THE VITAMIN C CONTENT OF BLOOMSDALE SPINACH
AS AFFECTED BY
HOME CANNING AND HOME COOKING

by

Dorothy Mae Johnson, B.S.

SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF ARTS

in the

GRADUATE SCHOOL

of the

UNIVERSITY OF MISSOURI

1930

Margaret C. Hendler
May 3, 1930

Table of Contents

Introduction	1
Historical	3
Human and Experimental Scurvy	11
Vitamin C	
Quantitative Determination	16
Properties	17
Occurrence and Requirement	26
Basal Diet	32
Antiscorbutic Food	34
Chemical Analysis of Foods	37
Experimental Animals	39
Experimental Procedure	40
Experimental Results and Discussion	42
Summary	47
Introduction to Appendix	48
Figures	49
Charts	58
Tables	61
Bibliography	67

Acknowledgment

The writer wishes to express her appreciation to her adviser, Dr. Margaret C. Hessler, for the selection and supervision of this problem.

Introduction

The subject of vitamins has assumed in the past years great prominence. The role which they play in nutrition is to-day generally recognized. Much of scientific and practical value has been learned regarding them although their chemical nature is still unknown.

Research workers have identified the deficiency disease with absence of the vitamins so clearly that our conception of them is intimately associated with that of the deficiency disease. Even a greater interest, however, may be attached to them as chemical substances occurring in food materials and having important functions in the normal process of nutrition.

"Seek the cure of scurvy neither in the armamentarium of the physician nor in the apothecary shops. The druggist will be of as little aid to you as the surgeon. On the other hand employ fresh vegetables, the juices of fresh antiscorbutic plants, oranges and lemons; in this way without other means you will be able to overcome this terrible disease", a physician declared over two hundred years ago. Since then we have advanced in our knowledge of the antiscorbutic vitamin in regard to its nutritional relationship, significance to health and distribution among foodstuffs.

Results of various investigations would indicate that raw spinach can be classified as a food exceptionally rich in its vitamin C content although the exact amount needed for protection against scurvy has not been agreed upon. Previous work in this laboratory has indicated that the spinach which has been grown in the middle, western, or southern states was not as rich in vitamin C as that grown in the east as reported by Eddy. A number of determinations must be made however before this can be considered conclusive. Since to what extent this difference can be accounted for by changes in variety, soil, and climate has not been determined, it would seem most desirable if a known variety of spinach was used throughout the experiment.

Since the value of a foodstuff as a vitamin source is not determined solely on the raw product but must be judged by a consideration of the protective amount in foods as consumed, it becomes necessary to test the vitamin content of foods in the forms in which they are eaten. For this reason a comparison of raw, home cooked, and home canned spinach was chosen for this study. The purpose of this investigation is to determine the loss of the antiscorbutic factor in raw spinach after exposure to heat at two temperatures and for two periods of time.

Historical

Centuries ago historians noted the ravages of an unknown disorder in people separated from adequate supplies of fresh foods. In the armies of the Crusaders of the thirteenth century, scurvy was very prevalent. The reports of the long sea voyages of the fifteenth and sixteenth centuries are filled with accounts of this life-taking disease. Scurvy became known as the calamity of the sailor because of its frequency on ship board. No country has ever been free from it and as might be expected it has been particularly prevalent in the northern countries where vegetation is scarce although the tropics have also suffered when crops have failed. The early American colonists were afflicted to such an extent that attempts at colonization had to be abandoned. In 1536 Jacques Cartier on a voyage to Newfoundland, after having lost most of his crew as victims from scurvy, was able to save the remainder by an infusion of pine needles (1a) (2a) (3a).

A high antiscorbutic power was for generations attributed to Kaffir beer which was in use on sea voyages as early as 1549 (4). Its value was believed to be due not only to the ingredients from which it was brewed but to the fermentation itself but with the elaboration and

perfection of the brewing process the antiscorbutic value was practically, if not always, eliminated. It was however among the antiscorbutics in requisition in the British navy until the introduction of lemon juice in 1794. With our present knowledge of the instability of vitamin C we can clearly understand that beer would possess antiscorbutic value only when freshly brewed and immediately consumed.

Kramer (5a), in 1720, wrote that neither medicine nor surgery could give relief in scurvy but "if you can eat green vegetables; if you can prepare a quantity of fresh antiscorbutic juices and give in quantities of three to four ounces you will without other assistance cure this dreadful disease".

In 1747 James Lind (6a) reports a specific cure for scurvy by the use of orange and lemon juices and recommended that concentrated lemon juice be carried by soldiers and sailors. He recognized at this time the futility of all medical treatment in vogue. He confirmed Kramer's report that scurvy can not be prevented by dried vegetables. Again in 1757, he states that although lemon juice retains its antiscorbutic property sufficiently well to make it useful for long voyages there is no hope of preventing scurvy by means of dried spinach since it has lost during its preparation something contained in the natural juices of the plant which no moisture could

replace.

Scurvy outbreaks continued until in 1772 Captain Cook undertook a three year voyage which resulted in no deaths from scurvy (3a). Its absence he attributed to sweetwort, an infusion of barley, which was prepared fresh and served in large quantities.

The British Navy in 1795 began a regular administration of lime juice to sailors. Its introduction reduced the mortality from scurvy seventy-five per cent.

The early view point regarding scurvy was revealed by Hood (7) in 1880 when he wrote, "it is a very curious and interesting circumstance that this condition which at various times has been regarded as a plague, as a mysterious infliction of Providence against which it would be useless or even impious to strive, or as a disease inseparable from long voyages should have been proved most conclusively to arise from causes which are strictly under our control and to be curable by means which are afforded by every habitable country. Scurvy occurs only when fresh vegetable food has been for some time partially or completely withheld".

Throughout history in connection with every war, we find evidences of scurvy epidemics. It has continued to be a menace to armies, appearing during the world war in practically every fighting troop. A lack of knowledge of the dietary properties of food linked with food depriva-

tion, the dominant factors in the development of scurvy seem to be the cause (2a).

Scurvy in animals was first reported by Theobald Smith (8). The diet of oats and bran produced in guinea pigs a subcutaneous extravasation of blood which he recognized as the same disease as human scurvy. Eijkman in 1897 was the first investigator, however, to focus attention on the dietary by producing positive rather than negative experimental evidence (9a). Many theories for the cause of scurvy have been suggested but have been discarded when found inadequate. Coplans and co-workers in 1904 declared that the condition known as scurvy in adults is not brought about by an absence of any particular kind of food from the diet but is more probably "a specific infection of bacterial origin". Foods, he states, may be the cause of the infection under conditions of dirty storage or preparation. Unsanitary habits or unwholesomeness of occupation are activators for this disease (10). The potassium deficiency and citric acid theories both were accepted for a short time but were soon rejected when scurvy could not be prevented or cured by their use (3b). Again Wright, in 1908, proposed the acid intoxication theory. To prevent the disease he claimed that the diet should supply sufficient alkaline radicals to yield a basic reaction but uncooked succulent foods were not requisites (11). That ptomaine poisoning or an acid

intoxication is not the cause of scurvy was strongly argued by Hutchinson (12) who speaks of the acid intoxication of diabetes with no development of scurvy. The cause of infantile scurvy he believed was the over sterilization of food.

Hopkins (13) was the first to clearly recognize the need of accessory food factors in nutrition. In 1906 he states, "no animal can live upon a mixture of pure protein, fat, and carbohydrate and even when the necessary inorganic material is carefully supplied the animal still cannot flourish".

From 1907-1912 Holst and Frölich (14) extensively studied experimental scurvy in guinea pigs and began the first qualitative determinations of antiscorbutic values of foods. Their results showed the disease to be due to a deficiency in the diet of a substance soluble in water and easily destroyed by heating, also more stable in acid than in neutral or alkaline mediums. Furst working in the same laboratory with mixtures of seeds concludes that "there is no advantage in numerous foods when none contain the needed substances". He reports the development of the antiscorbutic vitamin in the process of seed germination. The occurrence of infantile scurvy, according to Frölich["] is probably due to the use of proprietary foods and well sterilized milk.

The word vitamine was coined by Funk (5b) in 1911

when working with vitamin B to represent the substances referred to in 1906 by Hopkins. An analysis showed the presence of nitrogen in basic form and since their presence meant life and absence death the group of substances was called vitamines. In 1916 McCollum and Kennedy (15) suggested the term "unidentified dietary factors" and later added the phrase water-soluble B and fat-soluble A which was at that time the only basis of differentiation. Drummond in 1920 (16) suggested that the designations of Funk and McCollum be combined and that the final "e" be dropped so that the resulting word vitamin would carry no implication as to its chemical nature and that the antecedent statement of solubility be dropped for convenience. The water soluble vitamin C now becomes vitamin C.

Jackson with Moore and Moody report in 1916 the development of scurvy on diets of pasteurized, raw, boiled, skimmed, and condensed milk. The addition of calcium lactate to milk or its injection into guinea pigs on a milk diet did not prevent scurvy. An olive oil injection rendered no help to the scorbutic pig. A diplococcus of low virulence injected into the circulation of a guinea pig on normal diet gave rise in most cases to hemorrhages and other lesions in the bones, joints, muscles, gums and lymph glands. That the separation of the diplococcus from the tissues of a scorbutic pig after death was

possible led to the conclusion that this disease was due to bacterial infection (17) (18).

In 1917 McCollum and Pitz (19) published their conclusions that scurvy development was due to retention of feces and consequent absorption of toxins. The anti-scorbutic activity of orange juice they ascribed to the retardation of the development of bacteria by the action of the citric acid in the juice and to its content of sodium and potassium citrates which possess laxative properties. They also report the beneficial results obtained from an artificial orange juice consisting of citric acid, cane sugar, and inorganic salts in about the proportion in which these occur in the edible portion of the orange.

Hess in 1917 (20) ascribed the cause of infantile scurvy to the bacteriological condition of milk but soon abandoned this theory to accept the view that scurvy is a deficiency disease.

Doubtfulness as to the presence of a third vitamin was increased due to the relative immunity of the rat to scurvy. For a time it was thought that perfect health could be attained on a scorbutic diet but Harden and Zilva in 1918 (2) followed by Drummond in 1919 (22) have produced experimental evidence that the antiscorbutic factor plays a beneficial role in the nutrition of the rat.

From 1918-1920 Chick, Hume and Shelton, Harden and Zilva, Torrey and Hess, Cohen and Mendel and others have given us conclusive proof that scurvy is a deficiency disease prevented and cured by the administration of antiscorbutics or vitamin C containing food materials. Positive evidence of the existence of an antiscorbutic vitamin was demonstrated by Hess and Unger by the curative effects produced by an intravenous action of orange juice (23). Harden and Zilva however in 1918 report that subcutaneous injections of decitrated lemon juice failed to arrest the progress of well-pronounced scurvy in guinea pigs (24). These authors report that artificial orange juice did not show the slightest antiscorbutic potency (25). In the last ten years workers have responded to the need for systematic study of the occurrence of the antiscorbutic vitamin and its stability under different methods of treatment.

