The Influence of the Diet of the Cow and the Effect of Heat Upon the Antiscorbutic Potency of Milk

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THE INFLUENCE OF THE DIET OF THE COW AND THE
EFFECT OF HEAT UPON THE ANTISCORBUTIC
POTENCY OF MILK

BY

Lynn Copeland, B. S. (1922)

THESIS SUBMITTED TO THE GRADUATE FACULTY
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SCIENCE IN DAIRY
HUSBANDRY

BROOKINGS, SOUTH DAKOTA
MAY 1923
INTRODUCTION

For a long time there has been a belief among scientific investigators and medical men that certain diseases, both of man and animal were the direct result of a faulty diet. In other words, something which was needed by the body for maintenance of growth and health was thought to be frequently lacking in the diet of man and animal.

Attention was first called to these accessory substances thru investigations into the cause of the disease beri-beri. In 1884, the Japanese Navy, under the direction of Admiral Takaki, positively demonstrated that beri-beri was the result of a faulty diet, but it was Eijkman who first produced the disease experimentally in birds. Hopkins first spoke of food accessories about 1908. He had tried to feed young animals with diets of purified protein, carbohydrates, fat, and mineral and had met with failure. When the same diet was supplemented with small additions of milk, growth was promoted. It has just been stated how he called the unknown substances furnished by the milk, "accessory" food substances.

The name vitamine owes its origin to Casimir Funk. In attempting to isolate the unknown
substance, he found it in a nitrogen base. Since the factor was apparently vital and necessary, he coined the word "vitamine" (vital-amine). This name was at first applied only to the necessary substance, preventing the disease beri-beri.

In 1913 Osborne and Mendel found that rats when fed a diet of purified nutrients developed an eye disease which was speedily cured by the introduction of butter into the diet. It remained for McCollum and Simmonds to explain this eye disease (Xerophthalmia) on the basis of a lack of a specific unknown substance. This substance they termed Fat soluble A. McCollum also suggested the term water soluble B. as a temporary name for Funk's "vitamine."

A third factor, accessory, or vitamine was discovered when Holst and Froelich (1) of the University of Christianna in 1912 showed that the cause of scurvy was the absence of a certain unknown chemical substance from the diet. In 1917, Chick and Hume substantiated the work of Holst and Froelich and definitely added a third vitamine to the list.

Still more recently, another deficiency disease has been under investigation and Hess (2) has found in cod-liver oil a remedy for rickets, that he believes does not owe its deficiency to the
fat soluble A. His results suggest that the antirachitic vitamine is separate and distinct from any of those yet named, possibly vitamine D. Also the vitamine B is thought by some to be the name of a group of complexes rather than of a single entity.

However the vitamine hypothesis was not readily accepted at first. F. Rohmann (3) took vigorous exception and asserted that, "accessory food stuffs are not necessary for the continued maintenance of fully grown animals." Thus he says; "The assumption that some unknown substances are indispensable for growth is a convenient devise for explaining experiments that result in failure, a devise that becomes superfluous as soon as the experiment succeeds."

The name "vitamine," suggested by Funk has persisted, likewise the temporary nomenclature devised by McCollum. So we have the name Vitamine A, Fat soluble A, Fat soluble food accessory, antithalmic vitamine, etc. Similar terminology has been applied to the other unknown dietary essentials. Recently a compromise has been proposed which seems satisfactory to most investigators. The terminal (e) has been dropped from the word vitamine and the letters A, B, and C added to distinguish them. Thus we have;
Vitamin A
Vitamin B
Vitamin C
Vitamin D - ?

This method of nomenclature is used throughout the remainder of this paper.
VITAMIN A

It has been stated that Osborne and Mendel (4) reported their failure to induce growth in young rats on a diet of purified casein, starch, lard and inorganic salt mixtures. Working from this basis in 1912, McCollum found that growth could be produced if the lard was replaced by butterfat, but not if by olive oil, or vegetable oils of various kinds. Egg yolk and cod-liver oil however proved as effective as the butterfat. These results with those of Osborne and Mendel and those of Stepp showed that egg yolk, butterfat and cod-liver oil contained a growth factor which was missing in the other fats. McCollum named this unidentified dietary essential, fat-soluble A.

