THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Edith May Pierson for the degree of Master of Science. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science.

Alice Biester
Chairman

C. M. Jackson

L. D. Goetner

August 19 1922
~~1918~~

THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Edith May Pierson final oral examination for the degree of

Master of Science.

We recommend that the degree of

Master of Science.

be conferred upon the candidate.

Alice Biester
Chairman

C. M. Jackson

Date August 19, 1922

10-20 5M

THE ANTISCORBUTIC PROPERTIES OF SOME COMMON FOOD MATERIALS.

by

Edith M. Pierson

A thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the Degree of Master of Science.

1 9 2 2

TABLE OF CONTENTS

THE ANTISCORBUTIC PROPERTIES OF SOME COMMON FOOD MATERIALS

INTRODUCTION

Impetus to a systematic study of the occurrence of the anti-scorbutic vitamine in food materials and its stability under different treatments was given by the tremendous problem of rationing the civilian population and the armies during the World War. In spite of a better understanding of preventive and curative measures, scurvy was an important problem in the nutrition of troops and of the civilian population of central and eastern Europe during and after this war.

The urgent need of more exact knowledge of the anti-scorbutic values of food stuffs of practical war time utility led to a systematic study of the more common food stuffs with respect to their relative value as anti-scorbutics. Through the work of Holst and Froelich, Chick and her co-workers, Osborne and Mendel and others a beginning has been made in standardizing the various food stuffs and in rating them approximately according to their anti-scorbutic value.

The question of the value of meat as an antiscorbutic food is of importance chiefly in connection with Polar expeditions and with army rations, inasmuch as meat is practically always included in the dietaries of men so engaged. It is generally conceded that animal tissues are inferior to fruits and vegetables in anti-scorbutic properties, but there has been no accurate evaluation of meat as an anti-scorbutic food such as has been made for many fruits and vegetables. It would therefore seem of value to know to what extent meat may be used as a source of the anti-scorbutic vitamine.

4-22-8M

The object of this experimental work was to test the anti-scorbutic properties of raw lean beef, and so far as animal experiments may be relied on to determine the relative potency of beef as an anti-scorbutic food. In the course of the investigation the milk used as a part of the basal ration called attention to the influence of the ration of the cow on the anti-scorbutic value of milk. A few experiments have been included in which control and scorbutic animals were satisfactorily changed to a ration in which the anti-scorbutic vitamine was derived from rhubarb after the experiments in which the guinea pigs had been used originally had been terminated.

HISTORICAL

It is reasonable to suppose that scurvy has existed since earliest times, but the older descriptions of the disease are vague and leave much to individual interpretation. The earlier descriptions are not sufficiently reliable to warrant definite conclusions as to the place and time in which scurvy made its first appearance. The reference of Hippocrates to a large number of men in the Greek army who suffered with pains in their legs and gangrene of the gums which was accompanied by loss of teeth, seems sufficiently definite to identify the disease as scurvy. De Jounville's description of a disease which occurred among the Crusaders while in Egypt is also typical of scurvy.

In the sixteenth century with the development and spread of education the records of the occurrence of scurvy became more accurate. Magnus (1555) published "A History of Northern Nations", in which he describes the disease which flourished among soldiers in camps and in prisons. The Faculty of Medicine at Copenhagen published in 1645 a "consilium" treating of the cause, prevention and cure of scurvy, which was prevalent among the Danes and other northern countries. The French and English colonists of the northern part of America suffered so terribly with scurvy that they seriously considered abandoning their settlements.

Kramer (1721), Bachstrom (1734) and Lind (1757), physicians of the 18th century had an accurate conception of the nature and the cure for the disease. Kramer (1721) writing of a severe epidemic which he had encountered in the Austrian army says, "Scurvy is a terrible disease for which there is no cure. Medication does not help, neither does surgery. If one could only have available a supply of green vegetables or a sufficient amount of vital anti-scorbutic juices then I would be in a position to cure this dreadful disease without other help."

Bachstrom (1734) describes an epidemic which occurred in Russia in mid-summer. He calls attention to the fact that the disease was not caused by cold weather nor salted meat, but by a lack of fresh vegetables. The publication in 1757 of Lind's treatise on scurvy did much toward correcting the erroneous views held at that time concerning the etiology, symptomatology and treatment of scurvy.

It is impossible within the limits of this historical discussion to review all scurvy epidemics of which there are records. To do so would necessitate a review of the history of all wars; for Hess (1920) states that no war is omitted from this list. Explorers, early settlers in isolated regions, sailors and fishermen have been numbered amon the victims of scurvy.

In the Civil War in the United States there were 30,714 cases of scurvy; in the Franco-Prussian War (1870 - 1871) scurvy was prevalent in the siege of Paris; and in the Russo-Japanese War after the siege of Port Arthur it was found that one half of the garrison of 17,000 men were suffering with the disease (Hess, 1920). During the World War thousands of cases of scurvy were observed. It was most prevalent in Russia and the bordering countries. Borish (1919), chief surgeon of the Red Cross Station in Russia personally observed 1343 cases. In the Servian army Wiltshire (1918) reported 3000 cases in the first half of the year 1917. In France, Harvier (1917) found that 95 per cent of his 800 men were suffering with scurvy and Dyke (1918) reported that 40 per cent of the 1700 men of the South African Labor Corps were scorbutic. The reports of Vannutetli (1917) and Vallarde (1918) and others indicate that scurvy was quite prevalent among the Italian troops. Scurvy was not common among the British troops, although 7500 were lost from the Colonial troops in a period of 19 weeks. The American soldiers were comparatively free from the disease. The Surgeon-General's report shows but 5 cases occurring in Europe and the United States in 1917 and but 15 cases in 1918. (Hess, 1920).

The history of scurvy shows that land epidemics often follow failure in vegetable crops, especially the potato crop. A recent illustration of the importance of the potato as an anti-scorbutic food is furnished by Pickins (1917) and Harlan (1917), two British practitioners. In New Castle 16 cases were admitted to the Poor Law Infirmary in a period of three months and in Glasgow 50 cases appeared in a period of four months. In both cases the men were from the so-called model lodging houses where the potato was the chief vegetable in the diet. No case of scurvy made its appearance until the potato famine when bread was substituted for potato in the diet.

Barlow (1894) was the first to describe scurvy in infants. Infantile scurvy is not often mentioned by the earlier medical writers. This was probably because very little attention was paid to the artificially fed infant, who died of intestinal trouble or starvation before scurvy had time to develop.

About the middle of the nineteenth century the manufacture and sale of proprietary infant foods began to prosper and there was a pronounced increase in the number of scorbutic cases. In 1894, 106 cases were reported to the Academy of Medicine, New York City by various physicians, and in 1898 the American Pediatric Society reported 379 cases asthe result of an investigation.

Hess (1920) is of the opinion that statistics do not and cannot give an adequate idea of the incidence of a disease of this type which requires a period of six months or more to develop to the period where it is recognizable clinically. Thus many infants who are deprived for a month or more of their quota of antiscorbutic foods suffer with the subacute or latent type of the disease, but are saved from the form, recognized clinically, by a change in their dietaries.

ETIOLOGY

There have been many theories advanced as to the cause of scurvy. There are those who believe that scurvy is of an infectious nature. This conception has been brought forward from time to time in relation to epidemics of adult scurvy. Hess (1920) states that it is the view held by the majority of Russian physicians today and recently has been advanced by European army surgeons. The only work considering the infectious theory from an experimental standpoint is that of Jackson and Moore (1916) who conclude that scurvy may be of bacterial origin.

Those who support the toxic theory believe that the poisons either are consumed in the food or formed in the food by means of bacterial action. At one time McCollum and Pitz (1917) supported this theory. They held that scurvy in guinea pigs is induced by the physical character of a diet which results in retention of feces and intestinal putrefaction followed by damage to the walls of the alimentary tract and consequent toxemia. They ascribed the beneficial action of orange juice to the laxative properties of potassium and sodium citrate normally present in citrous fruits.

The potassium deficiency theory postulated by Garrod (1848) in which he associates the antiscorbutic properties of fruits and vegetables with their rich potassium supply has been disproved by experimental work which has shown that potassium salts neither prevent nor cure scurvy.

The citric acid theory advanced by Netter (1899) was based on the antiscorbutic potency of citrous fruits. This theory withstood neither the practical test nor chemical investigation.

Wright (1908) held that scurvy was due to acidosis. According to this theory there is a preponderance in the diet of the acid-forming foods over the base-forming foods.

The most generally accepted theory at the present time is the vitamine theory. In 1897 Eykman, a Holland physician, published data to show that an exclusive diet of polished rice produced in pigeons a disease very similar to human beri beri. This investigator also found that the unpolished rice did not produce the disease, and wherever the rice polishings were introduced into the diets of birds afflicted with the disease, recovery followed almost immediately. Funk (1911) isolated a crystalline substance from rice polishings which contained nitrogen and had basic properties. Because of their curative and "life giving" properties he called such substances vitamines and classified scurvy, infantile scurvy, beri beri and pellagra as deficiency diseases, due entirely to a lack of these substances the "vitamines" in the diet.

At the present time there are recognized three specific vitamines: Vitamine A is also known at the fat-soluble vitamine, fat-soluble A and the anti-xerophthalmic vitamine. When the intake of Vitamine A in inadequate, growth is inhibited and there is an increased susceptibility to an infection which manifests itself quite frequently but not always in the development of the eye disease variously known as opthalmia, xeropthalmia, and conjunctivitis. Vitamine B is also known as water-soluble B and the antineuritic vitamine. The absence of Vitamine B causes polyneuritis in fowls, beri beri in man and a similar pathological condition in other mammals. Vitamine C is the anti-scorbutic vitamine, the absence of which causes scurvy in man and in experimental animals such as the guinea pig and the monkey.

To this list McCollum, Simmonds, Becker and Shipley (1922) have recently suggested the addition of a fourth vitamine which prevents or cures rickets.

SYMPTOMATOLOGY AND PATHOLOGY

Holst and Froelich (1907) were the first to observe the development of scurvy in the guinea pig as the result of a restricted diet, and to show that the pathological changes which take place as a result of such a diet are identical in all essentials with those of human scurvy. They fed 65 guinea pigs on diets of ground and unground oats, barley, rye or wheat and water. During the experiments they observed that the animals became greatly emaciated losing on an average 40 per cent of their original body weight. As a rule the animals lost weight, then either increased or remained unchanged in weight for 3 to 14 days, after which they exhibited a sharp decline during the week or two preceding death (death occurring on an average within 30 days). Postmortem examinations revealed loosening of the molars, hyperemia of the gums, hemorrhages into the muscles about the joints of the fore and hind legs, enlargement of the costochondral junctions, and very often a severing between the epiphyses and the shafts of the long bones, especially the tibia.

In addition to these symptoms Chick and her co-workers (1918) observed that the first manifestation is a soreness of joints and limbs, especially in the shoulders and knees. As the disease develops the animals assume typical scurvy positions, lying on one side with the painful leg held off the floor, or curled up with the face pressed against the floor of the cage in the face ache position. Postmortem examination showed rarification of the long bones with fractures between the epiphyses and the shafts of the bones themselves. The bone marrow lost its lymphoid character at the diaphyseal ends, became poor in cells and sometimes presented a homogenous appearance ("Heller's Mark").

Baumann and Howard (1917), in a study of the mineral metabolism of scorbutic guinea pigs, found that the elimination of nitrogen, sulphur and phosphorus was decreased, sodium and chlorine were normal, potassium was retained, and the calcium and magnesium output was increased.

McCarrison (1919) found that the adrenal glands in guinea pigs, rendered scorbutic on a diet of oats and milk, were enlarged and congested, averaging in the cases reported approximately twice the weight of the normal glands. In spite of the size of the gland, the production of adrenaline was diminished to about one-quarter of the normal amount. La Mer and Campbell (1920) have confirmed the work of McCarrison.