We should bear in mind the statement of Hess (3e), "the world is leading a hand to mouth existence in regard to its quota of antiscorbutic foods", wisely choose them and after their selection prepare them in such a way as to conserve to the greatest possible extent the antiscorbutic vitamin - the most unstable of the group.

Human and Experimental Scurvy

Scurvy is recognized as a nutritional disease developing in infants, adults, and in certain experimental animals following the absence from the diet of the same class of foods (3d). Experimental scurvy can be accepted as human scurvy from its close analogy in etiology, symptoms, methods of cure, and post-mortem appearance following death (26). During the past twenty-five years, the main interest in this disorder has been in infantile scurvy (27).

Even with a decidedly deficient diet a considerable amount of time is necessary before the disease becomes clinically manifest. The length of time required for its development is the outstanding distinction between scurvy in man and in the experimental animal. Human scurvy usually requires from six to nine months to develop; the period varying mainly in proportion to the diet deficiency (28). Symptoms can be noticed in the guinea pig from the twelfth to the fifteenth day after it is placed on the restricted diet and from the twenty-eighth to the thirty-fourth day the animal succumbs to the disease (6b).

In 1895 Theobald Smith (8) reported the development of experimental scurvy in animals. "When guinea pigs are fed cereals without grass, clover, or succulent vegetables

such as cabbage, a peculiar disease chiefly recognizable by subcutaneous extravasations of blood carries them off in from four to eight weeks".

The guinea pig has been universally chosen as the experimental animal because of convenience, its susceptibility to the disease, and relative inexpensiveness. When the animal has been placed on the restricted diet, the initial weight will be maintained but seldom exceeded for a period of fifteen to eighteen days. It then gradually falls until at death the loss is twenty-five to thirty per cent of the original weight. The scorbutic pig is inactive and disinterested in the food. The eyes lose their luster, the ears droop, and a general roughening of the coat takes place (29). The first scurvy symptoms are soreness of joints and limbs especially of the shoulders and knees so that the animal will squeal when pressure is applied to these parts (26). Lameness can then be noted. The animal may adopt the scurvy position by resting on its side with the painful limb in the air which seems to indicate hemorrhage and consequent discomfort. The face-ache position is also indicative of scurvy. The animal may press the side of its head against the floor of the cage indicating hemorrhage of the fragile jaw with soreness and looseness of teeth. The post mortem appearance consist mainly of hemorrhages and bone lesions. The hemorrhages may occur in any part of the body with a

marked degree of severity and are especially found over the ribs and among the muscles of the upper and lower limbs. Hemorrhages in the viscera are frequent in which cases blood is passed with the feces. The costochondral junctions are enlarged and surrounded with hemorrhages. Beading of the ribs similar to the rachitic rosary is associated with the disease. Changes in the bone tissue are characteristic. Joints become swollen and upon dissection the tibial epiphyses are often found disorganized and broken across in which case the hind legs become helpless. The jaw is easily fractured and the teeth become brittle and loose. (3e).

The macroscopic changes of the alimentary tract are emptiness of the entire canal and areas of congestion in the stomach and intestines. A generalized edema may also be present. No typical changes are noted in the respiratory tract other than congestion and subserous hemorrhages.

McCarrison (30) noted hemorrhages in the adrenals thus greatly enlarging them which has also been confirmed by La Mer and Campbell (31). An enlargement of the heart and kidney has also been noted.

That the cellular structure of the blood vessels is altered during scurvy has been shown by Hess by the results of his capillary resistance test (3f). Eddy associates failure to properly utilize lime with an absence of the antiscorbutic vitamin (32a). Hess (3g) reports a

positive balance of calcium during the active stages of the disease. That a negative nitrogen balance is found in starvation and is not a direct scorbutic result has been reported. Shipp and Zilva in 1928 (33) declared that there were no indications of a disturbed absorption or retention of nitrogen during the disease.

Zilva and Wells (34) and Howe (35) have noted the injury to developing teeth produced by this disease and conclude that possibly the deficient dietary is the important factor in the great prevalence of tooth decay. Howe describes the injury as resembling pyorrhea. Since the mildest degree of scurvy, which could just be discovered at post mortem examination, produces well defined changes in the tooth structure, Höjer (36) has developed a method for determining the antiscorbutic value of food stuffs by means of a histological examination of the teeth.

In human scurvy such extreme scorbutic changes as has been noted in the experimental animal seldom occur since the diet is rarely entirely free from antiscorbutic food. The symptoms are identical however with but one exception. One of the first manifestations of the disease in man is the extreme hemorrhagic condition of the swollen, spongy gums which is seldom seen in the experimental animal.

Scurvy may develop, especially in the child, in its latent or sub-acute form and is associated with loss of

weight, irritability, secondary anemia, decline in resistance to infection, rheumatism, retarded growth, and slow healing of wounds (37).

Vitamin C

Quantitative Measurement

The amount of any foodstuff which when fed as a daily allowance just suffices to afford complete protection from scurvy to a standard guinea pig has been defined as containing the unit of vitamin C (9b). To translate guinea pig results, as Eddy explains (32b), into human values it is only necessary to know the protective dose of one food for a human and a guinea pig. All guinea pig tests can then be assumed as relative. Since the protective dose of orange juice has been determined for a guinea pig and an infant to be 1.5 cc. and 15 cc. respectively, it is obvious that the human requirement is ten times greater than that of a guinea pig. These results have been obtained by the vitamin C determination as described by Sherman, La Mer, and Campbell (38).

Recently Vitamin C determinations have been conducted according to Höjer's method (36) by means of a histological examination of the teeth. The results show this method to be more sensitive than the Sherman scoring system and would indicate that twice as much antiscorbutic is necessary for tooth protection as for the prevention of external symptoms of scurvy (39).

If continued research confirms this difference we can easily convert the Sherman values into tooth protective values. The requirements of the antiscorbutic vitamin will then be definitely raised for obviously the higher level will become the standard.

Properties

Little is known of the chemical and physical nature of vitamin C. Eddy (32c) in 1921 says, "concerning the "C" we know only that it is like "B", water soluble and we know somewhat of its properties but nothing of its chemical nature. "The vitamins are as yet a complete mystery to chemists", reports Kohman (40) in 1927, "their chemistry is not known and they have not been isolated".

The antiscorbutic vitamin can be distinguished from the group as being the most unstable, the least resistant to chemical and physical processes and therefore the most sensitive of the vitamins.

Drummond, McCollum and Pitz recognized its solubility when they termed it water-soluble C. Hess and Unger (41) have found it soluble in 95 per cent alcohol. Hart, Steenbock and Lepkovsky (42) have reported it soluble in 80, 90, and 100 per cent ethyl alcohol.

That this vitamin can pass, without appreciable loss, through dialyzing parchment was reported by Holst

and Frölich (9c). Zilva and Harden in 1918 (43) demonstrated that orange juice can be filtered through a Berkefeld candle without losing its activity. In a mixture of orange juice and yeast, they found it possible by means of fuller's earth to remove the antineuritic substance leaving the unchanged antiscorbutic factor.

Hess (3h) and Zilva (44) working with orange and lemon juices respectively have shown that exposure to ultra-violet light for six to eight hours does not destroy or increase the antiscorbutic potency of these juices. La Mer in 1919 (45) reported that the vitamin does not appear to be sensitive to ordinary light.

Zilva has concentrated that antiscorbutic vitamin to such an extent that a daily dosage of 0.00045 grams to a guinea pig weighing three hundred grams is protective against scurvy (32d). Partial isolation of the vitamin from lemon juice is reported in the work of Sipple and King (46). It would seem from their results that the active substance probably contains glucose, free nitrogen, an acid, and ammonium salts. The purification has resulted in a reduction of total solids from one hundred grams in the original juice to twenty-eight hundredths milligram in the final active substance.

In 1924 Connell and Zilva (47) reported that the membrane through which the antiscorbutic factor diffuses would indicate the size of the active molecule to be

"not far removed from that of a hexose". Delf in her comparison of the temperature coefficient of the heat destruction of the vitamin with toxins and ferments concluded that the antiscorbutic factor must possess a simple chemical structure.

"Scientific investigation has shown the necessity of knowing the condition under which a food has been secured, the process to which it has been subjected during preparation as well as its original store of vitamin to determine its practical value as an antiscorbutic (1b)".

That vitamin C sensitiveness is regulated by both time and temperature has been clearly shown. Hess and Fish (48) report a greater destruction resulting from heating at pasteurization temperatures for some time than boiling for a few minutes. Delf (49) reports that the effect of heat is increased only to a slight degree with a temperature increase. La Mer, Campbell and Sherman (50) report "that for short periods of time (one hour or less) the extent of influence of heating depends more upon the length of time that it does upon the intensity of the process while for longer periods (four hours or longer) the effect of intensity assumes greater prominence in comparison to the time than it did in shorter periods". Their results with tomato juice show increased destruction by raising the temperature

from 140° F. - 212° F. and that the destruction occurred more rapidly during the first hour than in the subsequent three hours heating. Heat destruction is possibly of the nature of an intramolecular oxidation and reduction such as occurs when aldehydes or sugars are heated. The velocity of the heat destruction decreased more rapidly than it would if the reaction followed either the unimolecular reaction law or the square root of Schutz. To prove whether or not the destructive process is of an oxidative nature and that the sole effect of heat is to increase the rate of reaction, tomato juice at hydrogen ion concentrations of $10^{-4.3}$ and $10^{-8.3}$ was heated while oxygen was bubbled through at 100° C. for an hour. Complete destruction was noted in both alkaline and acid preparations in which oxygen had been used. Partial loss of the vitamin occurred while the juice was heated in the absence of oxygen. Besides heat, therefore, oxidation apparently also plays a role in the inactivation of vitamin C. Zilva in 1924 (51) reports that oxidizing agents, such as potassium permanganate and hydrogen peroxide, destroy the vitamin at room temperature. The destruction of vitamin C by oxidation is an irreversible process. Reducing agents do not restore its activity (32d). Hess (52) and Eddy (32e) report that catalysts, such as copper, increase the heat destruction of the vitamin.

That the vitamin C value of foods may be protected

by measures that instead of eliminating the oxygen removes the factors which permit it to act on the vitamin has been suggested. When laccase, the oxidizing enzyme in potatoes, has been inactivated by dilute acids vitamin C is not destroyed (32e).