As the name given by McCollum indicates, Vitamin A is commonly found in the fatty extracts of cells and tissues of plants and animals, being in greatest abundance in cod-liver oil, butterfat and in the chlorophyll bearing parts of plants. Cabbage, spinach, and carrots contain this vitamin in fairly considerable quantities.

Vitamin A is an acknowledged growth promoting substance. At first it was believed, especially by Drummond (5) that this vitamin was not necessary for maintenance in adults. This is not generally regarded as fact at present, yet it
may be possible (altho not yet proven), that adults require somewhat less of this essential than do young growing animals.

Steenbäck and Boutwell (6) state the following symptoms as a result of the lack of vitamin A in the diet, "First in importance there is the failure of maintainance of life; second there is the often occurring inflammation of the eyes, a conjunctivitis or xerophthalmia, as pointed out by Osborne and Mendel; and third there may prevail the general condition of malnutrition of the skin as indicated by incrustations of the ears, warts of the nose, infection of the tail and feet and even sores on the body itself. All these occur singly or collectively." The pathologic condition, xerophthalmia has been demonstrated frequently in experimental animals. It occurs in man, especially children in the orient where the diet is restricted to a large extent. Mori (7) has described xerophthalmia in children in Japan as early as 1904.

Steenbäck and Boutwell have made statements on the relation of vitamin A to the yellow plant pigments. They found the carrot and yellow sweet potato to be especially rich as a source of this dietary essential. Their most important discovery was that yellow corn is fairly rich in vitamin A. They conclude, "yellow maize when fed in
maximum amounts as the sole source of vitamin A, contained just sufficient fat soluble vitamin to make normal growth possible. Reproduction was possible but was usually a failure. White corn does not contain any demonstrable amounts of the fat-soluble vitamin." Other investigators doubt the work of Steenbock and Boutwell (7) and apparently have discredited it. The question is still unsettled.

Nothing is known of the chemistry of vitamin A. It is stable to quite high temperatures, (8) and is easily destroyed by oxidization (9). It is soluble in, and can be extracted with alcohol, but not ether. Water is entirely ineffective in dissolving it.

**Vitamin B:** The first vitamin was reported and named by Funk in 1911. This dietary essential is now called "Vitamin B."

The ancient disease, beri-beri is a direct result of the lack of this vitamin in the diet. It is a form of neuritis, a nervous disorder, occurring in two forms, wet and dry. The beginning of beri-beri occurs with a feeling of lassitude and general depression. There is edema of the face and legs, loss of the ability to stand, and very frequently death follows. The disease has been so serious that much time was devoted to its study.
Among the early investigations of beri-beri the work of Takagi Kanehiro, of the Japanese Navy, is noteworthy. Beri-beri was a plague among the Japanese sailors whose diet consisted mainly of polished rice and fish. Takagi increased the protein allowance per sailor by supplying more meat and substituting barley for part of the polished rice. Beri-beri was diminished. While the Japanese officials then believed this result was due to the increased protein; it is now known that the addition of more meat and barley was the cause. Eijkman, in 1897, was the first to produce experimental beri-beri, or polyneuritis, by feeding poultry on polished rice. Later McCollum and Davis showed that the germ of grains were rich in this antineuritic substance. Prior to this, American scientists working in the Phillipines, and others, had added much to the knowledge of beri-beri. Funk gained most of the glory, when in 1911, he found by chemical fractioning of extracts of rice polishings, a small quantity of a crystalline substance which was highly curative of polyneuritis in birds. Suzuki, and others working in Japan soon reported substances similar to "Funk's Vitamine" and confirmed his work.

Vitamin B occurs very plentifully in the germs of grains, in yeast, vegetables of nearly all kinds, milk, eggs, and is found in many other
foods. It is lacking principally in diets of a limited variety, especially when polished rice or other starch forms the major portion. It cannot be synthesized by the body, consequently the amount of vitamine B in milk is influenced by the diet of the cow. (11) It was believed that plants cannot synthesize these vitamines. But if the higher plants cannot build these bodies, from where do they come? Bottomley (12) claimed that certain plants obtained these substances from the growth of microorganisms. Since then, others have claimed that these vitamines, especially water soluble B, are necessary for the growth of micro-organisms. However Nelson, Fulmer, and Cessna have apparently proved that yeast can synthesize vitamin B.

