diet alone as a negative control. Animal 1014 was discarded because of failure to become depleted of body store of vitamin G. The average gain per week for the remaining three animals in this series was 2.0 grams, which may indicate either that the constituents of the diet were not entirely freed from vitamin G or that coprophagy may have been a factor.

There were at least three possible sources for vitamin G in the basal diet: the casein, the cornstarch, and the white corn. Goldberger and his associates (21) showed that casein may still have pellagra-preventing value after being leached in acidulated water for a week with the water changed daily. Chick and Roscoe (11) believed that they could render their casein practically free from vitamin B2 only after prolonged extraction with 50 to 70% acidified alcohol, and drying and roasting it for three days at 120 degrees centigrade. Daniel and Munsell (13) and Bourquin and Sherman (4) used casein extracted with 60% alcohol. Prunty and Roscoe (42) used a commercial casein that was especially purified; Garcia, Peterson, and Steenbock (22) used acid extracted casein.

It is shown in Series II (Table II), in which the only possible sources of vitamin G were the leached casein and the dextrinized cornstarch, that the animals lost an average of four grams per week. Munsell (37) found white corn to be not entirely lacking in vitamin G, but suffi-
ciently devoid of it to produce vitamin G deficiency symptoms after a period of time.

**Series II.** Six animals were maintained on the modified Sherman and Spohn diet for five weeks. These animals lost an average of 4 grams per week. This would indicate that the method used for the purification of the casein and the treatment of the cornstarch satisfactorily eliminated vitamin G. From these results it would appear that the white corn was the only constituent which contained small amounts of vitamin G.

**Series III.** Four animals were placed in this series. Observations of the weight charts and tables (Table III) for this series showed that the addition of 0.5 gram of autoclaved yeast to the basal diet supplied a growth factor sufficient to produce practically normal growth. Average gains per week for the six weeks experimental period were 16.3 grams.

**Series IV.** Since this basal diet, supplemented with 0.5 gram of dried yeast, had been satisfactory for good growth, this series was introduced as a check against the other positive control group in which the basal diet A and 0.5 gram autoclaved yeast were used. The weekly gain of animals in this series was 20.0 grams, which indicated that this diet, supplemented with dried yeast, adequately supplied vitamin B and vitamin G. As this diet, when supplemented with vitamin B and vitamin G, supported good
growth, it also indicated that the diet was not deficient in other nutritive factors.

**Series V.** Frozen strawberries were added to the modified Munsell diet as a source of vitamin G according to the method outlined above. Observations of the summary tables showed that the negative control group (Series I) on the unsupplemented diet made an average gain per week of 2.0 grams. When 3.0 grams of frozen strawberries were fed daily in addition to the basal diet, the average gain per week was 5.3 grams, which was an average gain per week of 3.0 grams more than the animals in Series I.

It will be noted from Table V that rat 1002 made a gain not consistent with the other animals in the group, although the food consumption was similar.

On autopsy, these animals appeared normal in all respects other than that their organs were smaller than those of animals receiving larger supplement levels. They were, however, slightly larger than those of the basal diet alone.

**Series VI.** The growth records (Table VI) for this group were of value in comparison with those of the basal diet both unsupplemented and when supplemented with 3.0 grams of frozen strawberries. An average gain of 4.5 grams more per week was made by the animals in this series than by those in Series I.

Observations of average gains per week for those
animals that received 6.0 grams of frozen strawberries daily (Series VI, Table VI) and those that received 3.0 grams frozen strawberries daily (Series V, Table V) indicated that with double the amount of supplement a gain of only 1.3 grams more per week was made by the animals on the higher supplement level. On autopsy, the organs were found to be considerably larger than those in Series I and Series V. Slightly more body fat was found in this group than in either of the other series just mentioned.