That foods during the process of canning can be subjected to higher temperatures for longer periods of heating with a much smaller loss of the vitamin than that which takes place during the open kettle cooking period indicates the role played by oxidation in the destruction of the antiscorbutic vitamin. In the work by Eddy and associates (53) all tests with canned foods have shown this to be true. Twenty grams of cabbage when cooked in a pressure cooker or in open kettle is necessary to protect a guinea pig from scurvy where-as four grams of canned and one gram of raw is sufficient. The retention of the vitamin C content of bananas and potatoes when baked in their skins is possibly due to the exclusion of air. When apples are baked, apple sauce made, and when apples are canned without special treatment the vitamin is practically all lost. Kohman, Eddy and Carlsson (54) report the canning of apples with no apparent loss of the vitamin when the apples are immersed in salt solution long enough before canning to allow the respiratory process to use up all the cellular oxygen. Apples lose their antiscorbutic value when stored while no deterioration is

evident when the fruit is canned after special treatment and then stored for eight months. Canned food as emptied from the container can be reheated without apparent loss of the antiscorbutic vitamin due in all probability to its small oxygen content.

The antiscorbutic vitamin is greatly protected from the destructive effect of heat when it is associated with an acid. The resistance displayed by fruit juices is indicative of this retention. Zilva (55) has shown that decitrated lemon juice made N/20 alkaline (pH 12.5) and kept under anaerobic conditions for twenty-four hours did not deteriorate to a great extent while the same juice aerated for the same period of time failed to delay the scurvy symptoms in a guinea pig even when the dosage was increased five or six times the original. With increased hydrogen ion concentration, the stability of the lemon juice was more assured.

From experiments based on evidence that vitamin C is most stable in an acid medium workers have demonstrated that the addition of either alkali or acid to the vegetables when cooking is usually undesirable from a vitamin standpoint. Delf (49) has shown that the addition of 0.5% citric acid to the cooking water of lentils produced if anything a greater destruction of vitamin C. The addition of vinegar to carrots while being cooked produced no effect. Williams (56), working in this

laboratory in 1927, reported an appreciable protection of the vitamin by the addition of citric acid to apples before the open kettle cooking period. No change in the vitamin content of rhubarb was produced when acid was added.

"When the natural acidity of tomato juice ($C_H/10^{-4.3}$) is less than half neutralized, the juice boiled an hour, and then immediately cooled and re-acidified, 58% of the vitamin is lost. Upon greater neutralization $C_H/10^{-10.9}$ to $10^{-8.3}$) the loss rose to 61-65% while with omission of the re-acidification and the juice stored for five days in a refrigerator the destruction amounted to 90-95% (50)". Hess and Unger (57) point to the use of alkali in the preparation of proprietary foods for infants as possibly one reason for the loss of the antiscorbutic vitamin in these foods.

Drying and aging result in considerable loss of vitamin C but these losses are probably determined by the factors time, temperature, hydrogen ion concentration, and exposure to oxidation (9d). In studying the effect of drying on the retention of the vitamin, there are two additional destructive factors to be considered - storage and cooking before eating. A forty-eight hour aging of pasteurized milk reduces the potency of the vitamin C factor. Babies fed on commercial and home pasteurized milk have shown the supremacy of the home treated liquid.

The difference is accounted for in the aging and handling of commercial milk (31).

Anderson in 1929 (58) found the unit of vitamin C in raw Jonathan apples fed from September to December was 25 grams. Forty grams of the stored apples protected the animals from scurvy during the months from November to February and was considered as the unit.

That the destruction of vitamin C proceeds rapidly upon standing in an alkaline or neutral medium and also but more slowly in acid has been reported by Zilva and Harden (55). Aging has the least effect when the vitamin food has been dried. Hess and Fish (48) have found orange peel even after a three months storage to be an active antiscorbutic while storage of the juice resulted in vitamin C loss. When fresh fruit is subjected to a sulfur dioxide treatment before drying, the vitamin is conserved. The addition of an acid to potatoes before drying had no effect on the loss of vitamin C (59). A loss of 93% of the vitamin took place in cabbage leaves dried at room temperature and stored for five to six weeks. The residual amount seemed greater if the product was placed in boiling water before drying (60).

That vitamin C is produced by the plant in process of growth seems quite well proven (61). It is not present in mature seeds but develops as soon as germination takes place. Barley, oats, peas and lentils soaked in

water for twenty-four hours and allowed to germinate will show in two or three days distinct antiscorbutic properties. The vitamin C value of germinated seeds is five or six times that of dried seeds. While germinated peas are inferior to lemon or orange juice they are equal to vegetables such as green beans and potatoes and superior to carrots in their antiscorbutic value (62).

The degree of maturity of vegetables apparently affects the vitamin C content. Young peas have been found superior to the more mature ones. Hess and Unger report old carrots much inferior to young and to the freshly harvested vegetable (63). Tomatoes ripened to the most desirable degree for use are superior to the green food. When the tomatoes are allowed to ripen on the plant the maximum value is obtained (40), (70).

In the cooking of vitamin C, we cannot assume any fixed distribution between the vegetable and its cooking water. Delf (49) reports a greater proportion from the liquid from germinated cowpeas than from the same weight of the vegetable. Hess has reported that the vitamin C value of raw carrots cannot be found in the combined use of the cooked vegetable and water in which it was cooked.

Vitamin C since it is not stored in the body necessitates daily consumption. An administration of very concentrated doses of lemon juice before placing the experimental animal on a scorbutic diet fails to delay the

onset of scurvy report Harden and Zilva (64). Hess (3c) working with other vitamin food has confirmed their statement.

Eddy (65) writes, "the sensitiveness of vitamin C makes it necessary to scrutinize cooking methods very carefully in order not to ruin a good source by a poor method of preparation for the table". "The amount of vitamin C which the food contains at the beginning; its hydrogen ion concentration; the time and temperature of heating to which it is subjected; the extent to which and temperature at which, it is exposed to air; the presence and concentration of oxidizing substances or of substances which may catalyze the oxidative or other destruction of the vitamin; all of these are factors which may influence the antiscorbutic value of a preserved food and there may be other factors not yet recognized", as written by Sherman (9d) well summarizes what has been found to influence the antiscorbutic vitamin.

Occurrence and Requirement

"As the capacity of the body to store vitamin C is very limited and as this vitamin under certain conditions is very easily destroyed by heat and oxidation, the diet for all ages but particularly for children should include

an abundance of vitamin C containing foods (66)".

Sherman writes (9e), "it has been estimated that the daily amount of vitamin C that a man must have to protect him from scurvy is about the amount contained in one ounce of orange or lemon juice or canned tomato, or raw cabbage or onion, or in about one pound of cooked cabbage or potato, or in one pint of milk. But it is now becoming plain that the amount of vitamin C which we must have to protect us from scurvy is only a fraction of the amount which is really needed for full health and vigor; and instead of reliance upon any one of the items just mentioned as possible minimum sources of vitamin C, the equivalent of several of them should be supplied in each day's food".

Fresh fruits and vegetables have long been considered valuable as antiscorbutic foods. A great deal of investigation has been done upon lemon, orange, and tomato juices because they contain the antiscorbutic factor in the highest concentration - 1.5 to 2.0 c.c. daily will protect a guinea pig from scurvy. Subcutaneous injections of neutralized orange juice report Hess (23) and Spencer (67) give rapid relief in infantile scurvy. Orange and lemon juices may be dried with little loss of their antiscorbutic power (3j). Orange and sugar boiled to a syrup retains its antiscorbutic potency after six months storage (68).

Bassett and Smith in 1920 (69) prepared a concentrated lemon juice in the form of lozenges. It has been reported that one-fifth of a tablet prevented and cured scurvy in a guinea pig and is estimated that one tablet daily would be protective for a human. The lozenges have been found to retain their antiscorbutic properties for over a year.

Hess in 1920 (3k) states that in spite of the view held by some that tomatoes should not be given to very young children, infants a few weeks or a few months old are just as able to consume a small quantity of tomato juice as orange or lemon juice. Although canned foods are recognized as having smaller vitamin C content than the raw food, canned tomatoes are just as efficient as the raw fruit. Ripe tomatoes are richer in vitamin C than green tomatoes even when ripened in the dark after removal from the vines. Green house tomatoes are as rich in C as the same variety grown out of doors (70).

Fresh vegetables are a good source of vitamin C although they cannot, from a vitamin standpoint, be considered as a standard and uniform foodstuff since they vary in its content according to their freshness and age (63).

Sherman writes (6c), "in how far differences in the content of vitamin C are to be regarded as fixed for the species and varieties concerned and in how far they may have been due to differences in soil, climate, season,

handling or storage, it would at present be exceedingly difficult and probably impossible to decide". "When investigating the vitamin C content of a food we are not dealing with a constant and unvarying agent", reports Hess (31).

"In general", Sherman writes (9f), "the studies of the distribution of vitamin C in plant materials show it to occur most abundantly in the actively functioning and the succulent parts, in the fresh green leaves, the growing shoots, and the juicy stems, roots, tubers, bulbs and fruits". Vitamin C is produced in process of growth. It is not present in dry mature seeds, such as cereal grains or legumes, but develops as soon as they begin to sprout. Dehydrated fruits and vegetables have been found to lose their antiscorbutic properties. Potatoes are rich in vitamin C and especially are valuable as an antiscorbutic food because of the quantity consumed. Although meat has been found to be inferior in its antiscorbutic content, Stefansson living on an exclusive meat diet for twelve months was free from scorbutic symptoms (71). "Blood, the purveyor of vitamins to the tissues, might be thought to be vitamin C rich". Hess reports (3m) that the content varies with the diet of the individual and according to the interval elapsing after the ingestion of the antiscorbutic food. The opinion that fresh cow's milk is a valuable antiscorbutic is without basis, reports Chick,

Hume, and Skelton (26). This is confirmed by Hess who adds that a pint of milk a day is necessary for a child. To what extent the antiscorbutic value of milk depends on the diet of the cow has not been determined. In a comparison of the milk from cows on a vitamin C free diet with that of the milk of cows on pasture, Hughes and associates in 1927 concluded that the vitamin C content of the cow's ration has little, if any, influence on the vitamin C content of its milk (72). Hart, Steenbock, and Smith (73) state that summer milk is much richer in the vitamin C content than dry feed milk or winter produced milk. A seasonal variation in the antiscorbutic potency of cow's milk was reported by Barnes and Hume in 1919 (74) while MacLeod in 1927 (75) found no variation when the cows received the same diet all the year. Hess, Unger and Supplee (76) by the use of dried milk found that milk from cows receiving a feed low in vitamin C was practically of no antiscorbutic value while milk from cows on pasture was a fair source of the vitamin. Milk powders all of which are inferior sources of vitamin C show a variation in the vitamin content depending on the method by which the milk was dried (77).