Zilva and Wells (1919) compared the microscopic sections of teeth from normal guinea pigs and from guinea pigs which had received an insufficient amount of anti-scorbutic food and found that the tooth is the first part of the system to become affected. This change is in the nature of a fibroid degeneration of the pulp; the fine connective tissue of the normal tooth being replaced by a fibrous structure devoid of cells. Howe (1920) states that the teeth of pigs on scorbutic diets show a marked decalcification.

CHEMICAL AND PHYSICAL PROPERTIES OF VITAMINE C.

Vitamine C, or the anti-scorbutic vitamine, is distinguished by being the most unstable and the least resistant to physical or chemical changes of the three vitamines. According to Holst and Froelich (1907) and Hess and Unger (1918), Vitamine C is soluble in water and in alcohol. Hart, Steenbock and Lepkovsky (1922) have studied its solubility in a number of organic solvents and have found it soluble in 80 per cent, 95 per cent, and absolute ethyl alcohol and in methyl alcohol. It was found to be insoluble in butyl alcohol as well as in benzene, petroleum ether, acetone, ether chloroform and ethyl acetate. The behavior of this vitamine toward organic solvents and water indicates that it is not of fat or lipin character. Hart, Steenbock and Lepkovsky (1922) raise the question of Vitamine C being water-soluble in the absence of other compounds occurring with it in such materials as orange juice and fresh green tissues. Vitamine C dialyzes through parchment (Holst and Froelich 1907) and passes through a porcelain filter and is not adsorbed as is Vitamine B by Fuller's earth or Lloyd's reagent (Harden and Zilva 1918a). It is more stable in an acid than in a neutral medium and still less stable when the medium is alkaline (Harden and Zilva 1918c; Hess and Unger 1919; La Mer, Campbell and Sherman 1922). It is relatively stable to ultra violet light. (Zilva 1919).

The studies of heat destruction of Vitamine C with reference to time and temperature warrant the general view that there is less loss of the anti-scorbutic property through heating a short time at a higher temperature than at a lower temperature for a longer time. Delf and Tozer (1918) found in their experiments with cabbage that 70 per cent of the original anti-scorbutic value was lost after one hour's heating at 60 deg. C; and 90 per cent after the same

treatment at 90 deg. C. After 20 minutes heating at 90 to 100 deg. C, the loss was 70 per cent. Experiments with this vitamine as found in milk accord with Delf's results, demonstrating that the intensity of heat is not so important as the length of time of heating (Anderson 1921). This work agrees with clinical experience in that milk which has been boiled for a few minutes does not induce scurvy as readily as pasteurized milk which has been heated for 45 minutes at 145 to 165 deg. F. (Hess and Fish 1918).

Experimental evidence shows that vegetables dried according to the present commercial methods lose their anti-scorbutic value as the result of dehydrating and become still more impoverished as the result of ageing. Holst and Froelich (1912) found that potatoes, carrots, peas and lentils have practically no protective value after they are thoroughly dried. Hess and Unger (1919) fed various brands of commercially dehydrated carrots to guinea pigs and found that they afforded no protection. Givens and Cohen (1918) experimented with cabbage and with potatoes which had been dried in an air blast at 38 - 52 deg. C and at 65 - 70 deg. C. The cabbage dried in an air blast at 38 - 52 deg. C. retained very little of its protective properties. Cabbage heated in an oven for two hours at 75 - 80 deg. C. then dried at 65 - 70 deg. C. for several days gave no protection as did cabbage cooked for 30 minutes and then dried for two days at 65 - 70 deg. C. Potatoes cooked and dried for two days at 65 - 70 deg. C. failed, when consumed by guinea pigs to the extent of the equivalent of 5 grams of raw potato, to protect against scurvy. These results are in accord with those of Delf and Skelton (1918) who made an extensive study of the value of dried cabbage as an anti-scorbutic food.

While it appears that the anti-scorbutic factor is peculiarly unstable to drying there are exceptions to this rule. Hess (1920) points out that milk dried by the Hatmaker process loses none of its anti-scorbutic value. Givens

and McClugage (1919) dried orange juice in two different ways, first, in shallow dishes at 55 to 60 deg. C. for 50 hours and second according to the Hatmaker process in which quick drying at ~~75 to 80~~ 100 or more deg. C. is obtained. The first preparation lost a great deal of its potency while the second preparation was almost as active as the original juice. In the case of the milk and the orange juice dried by the Hatmaker process, the rapidity of dessication and subsequent protection from oxidative processes seem to be important factors in preventing the destruction of Vitamine C.

The chemical behavior of Vitamine C as regards its susceptibility to oxidation has been the subject of active investigation. Anderson (1921) found that milk pasteurized in a closed vessel or in the presence of carbon dioxide retained its anti-scorbutic potency, while milk pasteruized with air or oxygen bubbling through it or in the presence of hydrogen peroxide lost its anti-scorbutic potency. Hess and Unger (1921) added 4 cc. of normal solution of hydrogen peroxide to a liter of raw milk and incubated it over night. Eighty cubic centimeters of this milk fed with oats and straw failed to protect guinea pigs against scurvy. Orange juice treated in like manner lost its anti-scorbutic potency. Zilva (1921) found that decitrated lemon juice through which air had been bubbled for twelve hours at laboratory temperature, delayed but did not prevent guinea pig scurvy when fed in doses of 3 - 5 cc. daily, while the same amount of decitrated juice which had been boiled for one hour in the presence of air neither delayed nor prevented guinea pig scurvy. On the other hand, decitrated juice which had been heated in a medium of carbon dioxide retained its original potency. Dutcher, Harshaw and Hall (1921) have shown that Vitamine C in orange juice is unstable in the presence of hydrogen peroxide. The destructive action of the hydrogen peroxide is not as great at room temperature as at 63 to 100 deg. C.

It would seem from these investigations that the process by which Vitamine C is destroyed is purely oxidative and that the effect of the application of heat is to increase the rate of oxidation.

LABORATORY AND CLINICAL DATA RELATING TO MEAT AS AN ANTISCORBUTIC FOOD

Clinical experience and laboratory findings are not always in accord as to the relative antiscorbutic properties of food stuffs. This is well illustrated in the case of muscle tissue. Meat has long been regarded as a potent antiscorbutic food by physicians and Artic explorers. Lind (1771) states that a soup prepared from the flesh of green turtles was curative in human scurvy. Barlow (1894) classifies fresh uncooked meat as a good antiscorbutic and advocates the addition of meat or meat juice to the diet of an infant as a preventive measure when proprietary or sterilized foods are used.

The Artic explorers, and men who have lived in the Artic regions agree that the inhabitants are protected from scurvy during the winter months by their diet of fresh meat and fish. In 1877 the British Artic Survey Committee (Sherman and Smith 1922) in a report of an outbreak of Scurvy in the Polar Expedition of 1875 - 76 make the statement that"although scurvy was due to the absence of lime juice from the sledge dietaries, meat in large quantities is able to prevent the disease." That this conclusion was sound has been proved by the experiences of Nansen and Johansen who wintered safely in Franz-Josefland on a diet of meat and bacon.

The following extracts taken from a letter of Dr. H. J. Hunt to Dr. Hess (1920) are of interest. He writes, "For some years I was with the Smith Sound Eskimos on the northwest coast of Greenland. These people get nothing but animal food normally, and have lived that way for generations, yet are healthy, of good physique, and are normal in other ways. Scurvy was unknown to them as far as I could ascertain, certainly there was nonewhile I was among them. Much

of their meat is eaten raw, and the rest only partly cooked." "The last English expeditions to the South Polar regions were afflicted with scurvy which was entirely and quickly eradicated by the use of fresh seal meat in the place of salt meats andcanned foods..... Personally, during my four years in the Artic, I took no fresh vegetables whatever, or other commonly-called antiscorbutics, relying solely on rare or raw meat. I never was stronger and more healthy in my life. I did have dried fruits and vegetables and usually plenty of fresh bread. Usually the Eskimos eat their meat in a frozen condition and I can attest that it is extremely palatable."

Quite recently Stefanson (1918) has carried out successful Artic explorations depending entirely on fresh meat as an antiscorbutic food stuff, and making no provision for vegetable foods.

Hehir (1919) attributes the high incidence of scurvy among the Indian troops and the almost complete absence of it among the British troops to the use by the latter of fresh meat toward the end of the siege when bullocks, horses and mules were killed to supplement the diminishing food supplies. Willcox (1917) in commenting on the same outbreak attributes the greater immunity to scurvy of the white troops in part, to the slight antiscorbutic value of the fresh meat which they consumed, and in part to the fact that their diet had previously contained a liberal supply of antiscorbutic food, whereas the previous diet of the Indian troops had been very low in its antiscorbutic content - almost on the borderland.

When the experiments reported herein were carried out the only experimental work with fresh meat was that reported by Chick, Hume and Skelton (1918) who found that 10 cc. of raw beef juice would not protect guinea pigs from scurvy.

If guinea pig scurvy is a deficiency disease analogous (as regards etiology, symptomatology and treatment) to human scurvy, the antiscorbutic properties of

any food can be studied by adding that food in measured amounts to a scorbutic diet. In the experiments reported herein a cold water extract equivalent to 5, 10, 15 and 20 gms. respectively of raw beef was fed on the assumption that the most potent antiscorbutics, the juices of the orange, lemon, swede and cabbage contain this vitamine in water or dilute salt solutions. If raw beef contains this vitamine it should be found in a cold water extract.

EXPERIMENTAL

Part I. The Antiscorbutic Properties of Raw Beef.

Series I.

Experimental Procedure

The guinea pigs used in the experiments were young animals, weighing from 200 to 300 grams. They were kept under observation for a week or more. At the end of this time, those animals whose growth curves were not satisfactory were eliminated from the groups.

The animals were kept singly in cages of woven wire with removable metal trays. Fresh sawdust was used for bedding. The cages were cleaned weekly, and sprayed with cresol at the time of cleaning.

The autoclaved milk fed was placed in dishes, and water was usually withheld until the milk was taken. Except in the last stages of the disease most of the animals took their milk eagerly. The meat extract was fed by hand by means of medicine droppers. The animals were weighed every fourth day before feeding. Autopsies were performed as soon after death as possible. Where the animals were in a dying condition they were chloroformed and autopsied at once. The ankle joint and a costochondral junction were saved for histological examination.

The scurvy-producing diet of oats and water is open to the criticism that it is lacking in Fat soluble A, and possible adequate proteins and mineral salts. We, therefore, used a basal ration oats, water ad libitum and 25 cc. of whole milk (autoclaved for one hour at 120 deg. C.).

The animals were divided into groups of four and placed on diets as follows:

Group I. Basal ration: Oats, water ad lib., 25 cc. autoclaved milk.

Group II. Basal ration: 10 cc. meat extract.

Group III. Basal ration: 20 cc. meat extract.

Group IV. Basal ration: 30 cc. meat extract.

Group V. Basal ration: 40 cc. meat extract.

Group VI. Basal ration: Oats, water ad lib.

Lean beef, freed from all visible fat and gristle, was finely ground, weighed, thoroughly mixed with tap water, and allowed to stand from twelve to fifteen hours. The mixture was then put in a heavy muslin bag, and all possible liquid extracted by means of a hand press. The dry meat pulp was removed from the bag, again thoroughly mixed with water and allowed to stand from one to two hours, when a second extraction was made. A third extraction was prepared in a similar manner. The three extractions were combined, made up to volume, (2 cc, representing the water extractable material from one gram of meat), and kept on ice until used. Fresh extracts were made every third day.