Series VII. In comparing the growth of this group with those of previous groups, an average weekly gain for this series was 9.3 grams as compared with 2.0 grams for Series I. This was an average gain of 7.3 grams more per week for the animals in this series than for those in Series I; 4.0 grams more per week than for those in Series V; and 2.8 grams more per week than for those in Series VI. On autopsy, more body fat was observed in these animals, and their organs were much larger than in those fed three and six grams of the supplement (Series V and VI).

Series VIII. The twelve gram supplement level was too high. There was an average weekly gain of 8.8 grams (Table VIII) as contrasted with 9.3 grams in Series VII (Table VII). This seemed to indicate that there was a level at which the intake of frozen strawberries was so high that the amount of basal food eaten contained an insufficient quantity of nutrients for growth, or that anoth-
er factor or factors in the vitamin G complex were involved.

Some difficulty was experienced in getting the animals to eat all the berries. This group was fed first and the basal food withheld for two to three hours, depending upon the time required to care for the other animals. By the end of the first two experimental weeks this difficulty had been overcome.
DISCUSSION

It will be noted that increasing the amount of frozen strawberries up to a certain amount evidently supplied increasing amounts of vitamin G. Since weight increase is in proportion to the amount of frozen strawberries fed, it is possible to approximate a unit of vitamin G in frozen strawberries. As a unit of vitamin G is that amount which results in a gain of 3.0 grams per week (4), it would appear that three grams of frozen strawberries contain one unit of vitamin G. However, when the strawberries were fed at the six gram and the nine gram levels, gains were obtained which indicated that one unit is contained in approximately four grams. Therefore, it is wise to conclude that three to four grams of strawberries contain one Bourquin unit.

Symptoms of vitamin G deficiency recorded by investigators have been somewhat varied. Goldberger and Lillie (20) published their observations in 1926 in which they record: arrest of growth; a tendency for the eyelids to adhere together with often an accumulation of dried secretion on the margin of the lids; a loss of fur which usually began in irregularly distributed patches, often first noticed on the side or front of the neck or in the region of the shoulders, in some cases almost complete denudation; the development of dermatitis with or without the loss of fur on various parts of the body; a
definite reddening and thickening of the ears, with a yellowish incrustation of dried serum. In a few cases an ulceration was observed at the angles of the mouth. In a somewhat larger number of cases there was observed a lesion at the tip of the tongue. Diarrhea was noted in two cases. The dermatitis appeared in about seven weeks.

Chick and Roscoe (11) described the condition of the rats on a diet deficient in vitamin B₂ in the following manner: Young rats can often be maintained for three months or longer upon a diet devoid of vitamin B₂. During this period they will show no significant increase or loss in weight. They do not, however, appear to be ill or distressed. They have fair appetites and remain active and interested in their environment. The skin symptoms, which are symmetrically placed on the animals, develop at any time after the fifth or sixth week, but do not usually become severe until several weeks later. Symptoms at this time are dermatitis and loss of hair from the eyelids, front paws stained with blood, and wetting of the lower portion of the abdomen with blood stained urine.

Roscoe (48) states that the most consistent sign of a deficiency of vitamin B₂ is a failure of growth, but in addition to this, an inflammatory dermatitis, considered by some to be analogous to human pellagra, may develop. This dermatitis, however, is irregular in occurrence. Some authors have not observed it in any of their animals,
others in only a portion. Roscoe reported that dermatitis had occurred in 108 out of 181 rats fed a vitamin B₂ deficient diet in the past seven years, and that the average time for these symptoms to appear was ten weeks. György (24) gave as the results of vitamin B₂ deficiency a cessation of growth followed later by a gradual decrease in weight.

Morgan (35) used both the twenty-eight and the fifty-six day feeding periods with results indicating that either period is acceptable, but the growth increment per week in the shorter time was greater than the corresponding values for the longer period. The rats did not die within the longer experimental period of fifty-six days, nor did the weight decline below the initial figure. Severe dermatitis with loss of fur and sometimes regrowth of hair, nosebleed, tail scale, and blindness were seen in nearly all of forty-one such animals. Severe convulsions were common among these rats after several months.