Vitamin values of common foods have been determined and compared; the results are tabulated and are helpful to us in selecting valuable antiscorbutics. McCollum gives us this advice; "the place to get vitamins is in

the market, in the grocery store, from the milk man, and from the garden and not the drug store (32e).

Basal Diet

Obviously the basal diet must be antiscorbutic free while adequate in all other respects. Sherman, La Mer and Campbell (38) developed the following diet which has been in use in this laboratory for the past three years.

Oats	(finely ground)	59%
Skimmed Milk Powder	(heated)	30%
Butter Fat	(freshly prepared)	10%
Sodium Chloride		1%

Mature oats have been found to be of no antiscorbutic value. The milk powder was heated in an electric oven in open, shallow pans at 110° C. for two hours. The butter fat was prepared from fresh butter by decantation and filtration at a temperature below 40° C.

The constituents were thoroughly mixed thus making it impossible for the experimental animals to separate the ingredients.

The basal diet was prepared at least twice weekly and when not in use was kept in the refrigerator in airtight containers.

Negative control animals which were given only basal diet and water ad libitum were fed parallel with each group of experimental animals to demonstrate the scorbutic value of the diet. In all of these control experiments

the animals died of scurvy, none living longer than thirty-two days.

Antiscorbutic Food

Raw spinach is an excellent source of vitamins A, B, and C. Since temperature and length of time of heating are factors influencing the potency of the antiscorbutic vitamin and since spinach is consumed almost entirely in the cooked form it becomes necessary to quantitatively determine the vitamin content of the product, which has been subjected to various household methods of cooking, before we can evaluate the cooked vegetable as an antiscorbutic.

Eddy, Kohman, and Carlsson in 1925 (78) conducted an investigation on the vitamin C content of spinach. One variety of spinach was used for the raw and home-cooked determinations throughout the experiment.

Raw spinach was secured as fresh as possible from the New York markets during the months of June, July, and August. The home-cooked vegetable was washed, placed in a kettle with the water which adhered to the leaves, the kettle covered, and the material boiled for fifteen minutes. The contents were then cooled, stirred to a uniform mixture and an aliquot fed. The commercial canned spinach was subjected to a two minute water blanch, and sealed in No. 2½ cans which were then placed in a commercial retort and processed for seventy minutes at a

temperature of 115 C. They report that the antiscorbutic unit is not more than one gram of raw, four grams of commercially canned, and on a ten gram portion of home cooked spinach one of three guinea pigs developed scurvy.

The South Dakota Agricultural Experiment Station published in their annual reports of 1927 and 1928 the results obtained from an investigation of home canned spinach which had been blanched for two minutes and processed seventy minutes at fifteen pounds pressure. The spinach was not grown in South Dakota but was shipped in during April. Ten grams of home canned spinach were found to be too near the border line to be safe as a protective food while five grams of the commercially canned product were sufficient to protect the animals from scurvy (79).

Craig (80) working in this laboratory in 1927 reported that the unit of vitamin C for fresh raw spinach falls between five and six grams. Her results from the open kettle cooked spinach indicated that the unit is above fifteen grams. The amount of commercially canned spinach necessary to protect a guinea pig from scurvy for a period of ninety days was between seven and nine grams.

In this determination experiments were conducted upon raw, home cooked, and home canned spinach to try to determine the unit of vitamin C in each case. Bloomsdale spinach, grown in Chesterfield, Missouri was used in all

of the determinations. To secure comparable results from the spinach determinations the weight of the home cooked and the home canned portions were fed in terms of the raw spinach. Aliquot portions were fed daily with the exception of Sunday.

The raw spinach, washed and dried, was fed at levels of 1.5, 2, 2.5, 3, and 4 grams. The method of Eddy as used by Craig was followed in preparing the home cooked spinach which was fed in portions of 12, 12.5, 13 and 14 grams. The home canned spinach was prepared in lots according to Farmer's Bulletin No. 1471. The leaves were washed well and placed in a covered kettle for a five minute blanch with just enough water added to prevent burning. It was packed in one-half pint jars and immediately processed at ten pounds pressure for eighty minutes. The jars were stored until needed. The product was fed at levels equivalent to 9, 10, 11, and 12 grams of the raw material.

Chemical Analysis of Food

The chemical analysis for crude protein, moisture, crude fat, crude fiber, total ash, and carbohydrate was made. The tested samples of the basal diet were obtained from the regular laboratory mixing of the food. The spinach which was tested was a representative portion of the home canned product.

The methods (81) used for the determinations are as follows:

1. Crude protein - by the Kjeldahl - Gunning Arnold method.
2. Moisture - by drying to constant weight in a vacuum electric oven.
3. Crude fiber - by acid and alkali digestion and subsequent drying method.
4. Total ash - by oxidizing the material to a whitish gray ash over a slow flame.
5. Crude fat - by ether extraction in a Soxhlet apparatus.
6. Carbohydrate - by difference.

Chemical Composition of Foods

Basal Diet

Component Parts	Number of Determinations	Per Cent Composition
Protein	3	17.37
Moisture	3	5.12
Crude fat	2	12.85
Crude fiber	2	8.06
Total ash	2	5.63
Carbohydrate		50.97

Bloomsdale Spinach

Component Parts	Number of Determinations	Per Cent Composition
Protein	2	3.88
Moisture	4	87.27
Crude fat	3	0.75
Crude fiber	2	1.08
Total ash	2	1.97
Carbohydrate		5.05

Experimental Animals

The method of care and selection of experimental animals chosen for this investigation is that of Sherman, La Mer, and Campbell (38) which has been for the last three years in use in this laboratory.

The animals, young male guinea pigs, were obtained from Manhattan, Kansas. They were housed in groups of three in wire cages (Figure IX) during an acclimation period of at least two weeks. The majority of the experimental animals were placed in individual cages during the latter half of this period. During this time they were fed ad libitum basal diet, water and green vegetables.

Every precaution was taken in the care of the animals to secure favorable living conditions. The room temperature was kept as constant as possible with thermostat control. The cages were changed as often as necessary and the trays were cleaned daily. The basal diet was fed in cups sterilized weekly while fresh distilled water was always available in cups sterilized daily. The animals were weighed three times a week and the food record was determined weekly. Each animal was irradiated for five minutes daily, with the exception of Sunday, with a quartz mercury vapor lamp at a distance of thirty-two inches.

Experimental Procedure

The animals, averaging 298 grams when selected for the experiment, were placed in individual cages and fed the basal diet and water ad libitum. Since the anti-scorbutic vitamin is not stored in the body of the guinea pig a depletion period on a vitamin C free ration is not necessary and the experiment was begun at once by substituting the definite amounts of spinach to be tested for the green vegetables. To rule out any possibility that an original weight difference was affecting the results, the animals were distributed among the various portions in well matched groups. The raw and home cooked spinach experiments were run parallel followed by the home canned spinach determination.

Following the precedent of Sherman, La Mer, and Campbell and previous investigators in the laboratory the severity of autopsy findings were indicated by - (no difference from normal), ? (doubtful) and x, xx, xxx for increasing degrees of severity. The jaw, teeth, joints, and ribs were tested for weakness in the bony system and special notice was given to hemorrhages in the tissues, ribs, joints, and intestines. The total number of x signs given to an animal is spoken of as its scurvy score. Following this procedure the maximum scurvy score

which an animal can have is twenty-four. "The use of a scurvy score in judging the severity of scurvy necessitates a careful study on the part of the experimenter of the symptoms which are typical of the disease. These estimations can be made quite uniform however with practice (82).

The autopsy was performed as soon after death as possible. When the animal lived through-out the experimental period, ninety days, it was chloroformed and an autopsy made to determine the severity of the scurvy.

Experimental Results and Discussion

Although eighty-seven animals were used in testing the antiscorbutic content of spinach, Appendix-Table I, only sixty-seven of these are considered in the results of this investigation. Pigs living less than twenty days on the experimental period with the exception of negative controls were not included because scurvy was not found in these animals. It would seem that other factors caused death since negative control animals usually live longer than this and show extensive scurvy symptoms in this time. The average growth curves of animals fed raw, home cooked, and home canned spinach are shown in Charts I, II, and III. Tables II, III, and IV in the Appendix give the weight, per cent gain, food intake, and the scurvy scores of animals receiving the various portions of raw, home cooked, and home canned spinach. This information for negative and positive control animals is shown in Table V in the Appendix. The average results for animals receiving various portions of raw, home cooked, and home canned spinach can be found on page 42. Autopsy findings are reported in Table VI in the Appendix.

We can only estimate the vitamin unit from the results obtained from the raw and home cooked spinach feedings. The scurvy scores for these groups would

Summary of Results

Effect of Feeding Varying Amounts of
Raw Spinach
Animals Included Which Lived 20 Days or
More on Experiment

Spinach (Grams)	Number of Animals	Days Lived on Experiment	Initial Weight	Per Cent Gain in Weight	Grams Basal Per Day	Gram Basal Per Gram Pig Per Day	Scurvy Score
1.5	2	24	280	-30	12.8	0.056	6.8
2.0	5	33	313	-30	14.2	0.049	3.3
2.5	2	34	318	-33	12.3	0.044	4.0
3.0	4	32	322	-23	16.6	0.049	1.8
4.0	3	32	325	-24	13.1	0.045	2.0

Effect of Feeding Varying Amounts of Home Cooked Spinach

Spinach (Grams)	Number of Animals	Days Lived on Experiment	Initial Weight	Per Cent Gain in Weight	Grams Basal Per Day	Gram Basal Per Gram Pig Per Day	Scurvy Score
10.6	1	27	336	-36	15.0	0.034	6.0
12.0	4	28	325	-27	15.5	0.052	6.3
13.0	2	42	340	-41	15.8	0.046	4.3
14.0	2	44	326	-31	15.9	0.048	3.8

Effect of Feeding Varying Amounts of Home Canned Spinach

Spinach (Grams)	Number of Animals	Days Lived on Experiment	Initial Weight	Per Cent Gain in Weight	Grams Basal Per Day	Gram Basal Per Gram Pig Per Day	Scurvy Score
9.0	8	46	296	-29	12.1	0.046	5.0
10.0	6	50	295	-22	10.8	0.040	3.8
11.0	9	51	294	-16	13.0	0.047	2.6
12.0	8	58	298	-29	10.0	0.041	3.7

indicate that the animals did not die from scurvy although it is impossible to say whether or not the score would have been increased had the animals lived throughout the experimental period. The pigs started earlier in the experiment, on an average, lived longer since all of the animals apparently refused to eat the basal diet at about the same time. It is believed that the oats became musty after the experiment had been started.