Discussion and Interpretation of Results

The details concerning the animals in the above groups are set forth in Table I, and Charts I, II, III, IV, V, VI, and on the whole present uniform results. The charts show that there was either a loss of weight followed by a gain, or a gain, until the 10th or 12th day of the experiment, when scurvy symptons developed, after which there was a steady decline in weight until death occurred. Table I shows that the average number of days elapsing before the development of scurvy in the six groups was 12, 18, 14, 15, 15 and 18 days, respectively, and that the average length of life of the animals was 22, 25, 24, 26, 26, and 26 days, respectively.

In general our observations are in accord with those of Holst and Froelich (1907), Chick and Hume (1918), and Cohen and Mendel (1918) with regard to the

symptomatology of scurvy in guinea pigs. There were roughness of coat, tenderness of shoulders and ankles, swelling in the joints, general inactivity and loss of appetite, and frequently but not always, paralysis of the hind legs. Postmortem examinations revealed fragility of bones, hemorrhages in the muscles of the hind legs and the shoulders and quite often loose teeth. Histological examinations were made of joints and costochondral junctions of representative animals in each group and in every case our clinical observations were substantiated by the histological findings.

These results indicate that a cold water extract of meat, when fed in amounts equivalent to 5, 10, 15, and 20 grams of meat, neither delays the onset of scurvy nor prolongs the life of the animal. Our results confirm those of Chick and Hume (1918) who found that a guinea pig, fed on a diet of oats and bran plus 10 cc. fresh raw beef juice, developed scurvy on the 15th day, which resulted in death on the 30th day.

These results can be interpreted in one of two ways: either the antiscorbutic vitamine is not found in the cold water extract, or the feeding of meat extract to an herbivorous animal like the guinea pig, produced complications with resulting symptoms similar to those of scurvy. Before drawing final conclusions regarding the antiscorbutic properties of a cold water extract of lean beef, it was decided to repeat the experiment, under the most carefully controlled conditions.

Series II.

Experimental Procedure.

The basal ration used was oats, water ad libitum and 20 cc. of pasteurized milk, it having been found by Chick and Hume (1918) that the antiscorbutic vitamine in 30 to 50 cc. of milk influences but slightly the course of the disease. The possibility of Vitamine C. being present in the meat and not in the cold

water extract necessitated the feeding of raw lean beef also. The finely ground meat and rolled oats were combined as intimately as possible by means of a mortar and pestle. The mixture was offered to the animals twice daily, thus affording little chance of contamination. The groups of experimental animals were placed on the following rations:

Group VIII. Basal ration: oats, water, 20 cc. pasteurized milk.

Group IX. Basal ration: 5 cc. orange juice.

Group X. Basal ration: 5 cc. orange juice, 10 cc. meat extract.

Group XI. Basal ration: 10 cc. meat extract.

Group XII. 5 gm. raw beef, 10 gm. rolled oats, water.

Discussion and Interpretation of Results

The animals on the basal ration showed the first symptoms of scurvy in 24 days. Death occurred in two of the animals on the 39th and 40th days respectively. The third animal, which had a well developed case of scurvy was placed on a curative diet on the 40th day. (See Chart VIII, Table II.)

The four animals which received the 5 cc. of orange juice in addition to the basal ration grew normally for 60 days (Chart IX). At the end of this time they were used for another experiment. A study of Charts IX and X shows that the growth curves of the animals receiving the basal ration, 5 cc. of orange juice and 10 cc. of meat extract run almost parallel with the growth curves of the animals on the protective diet.

Of the animals on the basal ration and 10 cc. of meat extract, Pig 37 showed symptoms of scurvy on the 25th day, and death occurred prematurely on the 28th day. Pig 38 showed first evidences of scurvy on the 36th day, and death occurred on the 54th day. Pig 39 showed scurvy symptoms on the 29th day, resulting in death on the 54th day. Pig 40 is a good example of the

difference in resistance which some animals have for the disease. This animal showed scurvy symptoms on the 40th day, but lived for 69 days on the diet, at the end of which time raw rhubarb was substituted for the meat extract (Chart XI Table II).

The fact that guinea pigs were able to grow normally on a diet of oats, water ad lib. and 5 cc. of orange juice, further confirms the fact that guinea pig scurvy is not induced by the physical character of the diet. That the addition of 10 cc, of meat extract to a protective diet did not interfere with normal growth and the general welfare of the animals, removes the possibility that meat extract was responsible for the condition of the animals in the first series of experiments.

A comparison of the growth curves of the animals in the first series, (Charts II, III, IV, V) with the growth curves in the second series (Chart XI) shows that while the onset of the disease was delayed, and the life of the animal was appreciably lengthened, scurvy did not fail to develop.

In the second series of experiments with beef extract we have showed that meat extract when added to a protective diet did not interfere with normal growth in guinea pigs. With this factor eliminated we are able to conclude that meat extract when fed in an amount equivalent to an extract from 20 grams of meat does not contain a sufficient amount of the antiscorbutic vitamine to influence the course of scurvy in guinea pigs.

The diet of raw beef and rolled oats was not relished by the animals and in no case was the daily ration ever entirely consumed, but a fair idea of the average daily consumption was obtained by measuring back the uneaten portions. The average daily consumption of raw meat, per animal, for the first 15 days of the experiment was 2½ gm. As the disease progressed the average daily intake fell below this amount.

Two of the animals died on the 24th and 28th days, respectively, and though they showed no marked symptoms of scurvy during life, autopsies showed in both cases, beaded and hemorrhagic costochondral junctions. Pig 51 died on the 22nd day in an extremely emaciated condition. During life no scorbutic symptoms were manifest and postmortem failed to show any evidence of the disease. Pig 54 exhibited typical scurvy symptoms during life, but autopsy failed to reveal scurvy lesions (Chart XII Table II).

The fact that the meat ration was not well eaten by the animals, and that the average loss of body weight was 39 per cent, raises the question, To what degree is inanition responsible for the symptoms produced? Holst and Froelich (1907) and Cohen and Mendel (1918) have shown that guinea pigs on starvation diets do not exhibit tenderness and swelling of the joings, hemorrhages of the muscles and fragility of the bones. They are of the opinion that inanition can play only a minor role, if any, in the production of the commom symptoms of scurvy. We may conclude then that raw beef, in the amounts we have been able to feed does not influence the course of scurvy in the guinea pig.

We concluded at the close of our experiments in 1918 and 1919 that when water extracts of raw lean beef were fed representing 5, 10, 15, and 20 gms. of raw beef no difference could be noted in the time of onset of scurvy or in the length of life of the experimental animals. Since then the chemical behavior of Vitamine C has been studied with respect to its susceptibility to oxidation and it has been shown in a conclusive manner that oxidation has a decidely destructive action upon solutions containing the antiscorbutic vitamine.

The meat used in the experiment came from a local market. It was impossible to ascertain the method of preservation previous to the time of purchase. Such factors as enzyme action during the process of aging, or freezing and thawing, if the meat were preserved in the frozen state, might have an influence

upon the antiscorbutic content of beef. The processes involved in the preparation of the cold water extract of beef such as the grinding of the meat, the thorough mixing with tap water, the subsequent standing of the mixture for twelve hours or more, and the separation of meat pulp and extract, are processes which facilitate oxidation.

It may be possible that the explanation for the antiscorbutic properties of meat in the diet of Arctic explorers and inhabitants of the polar regions lies in the fact that the meat is obtained almost entirely from the seal and the bear and includes not only muscle tissue but other tissues as the liver; that it is eaten in large quantities and for the most part in the fresh raw state.

It has been demonstrated that vegetables and milk cannot be considered from an antiscorbutic standpoint as standard and uniform food stuffs. Hess and Unger (1919) have demonstrated the superiority of young over old carrots for the protection of guinea pigs against scurvy. Barnes and Hume (1919) working with two varieties of dried milk powders found the older variety to be much superior in its antiscorbutic properties. They attribute the superiority of the older milk powder to the fact that the milk from which the powder was prepared came from cows which were grass fed the year round. Steenbock and Ellis (1920) and Dutcher, Eckles, Dahle, Mead and Shaeffer (1920) have shown that the antiscorbutic potency of milk varies with the diet of the lactating animal.

It may be that meat is not a standard and uniform food stuff from the antiscorbutic standpoint and varies in its Vitamine C. content with certain factors, such as the ration of the animal, or it may be that Vitamine C. in the animal organism is concentrated in glandular tissues. Parsons (1920) has shown that the liver, spleen and kidney contain relatively large amounts of Vitamine C. so that 10 gm. of rat's liver per day is sufficient to protect guinea pigs from scurvy whereas the same animal's muscle tissue did not.

Funk (1922) deems it unjustifiable to determine the nutritive value of animal tissues by using animals that normally live on a vegetable diet, and he suggests that the variation in the anti-scorbutic value of meat in man and guinea pigs may be due to just such procedures. The results of Parsons(1920) with rats' livers would not be in accord with Funk's view.

The recent experimental investigation of Vedder (1922) of the United States Army at Fort Sam Houston, Texas, confirms our experimental evidence. He found that considerable amounts of erythrocytes, voluntary muscle and heart muscle all failed to prevent the development of scurvy or even prolong the depletion period. On the other hand various viscera - the liver, lungs, spleen and pancreas, were found to possess anti-scorbutic properties. This work with viscera confirms the experimental work of Parsons (1920).

Table I.

Series I.

Influence of Cold Water Extract of Raw Beef upon Scurvy in Guinea Pigs (2 Cc. from 1 Gm. of Beef). Basal Diet Consisted of Oats and Water ad Libitum and 25 Cc. of Autoclaved Milk.

Group and diet.	Animal No.	Initial weight.	Final weight.	Gain or loss.	Day on which first scurvy symptoms were noted.	Average time for group	Length of life.	Average life for group	Post-mortem examination	Remarks
		gm.	gm.	per cent		days	days	days		
I	1	304	201	-33.9	10	11.7	19	21.7	Scurvy	
Basal diet.	2	225	150	-33.3	7		23		"	
	3	280	160	-42.9	15		20		"	
	4	160	120	-25.0	15		25		"	
II	5	358	250	-30.2	23	18.2	28	25.2	" and pneumonia	
Basal diet + 10 cc. beef extract.	6	223	210	- 5.8	13		23		Scurvy	
	7	253	160	-36.8	22		25		"	
	8	189	160	-15.3	15		25		"	
III	9	345	240	-30.4	14	14.2	23	24.0	"	
Basal diet + 20 cc. beef extract.	10	240	157	-34.5	17		26		"	
	11	211	170	-19.5	13		25		"	
	12	174	130	-25.3	13		22		"	
IV	13	257	183	-28.8	15	14.6	25	25.6	" and pneumonia	

Table I.

Series I. (cont.)

Influence of Cold Water Extract of Raw Beef upon Scurvy in Guinea Pigs (2 Cc. from 1 Gm. of Beef). Basal Diet Consisted of Oats and Water ad Libitum and 25 Cc. of Autoclaved Milk.

Group and diet.	Animal No.	Initial weight.	Final weight.	Gain or loss.	Day on which first scurvy symptoms were noted.	Average time for group.	Length of life.	Average life for group	Post-mortem examination	Remarks
IV (cont.)		gm.	gm.	per cent.		days	days	days		
Basal diet + 30 cc/ beef extract.	14	330	245	-25.8	15		31		Scurvy	
	15	226	167	-26.1	14		21		"	
V	17	365	248	-32.1		18.6	30	26.0	Scurvy	
Basal diet + 40 cc. beef extract.	18	278	195	-29.9	17		25		"	
	19	233	200	-14.2	13		22		" and pneumonia	
	20	153	165	+ 7.8	26		27		Scurvy -	(Cured on orange juice; no histological evidence of scurvy.
VI	21	247	135	-45.3	20	17.5	25	26.0	Scurvy	
Oats and water.	22	183	120	-34.4	15		24		"	
	23	175	110	-37.1			29		"	

Table II.