The severe symptoms recorded of G- avitaminosis by these investigators have not been noticed in the laboratory. During the six weeks experimental period the only discernible reaction of a vitamin G deficiency has been the low rate of growth. Autopsies performed at the end of the experimental period showed no definite signs of a deficiency except that all organs appeared to be undersized on the lower levels of feeding and little, if any, body fat was discernible. The fact that symptoms as found by pre-
vious investigators were not obtained may be explained by the shorter experimental period or by the low vitamin G content in the white corn in the basal diet.
I. The vitamin G value of frozen strawberries has been determined.

2. Good growth was obtained when autoclaved yeast at 0.5 gram level daily supplemented the basal diet.

3. The basal diet was not entirely deficient in vitamin G, since white corn, used as a source of vitamin B, contained traces of vitamin G.

4. Increased amounts of frozen strawberries up to a nine gram level as a daily supplement to the modified Munsell diet resulted in increases in growth.

5. Under the conditions of this investigation it appears that from three to four grams of frozen strawberries contain one Bourquin unit of vitamin G.
<table>
<thead>
<tr>
<th>Sex and Exper. Period Numbers</th>
<th>Length of Period Days</th>
<th>Initial Weight Grams</th>
<th>Weight at End of Depletion Grams</th>
<th>Final Weight Grams</th>
<th>Gain in Weight Grams</th>
<th>Food Intake Basal Diet Grams</th>
<th>Gain Per Gm. of Food Grams</th>
<th>Gain per 100 Calories Grams</th>
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</thead>
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<tr>
<td>1003 ♀</td>
<td>42</td>
<td>55</td>
<td>68</td>
<td>88</td>
<td>20</td>
<td>229</td>
<td>0.09</td>
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<tr>
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<td>54</td>
<td>70</td>
<td>81</td>
<td>11</td>
<td>237</td>
<td>0.05</td>
<td>1.14</td>
</tr>
<tr>
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<td>54</td>
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<td>76</td>
<td>7</td>
<td>215</td>
<td>0.03</td>
<td>.30</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>54</td>
<td>69</td>
<td>82</td>
<td>13</td>
<td>227</td>
<td>0.06</td>
<td>1.36</td>
</tr>
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<td>123</td>
<td>38</td>
<td>265</td>
<td>0.14</td>
<td>3.51</td>
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FIGURE I (Series I)

GROWTH OF ANIMALS ON A VITAMIN C DEFICIENT DIET
<table>
<thead>
<tr>
<th>Sex and Numbers</th>
<th>Length of Exper. Period Days</th>
<th>Initial Weight at end of Depletion Grams</th>
<th>Final Weight Grams</th>
<th>Gain in Weight Grams</th>
<th>Food Intake Basal Diet Grams</th>
<th>Gain Per Gm. of Food Grams</th>
<th>Gain per 100 Calories Grams</th>
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</thead>
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<td>63</td>
<td>70</td>
<td>-8</td>
<td>45</td>
<td>-0.18</td>
<td>-4.44</td>
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<tr>
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<td>62</td>
<td>80</td>
<td>-2</td>
<td>58</td>
<td>-0.03</td>
<td>-0.86</td>
</tr>
<tr>
<td>1038 ♀</td>
<td>15</td>
<td>61</td>
<td>72</td>
<td>-11</td>
<td>45</td>
<td>-0.24</td>
<td>-6.11</td>
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<tr>
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<tr>
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<td>82</td>
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<td>-4.36</td>
</tr>
<tr>
<td>1040 ♂</td>
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<td>67</td>
<td>72</td>
<td>-12</td>
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<td>-4.02</td>
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FIGURE II (SERIES II)

GROWTH OF ANIMALS ON VITAMINS
B AND G FREE DIET

END OF
DEPLETION
### TABLES III - SERIES III

Growth of Animals on Vitamin G Deficient Diet Plus 0.5 Grams Autoclaved Yeast Daily