Animal 474, receiving three grams of raw spinach, is pictured in Figure VI on the 29th day of the experiment. Two days later it refused to eat the basal diet and died with no scurvy. The animals on the three and four gram portions gained well during the first part of the experiment which seemed to indicate that the dosage was too liberal. Animals were then placed on one and one-half and two and one-half gram portions.

The results would seem to indicate that the unit of vitamin C is obtained from two to three grams of the raw spinach. Two grams probably more nearly approaches the minimum protective dose.

Two animals receiving the equivalent of fourteen grams of raw spinach, which had been home cooked, lived 44 days on the experiment with scurvy scores of 3.5 and 4.0. Although the average score for the two animals on the thirteen gram portion was 4.3, the individual scores were 1 and 7.5. The unit of vitamin C must approach the

equivalent of fourteen grams of raw spinach.

Thirty-one animals are reported in the results of the home canned spinach. An average of eight animals which received the equivalent of nine grams of raw spinach shows that these pigs were not protected from scurvy. One animal receiving eleven grams of the spinach was completely protected for ninety days. Pig number 539 was accidentally smothered on the 88th day of the experiment. A 12 gram portion did not protect this animal since it was given a score of 8.

No appreciable difference was found in the average scurvy score of animals on the ten, eleven, and twelve gram portions. The score would indicate that these animals did not have scurvy and that the unit of vitamin C is contained in the equivalent of ten grams of raw spinach which was home canned.

Seven negative control animals were fed the basal food and water along to make certain the basal diet was free from vitamin C. These pigs lived an average of 21 days on the experiment with an average scurvy score of 6.8. Three of this number lived 32, 29, and 21 days on the experiment, an average of 27 days, and presented an average scurvy score of 9.8. It would seem from this evidence that the basal diet was vitamin C free. These animals became inactive, lost all interest in the food, their coats became rough, and at death had lost on an

average 33 per cent of their original weight. Tender and swollen joints were recognized in two of these animals.

To test the possibility that the animals dying so soon after being placed on the experiment were inferior and were not affected by vitamin shortage four positive control pigs were fed 6 c.c. of canned tomato juice. Two of these lost weight consistently and died in 13 and 15 days. The remaining two lived 35 and 44 days. This group did not die from scurvy since the average score was 1.3.

The average weight of the animals when placed upon the experiment was 298 grams. The average original weight of the pigs living over 20 days was 308. Although the intake of basal ration was not normal, it seemed to decrease when the larger portions of spinach were fed. The per cent loss in weight and reduced food intake was no indication of the severity of scurvy. The higher basal intake did not insure a smaller loss in weight.

Summary

The purpose of this investigation was to determine the amounts of Bloomsdale spinach, raw, home cooked, and home canned, necessary to furnish one unit of vitamin C. The method of Sherman, La Mer, and Campbell was followed in this determination.

The spinach which was fed raw was washed and dried before weighing. The cooked vegetable was boiled for fifteen minutes in an open kettle. The home canned spinach which was subjected to a five minute water blanch, was placed in one-half pint jars, and immediately processed at ten pounds pressure for eighty minutes. All portions of home cooked and home canned spinach were fed on the basis of the raw weight.

Raw spinach is a rich source of vitamin C since two to three grams was found to contain one unit. Fourteen grams was required for the minimum protective dose when the spinach was cooked in an open kettle which indicates that over 79 per cent of the vitamin was destroyed in the 15 minute cooking period. Home canning resulted in a destruction of over 70 per cent of the vitamin since the equivalent of ten grams of raw spinach was necessary to furnish one unit of the vitamin.

APPENDIX

Introduction to Appendix

Autopsy findings, Table VI, include records of all animals living over twenty days on the experimental period. The following symbols are used;

†, † †, † † † - varying degrees of deviation from normal.

- no indication of scurvy.

? value used as 0.5 in determining total scurvy score.

x, x †, x x x - varying degrees of scurvy.

Figure I. Negative control number 453, which was photographed on the 30th day of the experiment. It died two days later with a scurvy score of 13.5 and a 33 per cent loss in weight.

Figure II. Number 453 is pictured also in Figure I. Note the roughness of the coat, the drooping of the ears, and the position of the hind legs.

Figure III. The same animal as in Figure I and II.

Figure IV guinea pig, number 474, received 3 grams of raw spinach. The animal, on the 29th day of the experiment, shows every indication of health. Two days later it refused to eat the basal diet and died in ten days with a scurvy score of 0.5.

Weight at beginning of period	383 grams
Weight on 28th day of period	458 grams
Weight at death	269 grams

Figure V. Animal number 569 photographed on the 84th day of the experiment received 9 grams of home canned spinach. It died two days later with a scurvy score of 8.

Figure VI. Guinea Pig number 568 photographed on the 84th day of the experiment. The animal received ten grams of home canned spinach daily and lived through-out the ninety day experimental period.

Figure VII. Animal number 538 received eleven grams of home canned spinach. It lived through-out the experimental period.

Figure VIII. An animal prepared for autopsy. The jaw, teeth, ribs and joints will be broken as tests for weaknesses in the bony system. Hemorrhages will be searched for in the joints, ribs, tissues, and intestines.

Figure IX . Galvanized wire cage in which individual animals were housed through out the experiment.

ANGLO
AFRICA

Chart III

Average Growth Curves of Animals
Fed Home Canned Spinach
Experimental Period 20 Days or More

Table I
All Animals Used Through-Out the Experiment

Source of Vitamin	Animal Number	Days Animal Lived on Experiment	Weight			Per Cent Gain in Weight	Gram Food Per Gram Wt. Per Day	Scurvy Score
			Initial	Maximum	Final			
Raw Spinach 1.5 grams	467	21	259	265	169	-35	0.066	6.0
	464	27	301	369	227	-25	0.045	7.5
	302	51	330	400	250	-22	0.046	1.5
	473	32	336	359	242	-26	0.040	5.5
	504	16	319	319	223	-30	0.045	4.0
	451	24	249	249	195	-22	0.043	4.0
	491	25	302	276	166	-40	0.051	2.5
	462	24	320	347	216	-28	0.062	3.0
	471	43	316	338	190	-29	0.034	2.0
	474	41	300	301	240	-27	0.053	5.0
2.5	455	35	303	458	269	-29	0.067	0.5
	467	32	304	353	215	-29	0.049	3.5
	300	53	312	417	267	-12	0.051	2.5
	460	19	343	400	221	-26	0.042	2.0
3.0	466	35	331	430	249	-25	0.038	3.5
	459	24	314	345	278	-11	0.056	2.0
Home Cooked Spinach 10.6	480	27	326	341	214	-36	0.051	6.0
	503	18	323	354	266	-18	0.048	6.0
	494	23	306	402	249	-35	0.040	6.0
	476	24	300	360	225	-15	0.055	6.5
12.5	456	23	304	346	222	-27	0.034	4.5
	440	33	281	281	214	-24	0.046	2.0
	481	16	310	315	215	-31	0.067	8.0
	522	7	298	302	245	-18	0.048	2.0
13.0	463	17	282	292	216	-26	0.021	1.0
	470	31	322	355	230	-9	0.042	4.0
	468	19	307	324	189	-41	0.046	1.0
	472	32	356	398	251	-25	0.044	2.0
14.0	478	14	300	312	197	-40	0.045	7.5
	449	16	337	377	241	-29	0.039	1.5
	446	44	308	315	247	-20	0.041	3.5
	445	44	215	370	210	-33	0.040	2.0
Home Canned Spinach 9.0	573	9	280	220	156	-29	0.054	0.5
	527	27	228	242	172	-25	0.047	1.0
	553	25	337	337	199	-41	0.032	6.5
	580	28	325	352	220	-14	0.039	2.5
	544	60	315	324	197	-37	0.055	4.5
	566	64	290	290	192	-34	0.061	7.5
	569	66	360	360	236	-36	0.054	0.0
	582	17	232	232	159	-31	0.041	6.0
	542	55	260	260	228	-9	0.046	2.5
	546	26	291	295	173	-39	0.051	6.5
10.0	583	46	216	228	161	-25	0.050	3.0
	528	11	325	325	256	-24	0.047	1.5
	550	46	433	433	236	-46	0.021	2.0
	574	60	312	325	228	-27	0.044	0.5
	568	90	337	425	225	-37	0.045	3.0
	541	30	226	244	153	-35	0.037	6.0
	570	12	231	231	168	-27	0.026	3.0
	549	28	278	280	174	-25	0.036	1.0
	545	10	215	215	209	-25	0.033	1.0
	540	9	309	325	164	-24	0.025	0.0
11.0	563	32	345	354	211	-18	0.045	1.0
	543	39	204	258	158	-29	0.032	1.0
	564	36	323	331	193	-40	0.042	0.5
	572	13	280	280	203	-28	0.040	0.5
	533	26	246	246	187	-38	0.030	4.0
	559	8	246	246	166	-38	0.054	4.0
	536	27	286	286	179	-37	0.038	1.0
	567	90	246	429	471	-71	0.060	6.5
	557	69	368	421	240	-35	0.042	1.0
	530	15	303	377	222	-27	0.042	6.0
12.0	542	90	256	356	205	-20	0.036	2.0
	578	90	356	373	249	-30	0.042	2.0
	340	31	323	332	264	-25	0.040	2.5
	571	60	266	266	166	-36	0.040	6.0
	539	26	252	268	168	-35	0.036	3.5
	556	13	280	280	140	-50	0.039	8.0
	585	26	277	277	193	-31	0.023	2.5
	578	48	322	322	205	-36	0.033	0.5
	575	44	332	332	205	-39	0.035	2.0
	561	35	308	359	248	-26	0.041	1.5
Positive Control 6% Tomato juice	552	13	240	240	205	-15	0.050	1.0
	577	15	268	268	196	-27	0.054	1.0
	575	15	268	268	196	-27	0.055	2.5
Negative Control	453	33	392	423	263	-33	0.045	12.5
	499	16	301	301	208	-30	0.049	3.0
	555	29	226	236	162	-32	0.041	3.0
	564	14	306	306	189	-38	0.036	2.0
	581	21	312	312	225	-28	0.038	2.0
	576	16	310	310	189	-39	0.030	7.0
	454	16	286	286	206	-29	0.044	3.0

Table II
Animals Receiving 1.5 Grams Raw Spinach

Animal Number	Days Lived on Experiment	Weight in Grams				Grams Basal Eaten Per Day	Gram Basal Per Gram Fig Per Day	Scurvy Score
		Original	Maximum	Final	Per Cent Gain			
487	21	299	308	199	-33	11.0	0.046	6.0
484	27	301	309	227	-25	14.6	0.048	7.5
Average	24	300	317	198	-30	12.8	0.056	6.8