Series II.

Confirmatory Data Relative to the Influence of Beef Extract in the Presence ofOrange Juice.
Basal Diet Consisted of Oats and Water ad Libitum + 20 Cc. of Pasteurized Milk.

Group and diet.	Animal No.	Initial weight.	Final weight.	Gain or loss.	Day on which first scurvy symptoms were noted.	Average time for group	Length of life.	Average life for group.	Postmortem examination	Remarks
		gm.	gm.	per cent		days	days	days		
VIII Basal diet.	24	311	220	-29.3	29	24.6		39.5		Changed to curative diet on 40th day.
	25	286	180	-37.1	26		40		Scurvy	
	26	265	205	-22.6	19		39		"	
IX Basal diet + 5 cc. orange juice.	28	365	360	- 1.4						(Changed to another diet on 60th day.
	29	367	435	+18.5						" " "
	30	320	393	+22.8						" " "
	31	230	245	+ 6.5						" " "
X Basal diet + 5 cc. orange juice + 10 cc. beef extract.	32	265	333	+25.7						" " "
	33	235	360	+53.2						" " "
	34	283	380	+34.3						" " "
	35	312	305	- 2.2						" " "

Table II.

Series II. (cont.)

Confirmatory Data Relative to the Influence of Beef Extract in the Presence of Orange Juice. Basal Diet Consisted of Oats and Water ad Libitum + 20 Cc. of Pasteurized Milk.

Group and diet.	Animal No.	Initial weight	Final weight	Gain or loss.	Day on which first scurvy symptoms were noted.	Average time for group.	Length of life.	Average life for group.	Postmortem examination	Remarks
		gm.	gm.	per cent		days	days	days		
XI	37	295	294	- 0.3	25	31.5		54.6	Scurvy	Choked to death on 28th day.
Basal diet + 10 cc. beef extract	38	280	175	-37.5	36		54		"	
	39	268	199	-25.7	29		41		"	
	40	425	260	-38.8	36		69			Changed to another diet on 69th day.
XII	51	320	175	-45.3		20 (?)	22	26.0	General inanition	
Oats + 5 gm. chopped raw beef	52	325	225	-31.8			24		Scurvy	
	53	250	147	-41.2	20		30		General inanition	No scurvy lesions.
	54	271	180	-34.6			28		Scurvy	

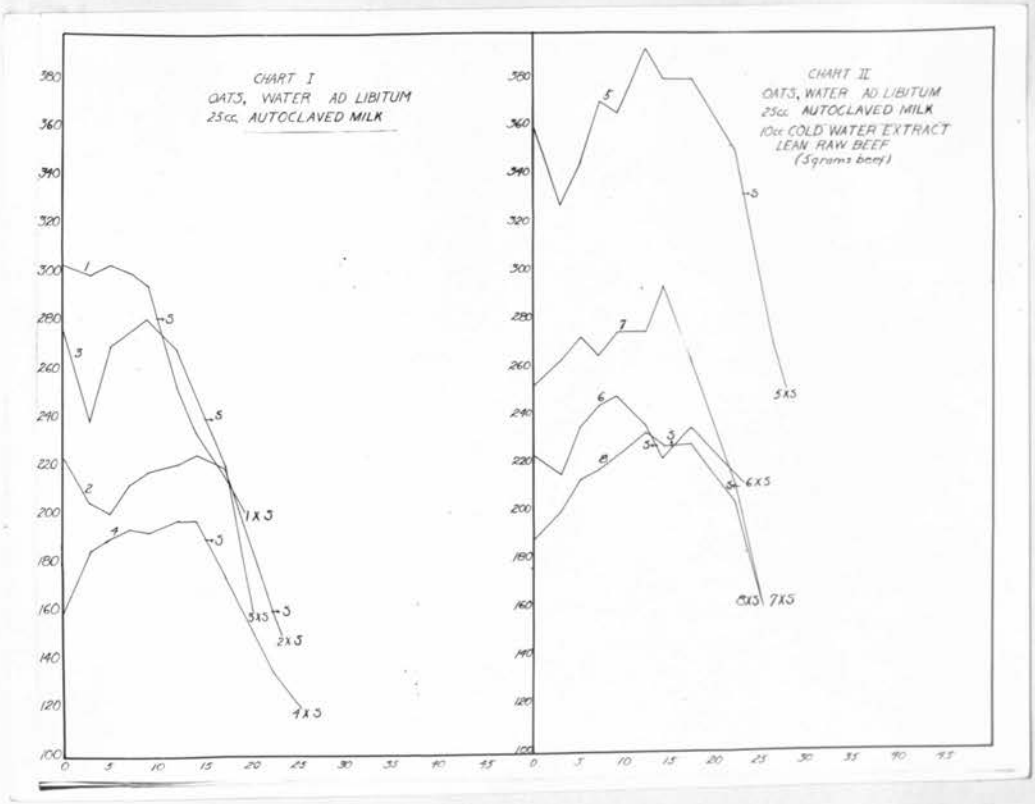
CHART I
OATS, WATER AD LIBITUM
25cc. AUTOCLAVED MILK
1X5
2X5
3X5
4X5
CHART II
OATS, WATER AD LIBITUM
25cc. AUTOCLAVED MILK
10cc COLD WATER EXTRACT
LEAN RAW BEEF
(5grams beef)
5X5
6X5
7X5
8X5

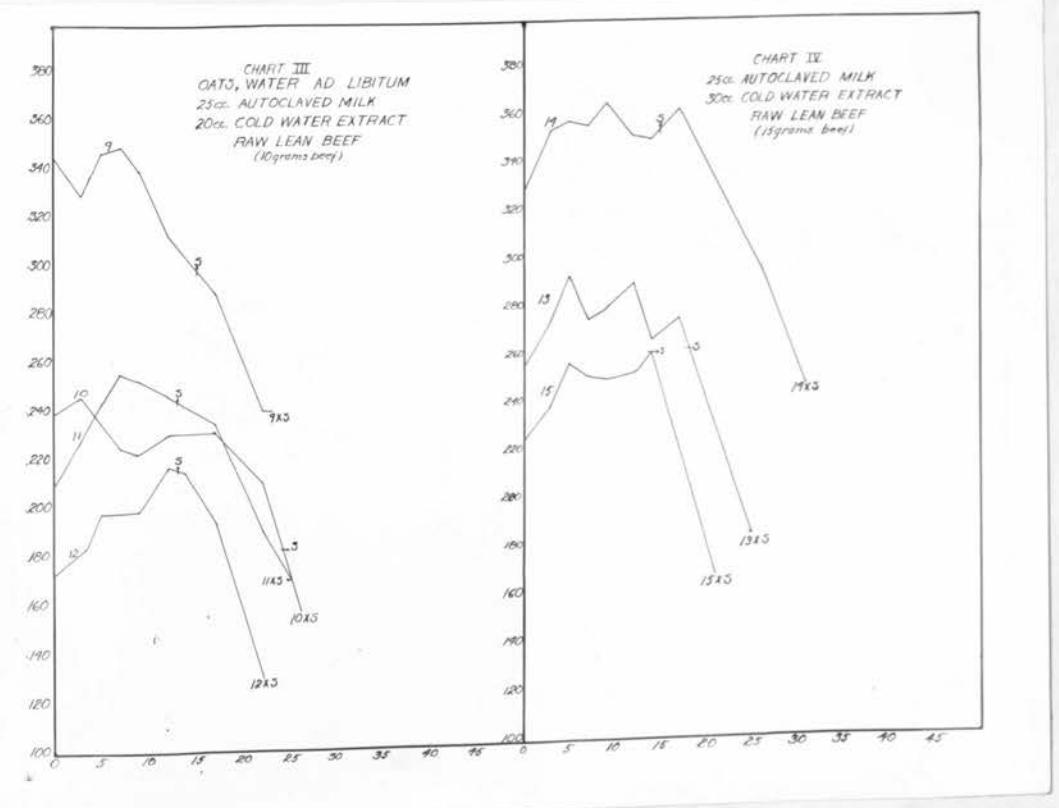
CHART III
OATS, WATER AD LIBITUM
25cc. AUTOCLAVED MILK
20cc. COLD WATER EXTRACT
RAW LEAN BEEF
(10grams beef)
9X5
10X5
11X5
12X5
CHART IV
25cc. AUTOCLAVED MILK
30cc. COLD WATER EXTRACT
RAW LEAN BEEF
(15grams beef)
14X5
13X5
15X5

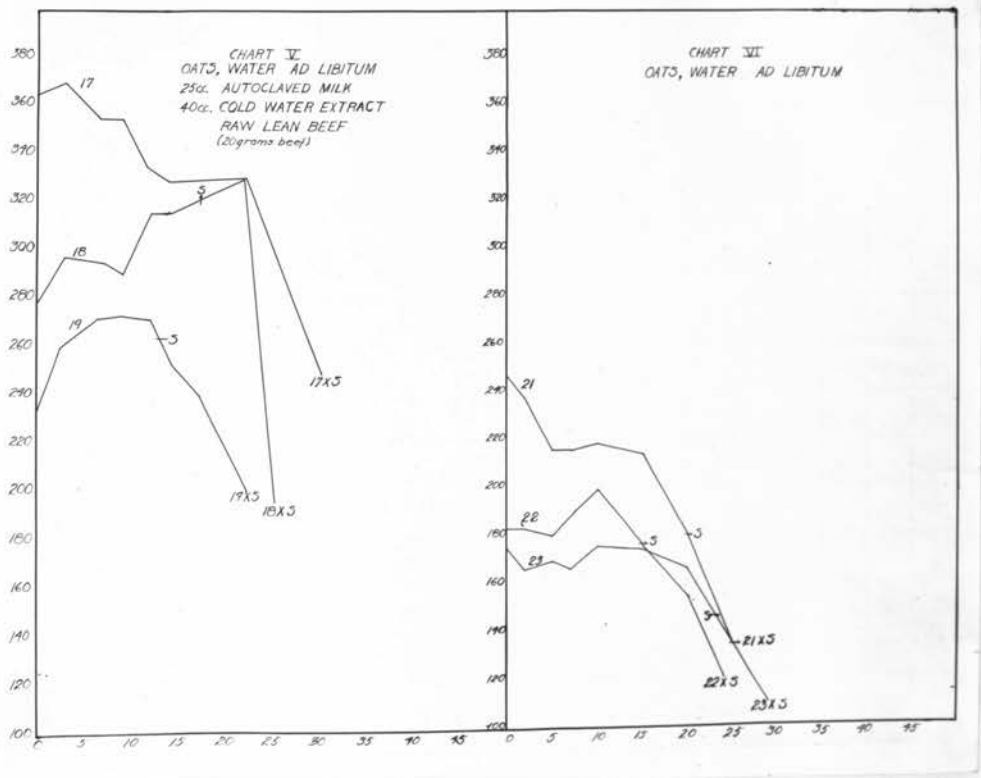
CHART V
OATS, WATER AD LIBITUM
25cc. AUTOCLAVED MILK
40cc. COLD WATER EXTRACT
RAW LEAN BEEF
(20grams beef)
17
18
19
17X3
18X3
19X3
CHART VI
OATS, WATER AD LIBITUM
21
22
23
21X3
22X3
23X3