<table>
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<tr>
<th>Sex and Numbers</th>
<th>Length of Exper. Period Days</th>
<th>Initial Weight Grams</th>
<th>Weight at end of Depletion Grams</th>
<th>Final Weight Grams</th>
<th>Gain in Weight Grams</th>
<th>Food Intake Basal Diet Grams</th>
<th>Gain Per Gram of Basal Diet Grams</th>
<th>Gain per 100 Calories</th>
</tr>
</thead>
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<tr>
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<td>42</td>
<td>64</td>
<td>80</td>
<td>208</td>
<td>128</td>
<td>417</td>
<td>0.31</td>
<td>7.24</td>
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<td>1007♂</td>
<td>42</td>
<td>52</td>
<td>83</td>
<td>202</td>
<td>119</td>
<td>485</td>
<td>0.25</td>
<td>5.82</td>
</tr>
<tr>
<td>1011♀</td>
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<td>58</td>
<td>75</td>
<td>163</td>
<td>88</td>
<td>379</td>
<td>0.23</td>
<td>5.47</td>
</tr>
<tr>
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<td>42</td>
<td>49</td>
<td>71</td>
<td>131</td>
<td>60</td>
<td>368</td>
<td>0.16</td>
<td>3.83</td>
</tr>
<tr>
<td>Average</td>
<td>56</td>
<td>77</td>
<td>176</td>
<td>98</td>
<td>412</td>
<td>0.25</td>
<td>5.59</td>
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GROWTH OF ANIMALS ON VITAMIN C DEFICIENT DIET

PLUS 0.5 GRAMS AUTOCLAVED YEAST DAILY

END OF
DEFICIENCY

GRAMS

DAYS

10
10
<table>
<thead>
<tr>
<th>Sex</th>
<th>Length of Exper. Period</th>
<th>Initial Weight Grams</th>
<th>Weight at end of Depletion Grams</th>
<th>Final Weight Grams</th>
<th>Gain in Weight Grams</th>
<th>Food Intake Basal Diet Grams</th>
<th>Gain Per Gm. of Food Grams</th>
<th>Gain per 100 Calories Grams</th>
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</thead>
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<tr>
<td>1005♂</td>
<td>42</td>
<td>58</td>
<td>74</td>
<td>217</td>
<td>143</td>
<td>477</td>
<td>0.30</td>
<td>7.26</td>
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<tr>
<td>1008♂</td>
<td>42</td>
<td>58</td>
<td>73</td>
<td>211</td>
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<td>490</td>
<td>0.28</td>
<td>6.83</td>
</tr>
<tr>
<td>1024 ♂</td>
<td>42</td>
<td>58</td>
<td>80</td>
<td>163</td>
<td>83</td>
<td>422</td>
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<td>76</td>
<td>197</td>
<td>121</td>
<td>463</td>
<td>0.26</td>
<td>6.28</td>
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</table>
FIGURE IV (SERIES IV)

GROWTH OF RATS ON VITAMINS B AND C
FREE DIET
PLUS 0.5 GRAMS DRIED YEAST DAILY

END OF DEPLETION

GRAND
AUG.
DEC.
<table>
<thead>
<tr>
<th>Sex and Numbers</th>
<th>Length of Exper. Period (Days)</th>
<th>Initial Weight (Grams)</th>
<th>Weight at end of Depletion (Grams)</th>
<th>Final Weight (Grams)</th>
<th>Gain in Weight (Grams)</th>
<th>Food Intake (Basal Diet Grams)</th>
<th>Gain Per Gram of Basal Diet</th>
<th>Gain per 100 Calories</th>
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<tbody>
<tr>
<td>1002 ♀</td>
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<td>58</td>
<td>73</td>
<td>83</td>
<td>10</td>
<td>219</td>
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<td>1.06</td>
</tr>
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<td>1010 ♂</td>
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<td>63</td>
<td>82</td>
<td>124</td>
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<td>279</td>
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<td>3.19</td>
</tr>
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<td>56</td>
<td>69</td>
<td>92</td>
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<td>223</td>
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<td>2.39</td>
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<td>42</td>
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<td>106</td>
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<td>112</td>
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<td>75</td>
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<td>261</td>
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</table>
FIGURE V (SERIES V)