Animals Receiving 2 Grams Raw Spinach

Animal Number	Days Lived on Experiment	Weight in Grams				Grams Basal Eaten Per Day	Gram Basal Per Gram Fig Per Day	Scurvy Score
		Original	Maximum	Final	Per Cent Gain			
502	31	330	400	256	-22	16.4	0.046	1.5
452	45	336	359	242	-28	12.7	0.040	5.5
475	22	319	319	223	-30	13.9	0.048	4.0
451	24	276	276	166	-40	10.7	0.051	2.5
491	25	302	307	216	-28	17.1	0.062	3.0
Average	33	313	332	221	-30	14.8	0.049	3.3

Animals Receiving 2.5 Grams Raw Spinach

Animal Number	Days Lived on Experiment	Weight in Grams				Grams Basal Eaten Per Day	Gram Basal Per Gram Fig Per Day	Scurvy Score
		Original	Maximum	Final	Per Cent Gain			
489	24	320	347	227	-29	10.5	0.054	3.0
462	43	316	336	198	-37	14.1	0.053	5.0
Average	34	318	343	213	-33	12.3	0.044	4.0

Animals Receiving 3 Grams Raw Spinach

Animal Number	Days Lived on Experiment	Weight in Grams				Grams Basal Eaten Per Day	Gram Basal Per Gram Fig Per Day	Scurvy Score
		Original	Maximum	Final	Per Cent Gain			
471	20	300	301	240	-20	16.3	0.057	0.5
474	41	303	438	269	-30	16.9	0.040	0.5
455	35	302	353	213	-29	15.0	0.049	3.5
467	32	304	417	267	-12	16.2	0.051	2.5
Average	32	322	362	246	-25	16.6	0.049	1.6

Animals Receiving 4 Grams Raw Spinach

Animal Number	Days Lived on Experiment	Weight in Grams				Grams Basal Eaten Per Day	Gram Basal Per Gram Fig Per Day	Scurvy Score
		Original	Maximum	Final	Per Cent Gain			
505	25	312	400	231	-26	7.0	0.042	2.0
486	25	331	420	249	-25	13.2	0.036	2.0
499	24	316	345	278	-11	19.2	0.056	2.0
Average	22	320	377	246	-24	13.1	0.045	2.0

Table III

Animals Receiving 12 Grams Home Cooked Spinach

Animal Number	Days Lived on Experiment	Weight in Grams				Grams Basal Eaten Per Day	Gram Basal Per Gram Pig Per Day	Scurvy Score
		Original	Maximum	Final	Per Cent Gain			
494	33	326	402	249	-35	14.8	0.040	5.0
496	34	300	300	255	-15	16.9	0.065	5.5
476	33	304	246	222	-27	11.1	0.034	4.5
440	33	310	315	213	-31	17.1	0.067	6.0
Average	33	325	341	235	-27	15.5	0.052	5.3

Animals Receiving 13 Grams Home Cooked Spinach

Animal Number	Days Lived on Experiment	Weight in Grams				Grams Basal Eaten Per Day	Gram Basal Per Gram Pig Per Day	Scurvy Score
		Original	Maximum	Final	Per Cent Gain			
470	31	322	375	189	-41	15.0	0.046	1.0
472	32	358	398	215	-40	16.5	0.045	7.5
Average	42	340	387	202	-41	15.8	0.046	4.3

Animals Receiving 14 Grams Home Cooked Spinach

Animal Number	Days Lived on Experiment	Weight in Grams				Grams Basal Eaten Per Day	Gram Basal Per Gram Pig Per Day	Scurvy Score
		Original	Maximum	Final	Per Cent Gain			
466	44	337	377	241	-29	14.8	0.041	3.5
446	44	315	370	210	-33	17.0	0.055	4.0
Average	44	326	374	226	-31	15.9	0.048	3.8
*482	27	326	341	214	-36	15.0	0.034	5.0

*482 which refused to eat 14 grams of spinach ate an average of 10.6 grams daily.

Table 27

Animals Receiving 9 Grams Home Canned Spinach

Animal Number	Days Lived on Experiment	Weight in Grams				Grams Bazel Notes Per Day	Grams Bazel Per Gram Pig Per Day	Survay Score
		Original	Maximum	Final	Per Cent Gain			
897	27	828	848	170	-22	12.0	0.027	1.0
898	28	807	807	190	-43	0.3	0.020	0.0
899	28	808	808	820	-16	12.0	0.020	2.0
900	28	819	804	109	-27	12.0	0.020	4.0
901	28	820	820	100	-24	12.0	0.021	7.0
902	28	820	820	821	-24	11.0	0.020	0.0
903	28	820	820	820	- 0	11.4	0.020	0.0
904	28	821	820	170	-20	11.1	0.021	0.0
Average	44	820	820	820	-22	12.1	0.020	0.0

Animals Receiving 10 Grams Home Canned Spinach

Animal Number	Days Lived on Experiment	Weight in Grams				Grams Bazel Notes Per Day	Grams Bazel Per Gram Pig Per Day	Survay Score
		Original	Maximum	Final	Per Cent Gain			
893	44	816	820	101	-20	10.1	0.020	2.0
894	44	424	420	200	-46	11.2	0.021	2.0
895	40	812	820	200	-27	11.0	0.021	2.0
896	20	827	425	425	724	12.5	0.045	2.5
897	20	828	844	188	-26	7.0	0.027	4.0
898	20	821	821	174	-20	2.4	0.020	2.0
Average	30	820	819	227	-22	10.6	0.040	2.6

Animals Receiving 11 Grams Home Canned Spinach

Animal Number	Days Lived on Experiment	Weight in Grams				Grams Bazel Notes Per Day	Grams Bazel Per Gram Pig Per Day	Survay Score
		Original	Maximum	Final	Per Cent Gain			
897	21	809	825	220	-18	12.9	0.045	0.5
898	22	845	844	211	-29	7.0	0.022	1.0
899	22	804	250	180	-22	0.1	0.042	2.0
900	22	825	821	180	-40	10.2	0.040	0.5
901	22	820	820	107	-20	12.5	0.044	4.0
902	27	804	806	179	-27	11.0	0.020	0.5
903	20	846	429	421	771	12.9	0.042	1.0
904	20	820	421	240	- 0	12.0	0.042	4.0
905	20	820	277	222	-27	12.2	0.040	2.0
Average	21	824	241	241	-18	12.0	0.047	2.6

Animals Receiving 12 Grams Home Canned Spinach

Animal Number	Days Lived on Experiment	Weight in Grams				Grams Bazel Notes Per Day	Grams Bazel Per Gram Pig Per Day	Survay Score
		Original	Maximum	Final	Per Cent Gain			
892	20	820	249	249	- 2	12.0	0.022	2.0
893	20	820	275	204	-20	11.0	0.020	2.0
894	21	820	820	220	-22	10.0	0.020	0.0
895	20	820	200	100	-20	0.0	0.020	2.0
896	20	820	200	100	-20	0.0	0.020	0.0
897	20	277	277	170	-20	7.0	0.020	0.0
898	20	820	820	220	-20	0.0	0.020	0.0
899	20	820	210	210	-20	12.0	0.021	2.0

Table V

Negative Controls

Animal Number	Days Lived on Experiment	Weight in Grams				Grams Basal Eaten Per Day	Gram Basal Per Gram Pig Per Day	Scurvy Score
		Original	Maximum	Final	Per Cent Gain			
434	16	298	298	206	-29	11.2	0.044	2.0
488	32	392	425	265	-33	14.7	0.046	12.5
490	17	301	301	200	-30	13.2	0.051	5.0
555	16	236	236	162	-32	6.6	0.041	5.0
564	29	306	309	189	-36	9.6	0.038	2.0
581	14	312	312	225	-28	10.6	0.036	5.0
576	21	310	310	189	-39	7.9	0.031	7.0
Average	21	307	312	206	-33	11.1	0.041	6.6

Positive Controls Fed 6 cc. Tomato Juice

Animal Number	Days Lived on Experiment	Weight in Grams				Grams Basal Eaten Per Day	Gram Basal Per Gram Pig Per Day	Scurvy Score
		Original	Maximum	Final	Per Cent Gain			
575	44	333	359	248	-26	12.6	0.041	1.5
561	35	302	315	233	-23	14.7	0.050	1.0
Average	40	318	337	241	-25	13.7	0.046	1.3