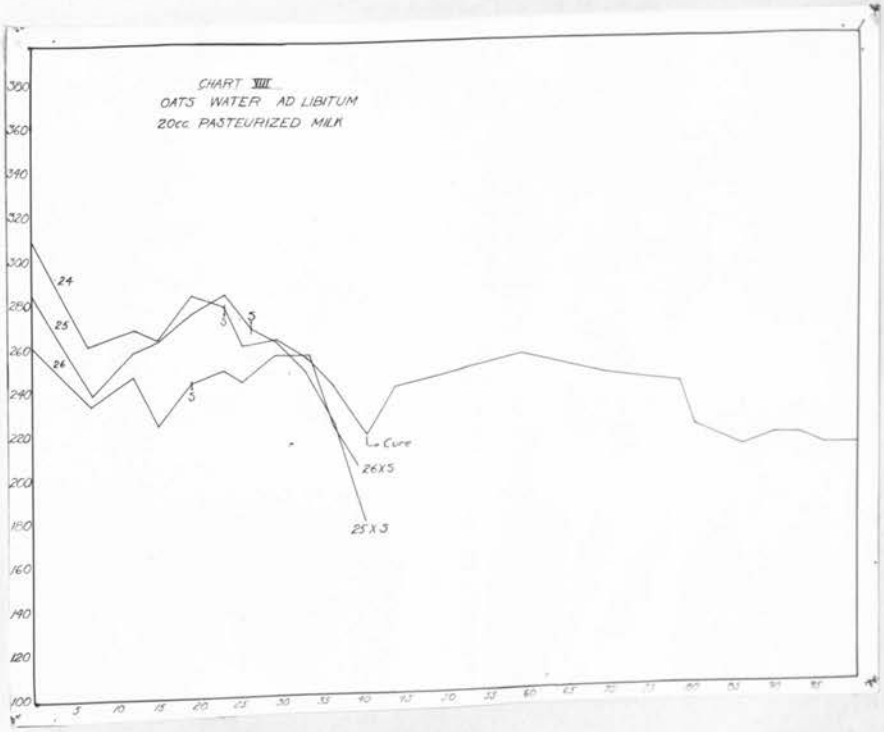
CHART VIII
OATS WATER AD LIBITUM
20cc. PASTEURIZED MILK
24
25
26
Cure
26X3
25X3

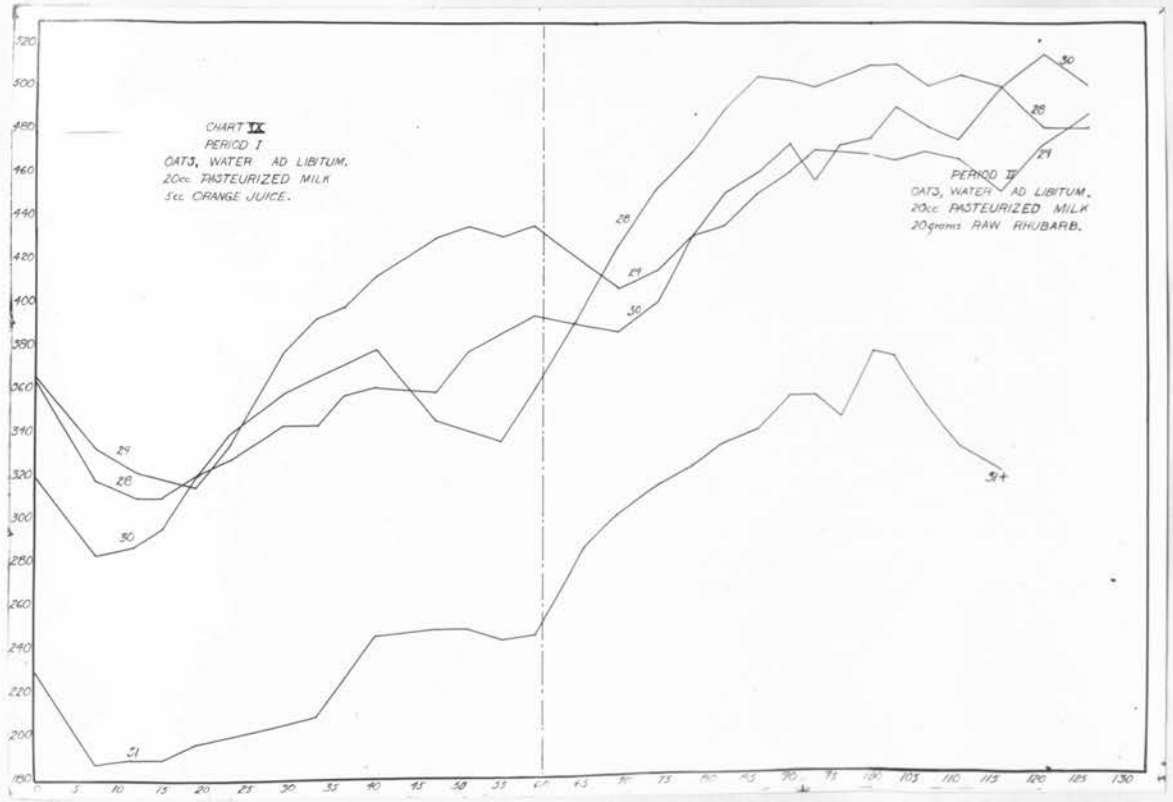
CHART II
PERIOD I
OATS, WATER AD LIBITUM.
20cc PASTEURIZED MILK
5cc ORANGE JUICE.
PERIOD II
OATS, WATER AD LIBITUM.
20cc PASTEURIZED MILK
20grams RAW RHUBARB.
29
28
30
31
28
29
30
30
28
29
31+

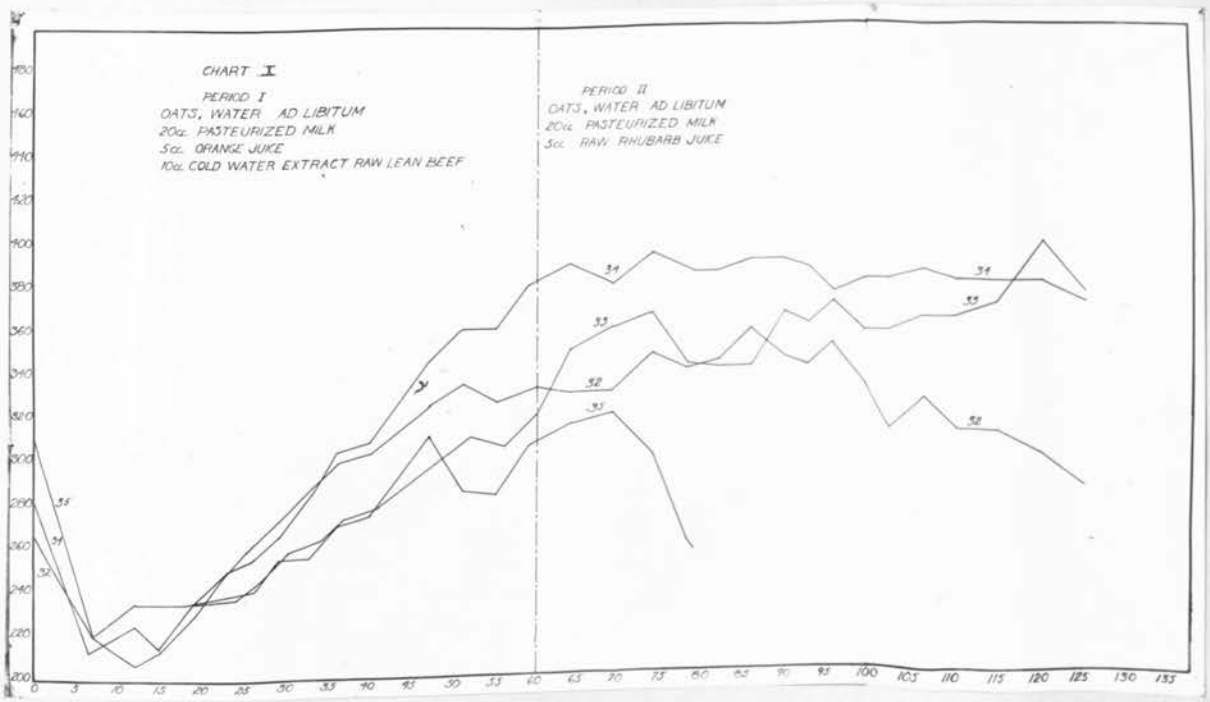
CHART I
PERIOD I
OATS, WATER AD LIBITUM
20cc PASTEURIZED MILK
5cc ORANGE JUICE
10cc COLD WATER EXTRACT RAW LEAN BEEF
PERIOD II
OATS, WATER AD LIBITUM
20cc PASTEURIZED MILK
5cc RAW RHUBARB JUICE
34
33
32
31
34
33
32
33
31
32

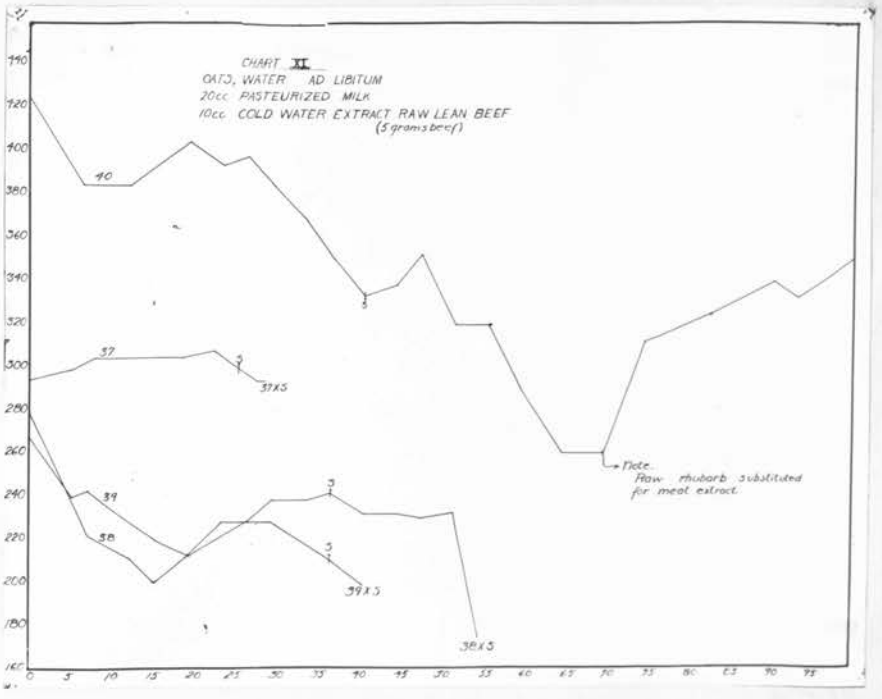
CHART XI
OATS, WATER AD LIBITUM
20cc PASTEURIZED MILK
10cc COLD WATER EXTRACT RAW LEAN BEEF
(5 grams beef)
40
37
37X5
39
38
39X5
38X5
Note
Raw rhubarb substituted
for meat extract

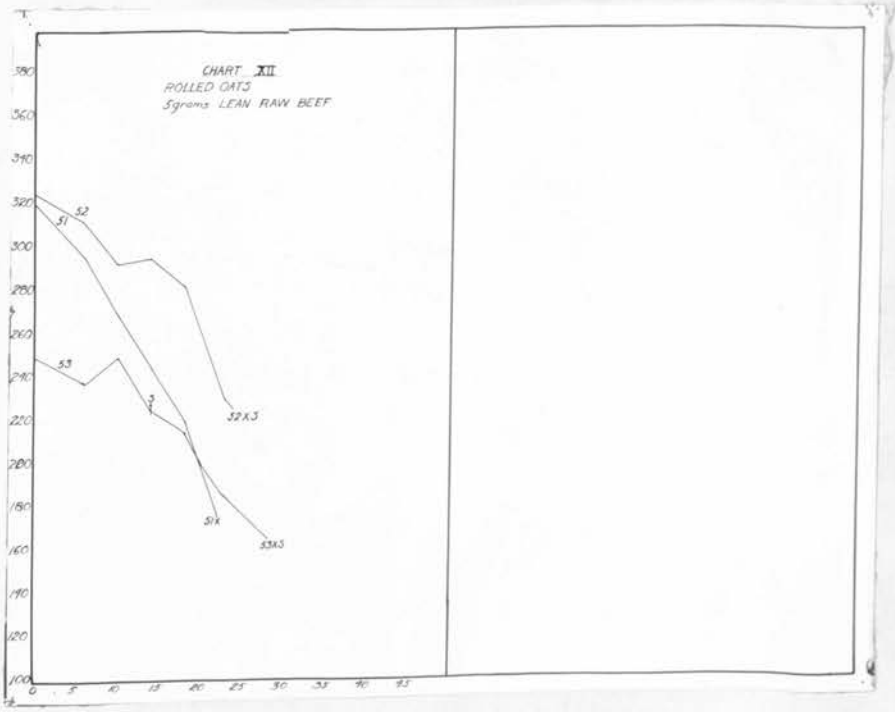
CHART XII
ROLLED OATS
5grams LEAN RAW BEEF
51
52
53
52X5
51X
53X5

Part II. The Antiscorbutic Properties of Summer Milk.