GROWTH OF ANIMALS RECEIVING VITAMIN G DEFICIENT DIET

PLUS 3 GRAMS FROZEN STRAWBERRIES DAILY

END OF DEPLETION

GRAMS

DAYS
### TABLE VI - SERIES VI

Growth of Animals Receiving Vitamin G Deficient Diet plus 6 Grams of Frozen Strawberries Daily

<table>
<thead>
<tr>
<th>Sex and Number</th>
<th>Length of Exper. Period Days</th>
<th>Initial Weight Grams</th>
<th>Weight at End of Depletion Grams</th>
<th>Final Weight Grams</th>
<th>Gain in Weight Grams</th>
<th>Food Intake Basal Diet Grams</th>
<th>Gain Per Gram of Basal Diet Grams</th>
<th>Gain Per 100 Calories Grams</th>
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<td>59</td>
<td>83</td>
<td>122</td>
<td>39</td>
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<td>3.56</td>
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<td>42</td>
<td>53</td>
<td>76</td>
<td>112</td>
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<td>274</td>
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<tr>
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<td>67</td>
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<td>3.66</td>
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<td>Final Weight Grams</td>
<td>Gain in Weight Grams</td>
<td>Food Intake Grams</td>
<td>Gain per Gram of Basal Diet Grams</td>
<td>Gain per 100 Calories Grams</td>
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<td>56</td>
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<td>0.17</td>
<td>3.65</td>
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</table>
FIGURE VII (SEE FIG. VII)

GROWTH OF ANIMALS RECEIVING VITAMIN C-DEFICIENT DIET

PLUS 9 GRAMS
FROZEN STRAWBERRIES DAILY

END OF DEPLETION

GRAMS

DAYS 5, 7, 10

10
<table>
<thead>
<tr>
<th>Sex and Number</th>
<th>Length of Exper. Period Days</th>
<th>Initial Weight Grams</th>
<th>Weight at end of Depletion Grams</th>
<th>Final Weight Grams</th>
<th>Gain in Weight Grams</th>
<th>Food Intake Basal Diet Grams</th>
<th>Gain Per Gram of Basal Diet Grams</th>
<th>Gain per 100 Calories Grams</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
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<td>3.28</td>
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<tr>
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<td>142</td>
<td>53</td>
<td>320</td>
<td>0.17</td>
<td>3.47</td>
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</tbody>
</table>
GROWTH OF ANIMALS RECEIVING VITAMIN DEFICIENT DIET

PLUS 12 GRAMS FROZEN STRAWBERRIES DAILY

END OF DEPLETION

GENHS

DAY 7

DAY 10
# TABLE IX - SUMMARY

The Effect of Feeding Graded Amounts of Frozen Strawberries on the Growth of Vitamin G Deficient Animals

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<tr>
<td>III.</td>
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<td>77</td>
<td>176</td>
<td>98</td>
<td>412</td>
<td>0.25</td>
<td>5.59</td>
</tr>
<tr>
<td>V.</td>
<td>6</td>
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<td>58</td>
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<td>106</td>
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</tr>
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<td>6 gms. Frozen Straw.</td>
<td>61</td>
<td>79</td>
<td>118</td>
<td>39</td>
<td>270</td>
<td>0.15</td>
<td>3.24</td>
</tr>
<tr>
<td>VII.</td>
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<td>9 gms. Frozen Straw.</td>
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<td>86</td>
<td>141</td>
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<td>3.65</td>
</tr>
<tr>
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<td>6</td>
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<td>12 gms. Frozen Straw.</td>
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<td>142</td>
<td>53</td>
<td>320</td>
<td>0.17</td>
<td>3.47</td>
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</table>
### TABLE X - SUMMARY

The effect of 0.5 Gram Dried Yeast on the Growth of Vitamins B and G-Free Animals

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BIBLIOGRAPHY