AUTOPSY FINDINGS

TABLE VI

Vitamin Portion Animal No.	Lungs (congested)	Gall Bladder (bloody)	Urinary Bladder (sediment)	Adrenals (enlarged)	Bony System				Musculoskeletal				Total Amino
					Teeth	Joints	Ribs	Spine	Joints	Clavicles	Ribs	Intestines	
New													
Spinach													
1.5 gms.													
487					?	XXX	X	?	-	-	X	-	5.0
488					-	X	X	XXX	-	-	X	-	7.5
5.0 gms.													
489		collapsed			-	-	-	-	-	-	X	?	1.5
490					-	-	X	-	-	-	X	?	3.5
491		stones			-	-	X	-	-	-	X	-	4.0
492		stones			-	-	X	-	-	-	X	-	3.0
7.5 gms.													
493		stones			-	-	X	-	-	-	X	-	5.0
494		stones			-	-	X	-	-	-	X	-	5.0
9.0 gms.													
495		stones			-	-	-	-	-	-	-	-	0.5
496		stones			-	-	-	-	-	-	-	-	0.5
497		collapsed			-	-	X	-	-	-	X	?	3.5
4.0 gms.													
498		stones			-	-	X	-	-	-	X	-	3.5
500					-	-	-	-	-	-	X	-	2.0
499					-	-	-	-	-	-	X	-	2.0
None													
Cooked													
Spinach													
10.0 gms.													
499					XX	X	X	-	-	-	XX	-	6.0
500					-	X	-	?	XXX	?	X	-	6.0
501					-	X	-	XX	-	-	-	XX	6.5
502					-	X	X	XX	-	-	X	-	4.5
15.0 gms.													
497		stones			-	X	X	XX	-	-	X	-	8.0
498					-	-	-	-	-	-	-	-	1.0
14.0 gms.													
499					-	-	X	-	XX	X	XX	-	7.5
500					-	-	X	-	-	-	XX	-	3.5
501					-	-	X	-	-	-	X	-	4.0
None													
Cooked													
Spinach													
9.0 gms.													
502					-	-	X	-	-	-	-	-	1.0
503					-	-	X	-	-	-	-	-	2.5
504		stones			-	-	X	-	-	-	-	-	2.5
505		stones			-	-	X	-	-	-	-	-	4.5
506		stones			-	-	X	-	-	-	-	-	7.5
507		stones			-	-	X	-	-	-	-	-	3.0
10.0 gms.													
508					-	-	X	-	-	-	-	-	6.5
509					-	-	X	-	-	-	-	-	3.0
510		stones			-	-	X	-	-	-	-	-	3.5
511		stones			-	-	X	-	-	-	-	-	3.5
512					-	-	X	-	-	-	-	-	3.5
11.0 gms.													
513					-	-	X	-	-	-	-	-	3.5
514					-	-	X	-	-	-	-	-	3.5
515					-	-	X	-	-	-	-	-	3.5
516					-	-	X	-	-	-	-	-	3.5
517					-	-	X	-	-	-	-	-	3.5
518					-	-	X	-	-	-	-	-	3.5
519					-	-	X	-	-	-	-	-	3.5
520		stones			-	-	X	-	-	-	-	-	3.5
521					-	-	X	-	-	-	-	-	3.5
522					-	-	X	-	-	-	-	-	3.5
523					-	-	X	-	-	-	-	-	3.5
524					-	-	X	-	-	-	-	-	3.5
525					-	-	X	-	-	-	-	-	3.5
526					-	-	X	-	-	-	-	-	3.5
527					-	-	X	-	-	-	-	-	3.5
528					-	-	X	-	-	-	-	-	3.5
529					-	-	X	-	-	-	-	-	3.5
530					-	-	X	-	-	-	-	-	3.5
531					-	-	X	-	-	-	-	-	3.5
532					-	-	X	-	-	-	-	-	3.5
533					-	-	X	-	-	-	-	-	3.5
534					-	-	X	-	-	-	-	-	3.5
535					-	-	X	-	-	-	-	-	3.5
536					-	-	X	-	-	-	-	-	3.5
537					-	-	X	-	-	-	-	-	3.5
538					-	-	X	-	-	-	-	-	3.5
539					-	-	X	-	-	-	-	-	3.5
540					-	-	X	-	-	-	-	-	3.5
541					-	-	X	-	-	-	-	-	3.5
542					-	-	X	-	-	-	-	-	3.5
543					-	-	X	-	-	-	-	-	3.5
544					-	-	X	-	-	-	-	-	3.5
545					-	-	X	-	-	-	-	-	3.5
546					-	-	X	-	-	-	-	-	3.5
547					-	-	X	-	-	-	-	-	3.5
548					-	-	X	-	-	-	-	-	3.5
549					-	-	X	-	-	-	-	-	3.5
550					-	-	X	-	-	-	-	-	3.5
551					-	-	X	-	-	-	-	-	3.5
552					-	-	X	-	-	-	-	-	3.5
553					-	-	X	-	-	-	-	-	3.5
554					-	-	X	-	-	-	-	-	3.5
555					-	-	X	-	-	-	-	-	3.5
556					-	-	X	-	-	-	-	-	3.5
557					-	-	X	-	-	-	-	-	3.5
558					-	-	X	-	-	-	-	-	3.5
559					-	-	X	-	-	-	-	-	3.5
560					-	-	X	-	-	-	-	-	3.5
561					-	-	X	-	-	-	-	-	3.5
562					-	-	X	-	-	-	-	-	3.5
563					-	-	X	-	-	-	-	-	3.5
564					-	-	X	-	-	-	-	-	3.5
565					-	-	X	-	-	-	-	-	3.5
566					-	-	X	-	-	-	-	-	3.5
567					-	-	X	-	-	-	-	-	3.5
568					-	-	X	-	-	-	-	-	3.5
569					-	-	X	-	-	-	-	-	3.5
570					-	-	X	-	-	-	-	-	3.5
571					-	-	X	-	-	-	-	-	3.5
572					-	-	X	-	-	-	-	-	3.5
573					-	-	X	-	-	-	-	-	3.5
574					-	-	X	-	-	-	-	-	3.5
575					-	-	X	-	-	-	-	-	3.5
576					-	-	X	-	-	-	-	-	3.5
577					-	-	X	-	-	-	-	-	3.5
578					-	-	X	-	-	-	-	-	3.5
579					-	-	X	-	-	-	-	-	3.5
580					-	-	X	-	-	-	-	-	3.5
581					-	-	X	-	-	-	-	-	3.5
582					-	-	X	-	-	-	-	-	3.5
583					-	-	X	-	-	-	-	-	3.5
584					-	-	X	-	-	-	-	-	3.5
585					-	-	X	-	-	-	-	-	3.5
586					-	-	X	-	-	-	-	-	3.5
587					-	-	X	-	-	-	-	-	3.5
588					-	-	X	-	-	-	-	-	3.5
589					-	-	X	-	-	-	-	-	3.5
590					-	-	X	-	-	-	-	-	3.5
591					-	-	X	-	-	-	-	-	3.5
592					-	-	X	-	-	-	-	-	3.5
593					-	-	X	-	-	-	-	-	3.5
594					-	-	X	-	-	-	-	-	3.5
595					-	-	X	-	-	-	-	-	3.5
596					-	-	X	-	-	-	-	-	3.5
597					-	-	X	-	-	-	-	-	3.5
598					-	-	X	-	-	-	-	-	3.5
599					-	-	X	-	-	-	-	-	3.5
600					-	-	X	-	-	-	-	-	3.5
601					-	-	X	-	-	-	-	-	3.5
602					-	-	X	-	-	-	-	-	3.5
603					-	-	X	-	-	-	-	-	3.5
604					-	-	X	-	-	-	-	-	3.5
605					-	-	X	-	-	-	-	-	3.5
606					-	-	X	-	-	-	-	-	3.5
607					-	-	X	-	-	-	-	-	3.5
608					-	-	X	-	-	-	-	-	3.5
609					-	-	X	-	-	-	-	-	3.5
610					-	-	X	-	-	-	-	-	3.5
611					-	-	X	-	-	-	-	-	3.5
612					-	-	X	-	-	-	-	-	3.5
613					-	-	X	-	-	-	-	-	3.5
614					-	-	X	-	-	-	-	-	

Bibliography

1. Rose, Mary Swartz; The Foundation of Nutrition. New York. The Macmillan Company. a. 212-215 b. 141 (1927)
2. McCollum, Elmer Verner, and Simmonds, Nina; The Newer Knowledge of Nutrition. Third Edition, New York. Macmillan Company. a. 171-180 b. 172 (1925)
3. Hess, Alfred Fabian; Scurvy Past and Present. First Edition, Philadelphia and London. L. B. Lippincott Company. a. 1-22, b. 23, c. 74, d. 117, e. 111-142, f. 212, g. 241-247, h. 67, i. 40-49, j. 155, k. 233, l. 77, m. 76 (1920)
4. Smith, Alice Henderson; Beer and Scurvy. Lancet 2: 813-815 (1918)
5. Funk, Casimir; The Vitamins. Baltimore, Williams and Wilkins Company. a. 27, b. 36-39 (1922)
6. Sherman, Henry Clapp, and Smith, Sybil Laura; The Vitamins. New York, The Chemical Catalog Company. a. 8, b. 145-146, c. 428 (1922)
7. Hood, C. Durant; Plain Talks and Common Sense. Medical Adviser. The Hood Medical Book Company, Chicago, Illinois. 473-478 (1880)
8. Smith, Theobald; Bacilli in Swine Disease. U. S. Dept. Agric. Bureau Animal-Industry Annual Report: 172 (1895-96)
9. Sherman, Henry Clapp; Chemistry of Food and Nutrition. Third Edition, New York. The Macmillan Company. a. 422, b. 425, c. 431, d. 437, e. 440, f. 427 (1926)
10. Coplans, Myer, et al; On the Etiology of Scurvy. Lancet 1: 1714-1716 (1904)

11. Wright, Sir Almroth E.; Causation and Treatment of Scurvy. Lancet 2: 725 (1908)
12. Hutchinson, Robert; Some Disorders of the Blood and Blood-Forming Organs in Early Life. Lancet 1: (1904)
13. Hopkins, F. Gowland; Feeding Experiments Illustrating the Importance of Accessory Factors in Normal Diets. J. of Physiol. 44: 449 (1912)
14. Holst, Axel, and Frölich; Ueber experimentellen Skorbut, Ztschr. f. Hyg. u. Infektionskrankh. 72: 1 (1912)
15. McCollum, Elmer Verner, and Kennedy, Cornelia; The Dietary Factors Operating in the Production of Polyneuritis. J. Biol. Chem. 24: 491 - 493 (1916)
16. Drummond, Jack Cecil; The Nomenclature of the So-Called Accessory Food Factors (Vitamins). Biochem. J. 14: 660 (1920)
17. Jackson, Leila, and Moore, J. J.; Studies on Experimental Scurvy in Guinea Pigs. J. Infect. Dis. 19: 478-510 (1916)
18. Jackson, Leila, and Moody; Bacterial Studies on Experimental Scurvy in Guinea Pigs. J. Infect. Dis. 19: 511-514 (1916)
19. McCollum, Elmer Verner, and Pitz, Walter; The "Vitamine" Hypothesis and Deficiency Diseases, A Study of Experimental Scurvy. J. Biol. Chem. 31: 229-255 (1917)
20. Torrey, John C., and Hess, Alfred Fabian; The Relation of the Intestinal Flora to the Scurvy of Guinea Pigs and of Infants. Soc. Exptl. Biol. and Med. 15: 74-78 (1917)
21. Harden, Arthur, and Zilva, Sylvester Solomon; Note on the Etiology of Scurvy in Guinea Pigs. Biochem. J. 12: 408-415 (1918)

22. Drummond, Jack Cecil; Note on the Role of the Antiscorbutic Factor in Nutrition. Biochem. J. 13: 77 (1919)
23. Hess, Alfred Fabian, and Unger, Lester J.; Experiments on Antiscorbutics. Report of an Antiscorbutic for Intravenous Use. Proc. Soc. Exptl. Biol. and Med. 15: 141 (1918)
24. Harden, Arthur, and Zilva, Sylvester Solomon; Antiscorbutic Factor in Lemon Juice. Biochem. J. 12: 258-268 (1918)
25. Harden, Arthur, and Zilva, Sylvester Solomon; Note on the Etiology of Scurvy in Guinea Pigs. Biochem. J. 12: 270-274 (1918)
26. Chick, Harriette; Hume, Eleanor M., and Skelton, Ruth F.; Antiscorbutic Value of Cow's Milk. Biochem. J. 12: 131-153 (1918)
27. Hess, Alfred Fabian; Chemistry in Medicine. Chem. Foundation Inc. New York. Chap. V. (1928)
28. Rosenau, Milton Joseph; Preventive Medicine and Hygiene. Fifth Edition, D. Appleton and Company, New York and London. 626-629 (1927)
29. Meyer, Arthur W., and McCormick, Lewis M.; Studies on Scurvy. Stanford University, California, Stanford University Press. 7-39 (1928)
30. McCarrison, R.; The Pathogenesis of Deficiency Disease. Ind. J. Med. Research 6: 275 (1919)
31. La Mer, Victor Kuhn and Campbell, H. Louise; Changes in Organ Weight Produced by Diets Deficient in Antiscorbutic Vitamin. Soc. for Exptl. Biol. and Med. 18: 32 (1921)
32. Eddy, Walter Hollis; Nutrition. Baltimore, The Williams and Wilkins Company a. 205, b. 165, c. 25, d. 180, e. 172-182 (1928)
33. Shipp, Henry Leigh and Zilva, Sylvester Solomon; Metabolism in Scurvy. Biochem. J. 22: 1449-1460 (1928)