This problem presented itself while conducting the second series of experiments with the meat extract. During this time it was observed that the animals in the control group (Group VIII) Series II (Part I) on the basal ration of oats, water and 20 cc. pasteurized milk were still gaining on the 26th day, a longer period of time than the average length of life of the animals in the control group (Group I) Series I. A brief summary of final results shows that:

1. The average time of the onset of scurvy in the animals of the control group Series I and II was 12 and 26 days respectively.

2. The average length of life of the animals in the two groups was 12 and 26 days respectively. The only variable factor in the diet of the two groups was the milk. The animals in the first series received 25 cc. of autoclaved milk while those in the second series received 20 cc. of pasteurized milk.

Upon inquiry as to the source of the pasteurized milk it was learned that it came from a dairy herd receiving the following ration.

Grain	Ground oats Ground corn Ground barley Wheat bran and screenings Linseed or oil ~~Alfalfa~~ meal	Roughage	Corn silage Alfalfa hay

On May 1, 1919 a part of this herd were put on green grass pasture. On this date the second series of experiments had been running eleven days. Thus these animals received for the major part of the experimental period pasteurized "green grass" milk. This fact suggested that possibly the explanation for the

delay of scurvy symptoms and for the prolongation of the life of the animals in Series II might be traced to the diet of the cow.

Andrews (1912) observed that infantile beriberi occurred when children were fed by mothers suffering with beriberi. McCollum and Simmonds (1918) found in their experimental work with rats that before Vitamines A or B appeared in the milk of the rat there must have been a goodly supply of these substances in her diet. If Vitamines A and B do not appear in milk unless they are supplied by the diet of the lactating animal it would seem reasonable to suppose that Vitamine C. would be supplied in this manner also.

Chick, Hume and Skelton (1918) who first work with milk quantitatively found that when 30 to 50 cc. of raw milk were taken daily, death from scurvy ensued in about 30 days. When 50 cc. were consumed daily, the life of one animal was prolonged to 75 days; when the daily intake of milk ranged from 85 to 135 cc. the animals remained in good health and were protected from scurvy. There are no data to show whether this milk was from cows on green pasture or on winter rations.

The difference in protective power of the two milks, the winter milk in Part I Series I and the summer milk in Part I Series II and the discrepancies in the results of other investigators as to the relative protective power of milk, led us next to investigate the antiscorbutic properties of milk from cows on summer pasture.

Experimental Procedure

The milk used was from a ~~Jersey~~ mixed herd pastured on green grass. Frequent Babcock tests gave an average fat content of 4 per cent. The separator skim milk was separated in a commercial separator at the college. Frequent fat tests of this milk never showed more than one tenth of one per cent butter fat.

20 cc. of milk were fed in order to make the results comparable quantitatively with those of the pasteurized milk in Series II, Part I.

The animals were divided into three groups with diets as follows:

Group I. Oats, water, 20 cc. raw summer milk.

Group II. Oats, water, 20 cc. autoclaved summer milk.

Group III. Oats, water, 19 cc. raw separator skimmed milk, equivalent to 20 cc. whole milk.

Discussion and Interpretation of Results

The results of the experimental work are set forth in Table III and Charts XIII, XIV, XV. The examination of the data in the table and charts shows that there is a marked similarity and a logical difference in the results of the three groups. The fact that scurvy symptoms appeared in the animals of Group II on an average of 25 days, as compared with an average of 37 and 32 days in Groups I and III respectively, and that the average length of life of the animals in Group II was 50 days as compared with an average of 63 and 71 days in Groups I and III respectively is accounted for by the unstable property of Vitamine C when subjected to heat in an autoclave.

In Groups I and III, the growth curves, the average time of onset, and the average number of days the animals lived, run closely parallel. These results are somewhat surprising for it was expected that the animals in Group I receiving whole milk would be in a better physical condition than the animals in Group III which received the skimmed milk. According to McCollum (1917) about one half of Vitamine A contained in milk is removed with the butter fat in skimming with the centrifugal separator, and one half remains behind in the skimmed milk. Hopkins (1920) has also observed experimentally the value of the skimmed milk as a source of Vitamine A and he is convinced that it contains much more of

this substance than its fat content would lead one to expect. It may be that this lack of Vitamine A would become manifest at a later period if the animals were on an otherwise adequate diet. As it was, the diets of the animals in both groups was deficient in Vitamine C. and after scurvy symptoms appeared, there followed a decline in weight of the animals in both groups. Barnes and Hume (1919) found that the separator skimmed milk seemed to agree with guinea pigs better than whole milk and that the two milks were of equal value as an anti-scorbutic. Vitamine requirements of animals vary (Harden and Zilva 1920) and it may be that a guinea pig's requirement for Vitamine A is not as great as that of other animals. It is possible that the guinea pig has the power to synthesize Vitamine A from its food as the rat does Vitamine C. (Parsons 1920)

The data from Part I and II which show the relative potency of winter and of summer milk as measured by the time of onset of scurvy and the length of life of the experimental animals have been summarized in Table IV.

Table IV

SUMMARY TABLE
Showing the Relative Antiscorbutic Properties
of Winter and of Summer Milks.

Group and Series of Experiment	Basal Ration	Average no. of days before onset of scurvy	Average length of life in days
Part I Series I, Group I	Oats, water, 20 cc. autoclaved winter milk	11.7	21.7
Part I Series II, Group VIII	Oats, water, 20 cc. pasteurized summer milk	24.6	39.5
Part II Group I	Oats, water, 20 cc. raw summer milk	37.0	63.0
Group II	Oats, water, 20 cc. autoclaved summer milk	25.0	50.0
Group III	Oats, water, 19 cc. raw separator skim milk	32.0	71.0

The fact that the animals in Groups I and III (Table II) developed scurvy at a later period and lived longer than the animals of Group VII Series II Part I can be accounted for by the difference in anti-scorbutic potency of raw and pasteurized milk. The results with autoclaved summer milk and pasteurized summer milk would indicate that they are of approximately equal rank in antiscorbutic value. Comparing the results obtained with animals on summer milk Group VIII (Series II, Part I) and Groups I, II, III (Part II) with the results obtained with the animals on winter autoclaved milk Group I (Series I, Part I) we find that pasteurized and autoclaved summer milk have about twice the potency that winter autoclaved milk, and that raw whole milk and raw separator skimmed milk have three times the antiscorbutic potency of winter autoclaved milk.

Since this work was done Hart, Steenbock and Ellis (1920) have reported studies of the antiscorbutic value of milk from cows which had been kept on dry feed for varying periods of time as contrasted with that of cows which were feeding on green pasture. Summer milk was found to be much richer in its antiscorbutic properties than winter milk - 15 cc. of summer pasture milk afforded protection against scurvy for one pig, but did not protect two others. On increasing the amount to 30 cc. two animals out of three were protected, the third developing scurvy in 8 weeks. 50 cc. were required daily for complete protection. In case of the dry feed milk 75 cc. daily were required for complete protection. Dutcher, Eckles, Dahle, Mead and Shaeffer (1920) have experimented with milk of cows on winter rations and on summer feed. They concluded that 20 cc. of summer milk were superior to 60 cc. of winter milk in anti-scorbutic value. Hess, Unger and Supplee (1920) have also shown that there is much more antiscorbutic vitamine in the milk of cows on pasture than on winter rations.

The results of these investigators fully confirm our results. Thus it appears fully demonstrated that the ration of a milk producing animal is a very

large factor in the relative anti-scorbutic potency of the milk produced, some samples of milk having at least three times as much anti-scorbutic vitamine as others, when the only variable factor of importance appears to be the food of the cow.

Table III,

Data on the Antiscorbutic Properties of Summer Milk. Basal Diet Consisted of Oats and Water ad Libitum.

Group and diet.	Animal No.	Initial weight.	Final weight.	Gain or loss	Day on which first scurvey symptoms were noted.	Average time for group.	Length of life	Average life for group.	Postmortem examination	Remarks
		gm.	gm.	per cent		days	days	days		
I	41	293	210	-28		37	37	63	Intestinal trouble.	
Basal diet +	43	280	220	-28	30		58			Put on curative diet.
20 cc. raw	44	265	222	-16			16		Intestinal trouble.	
summer milk.	45	230	179	-22			16		" "	
	50	290	250	-14	52		68		Scurvy	
	92	245	223	- 8	50		56		Scurvy	
	93	216	236	+ 8	17		66		Scurvy	
	94	187	196	+ 4	36		64		Scurvy	
II	46	310	275	-11	21	25	50	50		Put on curative diet.
Basal diet +	47	250	290	+14	25		30			" " " "
20 cc. raw	48	283	211	-25	30		51		Scurvy	
summer milk autoclaved.	49	290	220	-24			16		Intestinal trouble.	
III	55	239	270	+11		32	25	71		Choked to death accidentally
Basal diet +										
19 cc. raw	56	221	225	+ 2			20			" "
separated	57	222	235	+ 5	40		80		Scurvy	
milk	89	215	184	-14	31		76		Scurvy	
	90	245	255	+ 4	40		54		Scurvy	
	91	208	203	- 2	17		71		Scurvy	Put on an adequate diet

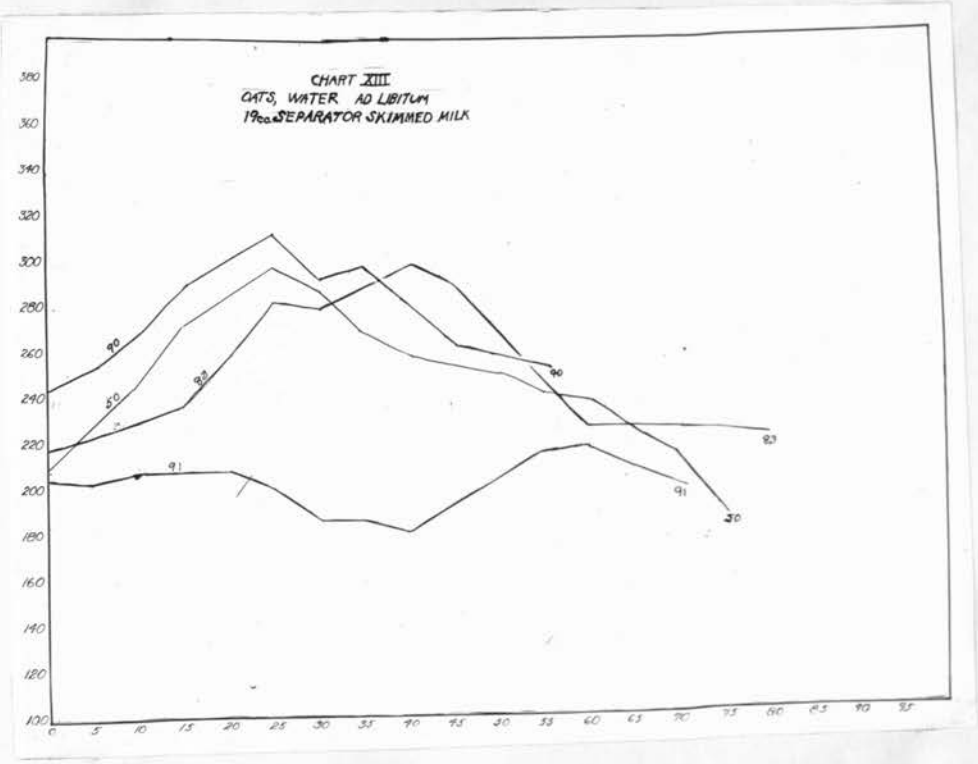
CHART XIII
OATS, WATER AD LIBITUM
19cc SEPARATOR SKIMMED MILK
90
50
83
91
90
83
91
30

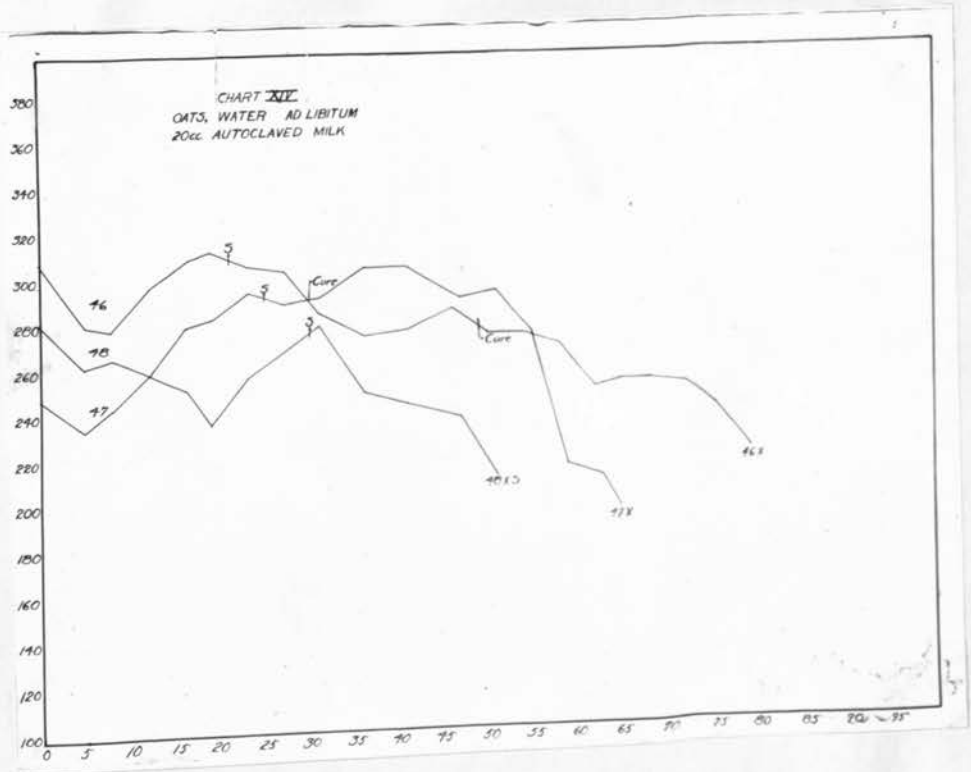
CHART XIV
OATS, WATER AD LIBITUM
20cc AUTOCLAVED MILK
16
18
17
5
5
3
Cure
Cure
46
40.5
41.8

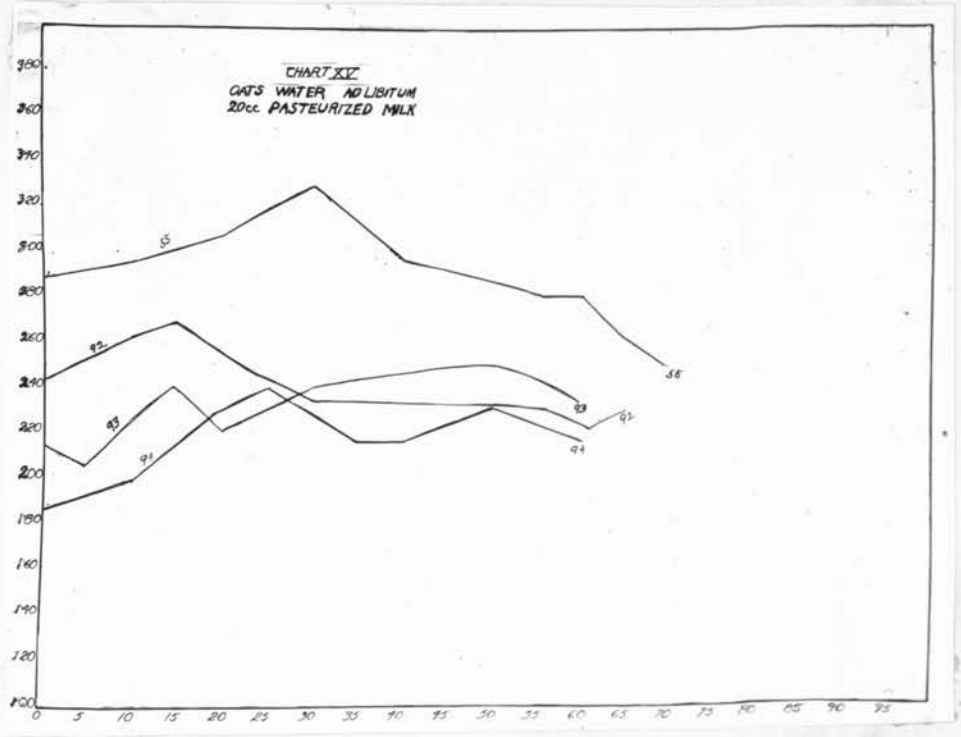
CHART XV
CATS WATER AD LIBITUM
20cc. PASTEURIZED MILK
350
340
330
320
310
300
290
280
270
260
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95
55
92
93
91
58
93
97
94

Part III. The Antiscorbutic Properties of Raw Rhubarb.

Experimental Procedure

The animals in Group IX, Series II Part I which had been on a protective diet of oats, water, 20 cc. of pasteurized milk and 5 cc. of orange juice were transferred from this diet on the 60th day to a diet consisting of oats, water, 20 cc. of milk and 20 gm. of fresh raw rhubarb. The animals in Group X which had been on a diet of oats, water, 20 cc. of pasteurized milk, 5 cc. of orange juice and 10 cc. of beef extract were placed on a diet consisting of oats, water, 20 cc. pasteurized milk and 5 cc. of raw rhubarb juice.

The rhubarb was picked fresh daily, weighed, cut in small pieces and placed in the cages. The animals ate it with apparent relish and very seldom left uneaten portions. The rhubarb juice was prepared by putting the rhubarb through a meat grinder and then straining it through a cloth. This was fed by hand by means of a large medicine dropper.

Discussion and Interpretation of Results

The details of the results are set forth in Table V and Charts IX and X. Three of the animals in Group IX made normal growth and at the end of the 70 days were in fine physical condition. Animals 28 and 29 were killed on the 70th day. Postmortem examination showed no evidences of scurvy. The muscle tissue was firm and pink, the intestinal tract free from any sign of congestion, and the costochondral junctions were clean cut. Pig 30 was placed on a normal mixed diet on the 70th day. Pig 31 grew normally for 47 days when it became "dumpy" and refused to eat. Death occurred on the 61st day. Postmortem examination showed a greatly distended bladder which contained in addition to

the urine a white putty like substance about the size of a pea. The alimentary tract was slightly congested. No scurvy symptoms were observed.

The animals in Group X on 5 cc. of rhubarb juice for the protective agent did not progress as favorably as did the animals in Group IX. Pig 33 was the only one of the group to make a gain in weight. Pig 35 never seemed to be a very strong and rugged animal. By referring to Table II, Series II, Group X, it will be noted that the original weight of this animal was 312 gms., that there was a steady decline in weight while on the orange juice diet. After he was transferred from the orange juice to the rhubarb juice the animal continued to lose in weight until death occurred on the 17th day of the rhubarb diet. Post mortem examination showed a stoppage in the duodenum just below the pylorus. The cecum and lower bowels were hemorrhagic, but there were no muscular hemorrhages or beaded ribs. Death was clearly due to intestinal trouble. Pig 24 from Group VIII Table II on a scurvy diet of oats, water and 20 cc. pasteurized milk showed first symptoms of scurvy on the 29th day. On the 37th day scurvy was so well developed that the animal moved with difficulty around its cage and squealed whenever touched. On the 40th day, 5 cc. of raw rhubarb juice were added to the diet. From this time on the animal showed signs of improvement. On the 42nd day of the curative diet he was eatingwell, he moved around the cage in a normal manner, and no longer squealed when handled. Soon after this the animal refused to eat, and there followed a decline in weight until death occurred on the 70th day of the curative diet. Postmortem examination showed old scurvy lesions. The alimentary tract was not badly congested. It is probable that the scurvy had produced such profound changes in the animal's system that he was unable to recover even on a curative diet. The writer has observed death to occur in scorbutic animals after having been placed on curative diets of orange juice and green vegetables. The disease had apparently

progressed too far for them to recover.

Pig 40 (Table II Series II Group XI) was given 5 cc. of cooked rhubarb juice on the 69th day. At this time scurvy had progressed to the extent that both front ankles were badly swollen and the hind legs partially paralyzed. Improvement in the animal was noted shortly after this addition to the diet. At the end of 70 days the animal was in fine physical condition having made a gain in weight of 155 gms. He was transferred at this time to a mixed diet.

This work with rhubarb is of a preliminary nature and does not warrant us in drawing conclusions as to the relative value of rhubarb as an anti-scorbutic food. The results are sufficiently definite to warrant us to predict, however, that raw or cooked rhubarb has anti-scorbutic properties.

Table V.

Data on the Antiscorbutic Properties of Raw and Cooked Rhubarb
Basal diet, oats, water ad libitum, 20 cc. pasteurized milk

Pigs used in this experiment transferred from Groups VIII, IX, X, XI, Part I, Series II

Group and diet	Animal No.	Initial weight	Final weight	Gain or loss	No. of days on diet	Postmortem examination	Remarks
		gm.	gm.	percent			
IX	28	390	500	+ 30	70	No scurvy	Killed on 70th day
Basal diet, 20 gm.	29	435	490	+ 12	70	No scurvy	" " " "
raw rhubarb	30	385	505	+ 31	70	No scurvy	Placed on mixed diet on 70th day
	31	245	300	+ 22	62	Intestinal trouble	
X	32	333	305	- 8.4	70	No scurvy	Killed on 70th day
Basal diet, 5 cc.	33	350	385	+ 10	70	No scurvy	" " " "
raw rhubarb	34	390	363	- 7	70	No scurvy	" " " "
juice	35	315	255	- 20	19	Intestinal trouble	
VIII	24	220	165	- 25	70	Evidence of old scurvy lesions	Cause of death unknown
Basal diet, 5 cc. raw rhubarb juice							
XI	40	260	415	+ 37	70		Placed on mixed diet on 70th day
Basal diet, 5 cc. cooked rhubarb juice							

SUMMARY

Water extracts of raw lean beef representing 5, 10, 15 and 20 gm. of raw beef, when fed with a basal diet of oats, water ad libitum and autoclaved milk failed to delay the onset of scurvy or to protect guinea pigs from scurvy. 5 gm. of raw lean beef fed daily to guinea pigs failed to protect from scurvy.