34. Zilva, Sylvester Solomon, and Wells, F. M.; Changes in the Teeth of the Guinea Pig Produced by a Scorbatic Diet. Proc. Roy. Soc. London. E 90: 505 (1919)
35. Howe, Percy R.; Food Accessory Factors in Relation to the Teeth. J. Home Econ. 12: 482 (1920)
36. Hojer, Axel; Method for Determining the Antiscorbatic Value of a Foodstuff by Means of Histological Examination of the Teeth of Young Guinea Pigs. Brit. J. Exptl. Path. 7: 356-360 (1926)
37. Hess, Julius H.; Feeding and Nutritional Disorders in Infancy and Childhood. E. A. Davis Company, Philadelphia, Pa. Chap. VII. (1928)
38. Sherman, Henry Clapp; La Mer, Victor Kuhn, and Campbell, H. Louise; The Quantitative Determination of the Antiscorbatic Vitamin. J. Am. Chem. Soc. 44: 165-172 (1922)
39. Eddy, Walter H.; An Improvement in the Quantitative Assay of the Antiscorvy Vitamin (C). Am. J. Pub. Health 19: 1309-20 (1929)
40. Kohman, E. F.; Vitamins in Canned Foods. National Canner's Assoc. Wash. D. C. Bul. No. 19L (1927)
41. Hess, Alfred Fabian, and Unger, Lester J.; The Scurvy of Guinea Pigs. J. Biol. Chem. 35: 479 (1918)
42. Hart, E. B.; Steenbock, H., and Lepkovsky, S.; The Antiscorbatic Vitamin. I. A Study of Its Solubility from Dessicated Orange Juice. J. Biol. Chem. 52: 241 (1922)
43. Zilva, Sylvester Solomon, and Harden, Arthur; Antiscorbatic Factor in Lemon Juice. Biochem. J. 12: 93-105 (1918)
44. Zilva, Sylvester Solomon; Conditions of Inactivation of Accessory Food Factors. Biochem. J. 16: 42-48 (1922)
45. La Mer, Victor Kuhn; Vitamins from the Standpoint of Physical Chemistry. J. Ind. and Eng. Chem. 13: 1108-1110 (1921)

46. Sipple, H. L. and King, C. G.; A Study of the Preparation and Properties of Vitamin C Fractions from Lemon Juice. J. Am. Chem. Soc. 52: 420-423 (1930)
47. Connell, Sidney James Buchanan, and Zilva, Sylvester Solomon; The Differential Dialysis of the Antiscorbutic Factor. Biochem. Jr. 18: 641-646 (1924)
48. Hess, Alfred Fabian, and Fish, M.; Infantile Scurvy: The Blood, The Blood Vessels, and the Diet. Am. J. Dis. Child. 8: 386 (1924)
49. Delf, Ellen Marion; The Antiscorbutic Value of Cabbage. I. The Antiscorbutic and Growth Promoting Properties of Raw and Heated Cabbage. Biochem. J. 12: 416 (1918)
50. La Mer, Victor Kuhn, Campbell, H. Louise, and Sherman, Henry Clapp; The Effect of Temperature and the Concentration of Hydrogen Ion Upon the Rate of Destruction of Antiscorbutic Vitamin. J. Am. Chem. Soc. 44: 172-181 (1922)
51. Zilva, Sylvester Solomon; A Note on the Conservation of the Potency of Concentrated Antiscorbutic Preparation. Biochem. J. 18: 632-637 (1924)
52. Hess, Alfred Fabian, and Weinstock, Mildred; Catalytic Action of Minute Amounts of Copper in the Destruction of the Antiscorbutic Vitamin in Milk. J. Am. Med. Assoc. 82: 952-956 (1924)
53. Eddy, Walter Hollis et al; The Effect of Cooking on the Vitamin Content of Cabbage. J. Home Econ. 15: 15-30 (1923)
54. Kohman, E. F., Eddy, Walter Hollis, and Carlsson, Victoria; Vitamins in Canned Foods Vitamin C Destructive Factor in Apples. J. of Ind. and Eng. Chem. 16: 1261-63 (1924)
55. Zilva, Sylvester Solomon; The Influence of Reaction on the Oxidation of the Antiscorbutic Factor in Lemon Juice. Biochem. J. 17: 410-415 (1923)

56. Williams, Zulu Ethel; A Study Comparing the Heat Destruction of Vitamin C in Apple and in Rhubarb as Related to Changes of Hydrogen Ion Concentration. Unpublished Report, University of Missouri (1927)
57. Hess, Alfred Fabian, and Unger, Lester J.; The Deleterious Effect of the Alkalinization of Infant's Food. J. Am. Med. Assoc. 73: 1353 (1919)
58. Anderson, Ruth; Vitamin C Content of Jonathan Apple. Thesis University of Missouri (1929)
59. Givens, M. H. and McClugage, H. B.; An Experimental Study of Raw and Dry Potatoes. J. Biol. Chem. 42: 491 (1920)
60. Delf, Ellen Marion, and Skelton, Ruth F.; The Effect of Drying on the Antiscorbutic and Growth Promoting Properties of Cabbage. Biochem. J. 12: 448 (1918)
61. Heller, V. G.; Vitamin Synthesis in Plants as Affected by Light Source. J. Biol. Chem. 76: 499-511 (1928)
62. Chick, Harriette and Delf, Ellen Marion; The Antiscorbutic Value of Dry and Germinated Seeds. Biochem. J. 13: 199-217 (1919)
63. Hess, Alfred Fabian, and Unger, Lester L.; The Scurvy of Guinea Pigs. III. The Effect of Age, Heat, and Reaction on Anti-scorbutic Foods. J. Biol. Chem. 38: 293 (1919)
64. Harden, Arthur, and Zilva, Sylvester Solomon; Antiscorbutic Factor in Lemon Juice. Biochem. J. 12: 258-268 (1918)
65. Eddy, Walter Hollis; The Vitamin Manual. Baltimore, Williams and Wilkins Company. 88 (1922)
66. Smith, Sybil L.; Vitamin in Food Material. U. S. Dept. of Agric. Cir. No. 84: 5 (1929)
67. Spencer, H. A.; Scurvy; Its Treatment with Raw

- Orange Juice Intravenously; Physiol. Review
12: 36 (1927)
68. Vaglino, S.; Antiscorbutic Properties of Preserved Orange Juice. J. Am. Med. Assoc. 85: 1518
(1925)
69. Bassett, P. W. and Smith, Clarence Beaman; Scurvy. Lancet 1: 1102-1105 (1920)
70. House, Margaret C. Nelson, P. M., and Haber, E. S.; The Vitamin A, B, and C Content of Artificially versus Naturally Ripened Tomatoes. J. Biol. Chem. 81: 495-504 (1922)
71. Lieb, C. W.; The Effect on Human Beings of a Twelve Months Exclusive Meat Diet Based on Intensive Clinical and Laboratory Studies on Two Arctic Explorers Living Under Average Conditions in a New York Climate. J. Am. Med. Assoc. 93: 20-22 (1929)
72. Hughes, J. S.; Fitch, J. B.; Cave, H. W.; and Riddell, W. H.; Relation Between the Vitamin C Content of a Cow's Ration and Vitamin C Content of It's Milk. J. Biol. Chem. 71: 309-316 (1927)
73. Hart, Edwin Bret; Steenbock, Harry; and Smith, D. W.; Effect of Heat on the Antiscorbutic Properties of Some Milk Powders. J. Biol. Chem. 38: 305-325 (1919)
74. Barnes, R. E. and Hume, E. M.; Comparison Between the Antiscorbutic Properties of Fresh, Heated, and Dried Cow's Milk. Lancet 11: 323 (1919)
75. MacLeod, Florence; Antiscorbutic Vitamin Content of Milk of Stall-fed Cows Through-out a Year. J. Am. Med. Assoc. 88: 1947-49
(1927)
76. Hess, Alfred Fabian; Unger, Lester A. and Supplee, A. C.; Relation of Fodder to the Antiscorbutic Potency and Salt Content of Milk. J. Biol. Chem. 45: 229 (1920)
77. Supplee, G. C. and Dow, O. D.; Variations in Antiscorbutic Properties of Dry Milk. Am. J.

- Dis. Child. 31: 41-50 (1926)
78. Eddy, Walter Hollis, and Kohman, E. F.; Vitamin C in Canned Foods. J. Ind. and Eng. Chem. 17: 69 (1925)
79. South Dakota Agric. Expt. Station Ann. Report (1927-28)
80. Craig, Georgia S.; The Vitamin C Content of Apple and Spinach. Thesis, University of Missouri. (1928)
81. Mahin, Edward G., and Carr, Ralph H.; Quantitative Agricultural Analysis. First Edition Third Impression. New York, McGraw-Hill Book Company Inc. 142-149
82. Kenny, Christopher L.; A Study of the Thermostability of Vitamin C. New York. Dissertation, Columbia University 26 (1926)

Film
Neg.
Reel 73
T 749-759

M.A. THESIS

JOHANN - JOHNSON

University of Missouri - Columbia

010-103734069

N. Y.

University Libraries
University of Missouri

Digitization Information for Theses and Dissertations project.
Scanned from microfilm; no subsequent editing done.

Local identifier JohnsonDorothy1930

Source information

Identifier	Film Neg. Reel 73 T 749-759
Format	Microfilm
Content type	Text
Notes	

Capture information

Date captured	2018
Scanner manufacturer/model	ScanPro 3000 /e-ImageData
Scanning software	PowerScan 3000(TM) v5.341
Optical resolution	300 dpi
Compression	LZW
Color settings	bitonal
File types	tiff
Notes	Scanned from microfilm; no editing done.

Derivatives - Access copy

Editing software	Photoshop
Resolution	300 dpi
Color	grayscale
File types	pdf
Notes	Converted from tiff to pdf using Adobe Acrobat DC.