20 cc. of raw summer milk (either whole or separator skimmed) has three times the protective potency of 25 cc. autoclaved winter milk. 20 cc. of pasteurized summer milk and 20 cc. of autoclaved summer milk have twice the potency of 25 cc. of autoclaved winter milk.

20 gm. of raw rhubarb when fed daily, afforded protection from scurvy to 3 guinea pigs for a period of 70 days. 5 cc. of raw rhubarb juice, fed daily, protected 3 guinea pigs from scurvy for a period of 70 days. 5 cc. of raw rhubarb juice cured one pig of scurvy. 5 cc. of cooked rhubarb juice cured one scorbutic pig.

4-22-8M

ACKNOWLEDGEMENTS

The author takes this opportunity to express her appreciation to Professor R. A. Dutcher, who suggested the investigation and under whose supervision the work was carried on.

The writer also wishes to thank Professor Alice Biester for the advice and help given in writing this thesis.

LITERATURE CITED

American Pediatric Society

1898, Collective investigations on infantile scurvy in North America. Arch. of Ped. 15, 48

Andrews, V. L.

1912, Infantile beri beri. Philippine Jour. Sci., Sec. B, 7, 67 - 88.

Anderson , E. V.

1921, Influence of heat and oxidation upon the nutritive and anti-scorbutic property of cow's milk. A thesis presented to the Graduate School, University of Minn.

Anderson, E. V., Dutcher, R. A., Eckles, C. H., and Wilbur, J.W.

1921, Influence of heat and oxidation on the nutritive property of cow's milk. Science 53, 446

Bachstrom,

1734, Observetiones circa scorbutum eiusque indolem, causas, signa et curam Cited by Hess 1920

Barlow, T.

1894, Infantile scurvy and its relation to rickets. Lancet, London, 2,1073

Barnes, K.E. and Hume E.M.

1919, Relative antiscorbutic value of fresh, dried and heated cow's milk. Biochem. Jour. 13, 306

Baumann, L. and Howard, C.P.

1917, Mineral metabolism of experimental scurvy of guinea pig. Am. J. Med. Sci., 153, 650 - 666

Borish, R.

1919, Ueber Skorbut, Archiv. f, Klin. Med. 130, 151, Cited by Hess, 1920

Chick, H., Hume, E.M., and Skelton, R.F.

1918, The antiscorbutic value of cow's milk. Biochem. J. 12, 131 - 153.

Cohen, B., and Mendel, L.B.

1918, Experimental scurvy of the guinea pig in relation to the diet. J. Biol. Chem. 35, 427 - 453.

Delf, E. M., and Tozer, F. M.

1918, The antiscorbutic value of cabbage. I. The antiscorbutic and growth-promoting properties of raw and heated cabbage. Biochem. J. 12, 416 - 447.

Delf, E. M., and Skelton, R. F.

1918, The antiscorbutic value of cabbage. II. The effect of drying on the antiscorbutic and growth-promoting properties of cabbage. Biochem. J. 12, 448 - 463.

Dutcher, R. A., Eckles, C. H., Dahle, C.D., Mead, S.W., and Schaeffer, O.G.

1920, Vitamine Studies VI. The Influence of thediet of the cow upon the nutritive and antiscorbutic properties of cow's milk. J. Biol. Chem. 45, 119 - 132.

Dutcher, R. A., Harshaw, H. M., and Hall, J. S.

1921, The effect of heat and oxidation upon the antiscorbutic vitamine. J. Biol. Chem. 47, 483 - 488.

Dyke, H. W.

1918, A report on an outbreak of scurvy in the South African native labour corps. Lancet, 2, 513 - 515.

Eykman, C.

1897, An attempt to control beri beri. Arch. Path. Anat.(Virchow's) 149,187.

Funk, C.

1911, The chemical nature of the substance which cures polyneuritis in birds induced by a diet of polished rice. J. Physiol. 43, 395 - 400.

Funk, C., and Dubin, H. E.

1922, The Vitamines, Williams and Wilkins, Baltimore.

Garrod, A. B.

1848, On the nature, cause and prevention of scurvy. Month. J. Med. Sci. 8, 457.

Givens, M. H. and Cohen, B.

1918, The antiscorbutic property of dessicated and cooked vegetables. J. Biol. Chem. 36, 127 - 145.

Givens, M. H., and McClugage, H.B.

1919, The antiscorbutic property of fruits. I. An experimental study of dried orange juice. Am. J. Diseases Children 18, 30 - 41.

Harden, A.

1919, The antiscorbutic properties of concentrated fruit juices. Biochem. J. 14, 171 - 177; 15, 521, 522.

Harden, A., and Zilva, S. S.

1918a, The differential behavior of the antineuritic and antiscorbutic factors towards adsorbents. Biochem. J. 12, 93 - 105.

1918b, The antiscorbutic factors in lemon juice. Biochem. J. 12, 259 - 269.

1918c, Note on the susceptibility of the antiscorbutic principle to alkalinity. Lancet 2, 320.

1920, The antiscorbutic requirement of the monkey. Biochem. J. 14, 131 - 134.

Harlan, G. P.

1917, Land scurvy in England. Brit. Med. Jour. 2, 46.

Hart, E.B., Steenbock, H. and Ellis, N. R.

1920, The influence of the diet on the antiscorbutic potency of milk. J. Biol. Chem. 42, 383 - 396.

Hart, E. B., Steenbock, H. and Lepkovsky, S.

1922, The antiscorbutic vitamine. I. A study of its solubility from dessicated orange juice. J. Biol. Chem. 42, 241 - 250.

Harvier, P.

1917, Epidemie de scorbut, Paris Med. 7, 394. Cited by Hess 1920.

Hehir, P.

1919, Scurvy, Indian. J. Med. Research. Spec. Indian Sci. Cong. No. 79 A 44-59, 79 - 82. Cited by Sherman and Smith 1922.

Hess, A. F.

1920, Scurvy, Past and Present. J. B. Lippincott Co., Philadelphia.

Hess, A. F. and Fish, M.

1914, Infantile Scurvy. The blood, the blood vessels, and the diet. Am. J. Dis. Child. 8, 386 - 405.

Hess, A. F. and Unger, L. J.

1918, The scurvy of guinea pigs. I. The experimental dietary. II. Experiments on the effect of the addition of fruits and vegetables to the dietary. J. Biol. Chem. 35, 479 - 486.

Hess, A. F. and Unger, L. J.

1919, The scurvy of guinea pigs. III. The Effect of age, heat and reaction on antiscorbutic foods. J. Biol. Chem. 38, 295 - 304.

1921, The destructive effect of oxidation on antiscorbutic vitamine. Proc. Soc. Exptl. Biol. Med. 18, 143.

Hess, A. F., and Unger, L. J., and Supplee, A. C.

1920, Relation of fodder to the antiscorbutic potency and salt content of

milk. J. Biol. Chem. 45, 229.

Holst, A., and Froelich, T.

1907, Experimental studies relating to ship beriberi and scurvy. J. of Hyg. 7, 634, 671.

1912, Uber Experimentellen Skorbut, Ztschr. f. Hyg. u. Infektionakrankh, 72.1

Hopkins, F. G.

1920, The present position of vitamines in clinical medicine. Brit. Med. J. 2, 147 - 160.

Howe, P. R.

1920, Food accessory factors in relation to the teeth. J. Home Econ. 12, 482.

Jackson, L., and Moody, A. M.

1916, Bacteriologic studies on experimental scurvy in guinea pigs. J. Infect. Diseases 19, 511 - 514.

Jackson, L., and Moore, J. J.

1916, Studies of experimental scurvy in guinea pigs. J. Infect. Diseases 19, 478 - 510.

Kramer

1721, Medicina Castrensis. Cited by Funk, 1922

La Mer, V. K., and Campbell, H. L.

1920, Changes in organ weight produced by diets deficient in antiscorbutic vitamine. Proc. Soc. Exptl. Biol. Med. 18, 32.

La Mer, V. K., Campbell, H. L., and Sherman, H.C.

1922, The effect of temperature and of the concentration of hydrogen ion upon the rate of antiscorbutic vitamin (Vitamine C). J. Am. Chem. Soc. 172 - 181.

Lind, J.

1757, A Treatise on Scurvy, London second edition.

1771, An essay on diseases incidental to Europeans in hot climates, second edition. 206.

McCarrison, R.

1919, The influence of the scorbutic diet on adrenal gland. Ind. J. Med. Research 7, 188 - 194.

McCollum, E. V.

1917a, Supplementary dietary relationships among our natural food stuffs. J. Amer. Med. Assoc. 68, 1379 - 1386.

1922, The Newer Knowledge of Nutrition. Macmillan Co., New York, second edition.

McCollum, E. V., and Pitz, W.

1917, The vitamine bypothesis and deficiency diseases, A study of experimental scurvy. J. Biol. Chem. 31, 229 - 253.

McCollum, E. V., and Simmonds, N.

1917, The minimum requirements of the two unidentified dietary factors for maintenance as contrasted with growth. J. Biol. Chem. 32, 181 - 193.

McCollum, E. V., and Simmonds, N.

1918, The nursing mother as a factor of safety in the nutrition of the young. Am. J. Physiol. 46, 275 - 313.

McCollum, E. V., Simmonds, N., Becker, J. E., and Shipley, P. G.

1922, Studies on experimental rickets 21. An experimental demonstration of the existence of a vitamine which promotes calcium deposition. Johns Hopkins, Hospital Bull. 33 - 229.

Magnus, O.

1555, History of the Northern Nations. Cited by Hess, 1920.

Medical and Surgical History of the

1888, War of the Rebellion. Washington, Part 3, Chap. 8. Cited by Sherman and Smith, 1922.

Nansen and Johansen

Cited by Hess 1920.

Netter

1899, Le Scorbut Infantile, La Semaine Medicale. No. 8. Cited by Hess 1920

Parsons, H. T.

1920, The antiscorbutic content of certain body tissues of the rat, the persistance of the antiscorbutic substance in the liver of the rat after long intervals on a scorbutic diet. J. Biol. Chem. 44, 587 - 602.

Pickens, R. M.

1917, A death from scurvy. Lancet, London 2, 21.

Sherman, H. C., and Smith, S. L.

1922. The Vitamin. The Chemical Catalog Co., New York

Stefansson, V.

1918, Observations on three cases of scurvy. J. Am. Med. Assn. 71, 1715 - 1918.

Vanmutelli, F.

1917, Un epedemia di porpora infettiva con manifestazione emorragico - scorbutiche tra la truppe combattenti, Il Policlinico, Sez Pratica, 24, 873. Cited by Hess, 1920.

Vallardi, C.

1918, Reforma, Med. 34, 793. Cited by Hess, 1920.

Vedder, E. B.

1922, The etiology of scurvy. IV. Observations concerning the physiologic action of the antiscorbutic vitaliment. Mil. Surgeon 50 - 534. Cited

from J. Amer. Med. Assoc. 78, 25.

Willcox, W. H.

1917, Rations in relation to disease in Mesopotamia. Lancet 2, 677.

Wiltshire, H. W.

1918, A note on the value of germinated beans in the treatment of scurvy and some points in its prophylaxis. Lancet 2, 811.

Wright, A. E.

1918, The causation and treatment of scurvy. Lancet 2, 725.

Zilva, S. S.

1919, Action of ultra violet rays on accessory food factors. Biochem. J. 13, 164.

1921, The influence of aeration on the stability of the antiscorbutic factor. Lancet, London, 478.

Zilva, S. S., and Wells, F. M.

1919, Changes in the teeth of the guinea pig produced by a scorbutic diet. Proceed. of the Royal Soc. London, B, 90, 505. Cited by Sherman and Smith, 1922.