It is, like vitamin A, also a growth promoting substance and is specific in its prevention and cure of beri-beri or polyneuritis. Not much is known of its chemistry. Funk's crystalline compound was organic, basic in nature, partly soluble in alcohol, but more so in water. He found in his compound a nitrogen base and when concentrated and analyzed was found to contain nicotinic acid. However nicotinic acid is not the active principle. It is free from phosphorous and is quite stable to high temperatures. McCollum (13) says, "It has been the experience of all investigators that the farther the substances possessing the curative pro-
properties are purified, the less effective they become. This suggests that the important substance sought is not a crystallizable body, and that the crystals of definite and identifiable compounds thus far obtained are merely contaminated with the nutritive principle."

VITAMIN - D? For a long time doctors have recognized cod-liver oil as a specific for curing rickets in children. When vitamin A was discovered it was believed that this was the substance in the cod-liver oil responsible for the cure. However it has been shown that other substances known to be rich in fat-soluble vitamin will not prevent or cure rickets.

McCollum and others at John Hopkins University have long experimented with ricket producing diets. They found the main deficiency in these diets to be calcium salts and vitamin A, but they do not believe that the disease as yet can be traced to any one factor. Hess (2) has definitely shown that cod-liver oil is not replacable by other rich sources of vitamin A as a cure for rickets. Whether the preventive and cure for rickets is an entirely separate dietary factor than vitamin A is yet very much in doubt. Some have proposed a new name, vitamin D, for this essential. Zilva and Minra (14) have shown that cod-liver oil is about
two hundred and fifty times as rich in vitamin A as butter fat, and so conclude that the fat soluble vitamin is the antirachitic factor.

Recently Hess and others have stated that sunlight as well as ultra-violet rays possess remarkable curative action for rickets. And yet people who live in the arctic regions where there is darkness for a greater part of the year rarely have the disease. It seems that nature has provided a substitute for light in the form of some organic substance known to be present in cod-liver oil. As yet this antirachitic substance needs much investigation.

VITAMIN - C: Since the experimental work of this thesis deals entirely with the antiscorbutic vitamin, its discussion is placed after that of A, B, and D. Before considering this vitamin it is well to take up the disease scurvy, its history, etiology and symptoms.

SCURVY: This disease is known to have occurred among the armies of the Crusaders, in the thirteenth century. It was prevalent among soldiers and prisoners. When America was discovered, long voyages lasting months were undertaken and scurvy then became a plague among sailors. McCollum (15) cites a notable incidence in the history of Scurvy. It was during the voyage of Jacques Cartier in 1536. While in the St. Lawrence River he lost twenty-six of his
party but saved the rest with the use of a beverage made from an infusion of pine needles. Kramer in 1720 stated that medicine could not cure scurvy but that fresh leaves or fresh fruit would, and later in 1757 Lind said, if leaves were dried, the antiscorbutic value could not be restored by moistening. Thus from common experience, the efficiency of fresh vegetables and fruits as a preventive or cure of scurvy became known and the English Navy in 1804 began daily doses of lime juice to all sailors. The result was that scurvy practically disappeared from the service.

However in nearly all wars, even in the last, scurvy is a menace. During the Civil War in the United States over 30,000 cases occurred, because of the use of dried vegetables in winter. There were thousands of cases among the Russinas during the Russo-Japanese War. During the late war many cases were observed among the Indian troops in Mesopotamia, and among Russian soldiers. Scurvy was also common in many prison camps.

Among civilians scurvy most frequently makes its appearance following crop failures, especially among potato eating peoples. In England in 1917, several cases of scurvy occurred in poorhouses in Glasgow and in Newcastle, which could be attributed to the lack of potatoes. The British Medical Journal at this time emphasized the danger of substituting rice and bread for potatoes.
The symptoms of scurvy in man are marked with general debility and muscular weakness. The tissues lose the firmness of health and as a result the gums are spongy and bleed, and the teeth loosen. Patches of discoloration appear on the body and limbs, due to subcutaneous hemorrhages or petechiae. There is also hemorrhage under the periosteum of the bone. The bones become fragile and there is enlargement and disruption of the rib junctions. In advanced stages the whole body and limbs are very tender.

In infants the first signs are anemia, sallowness, the child is fretful, and growth is retarded. These occur before any marked physical symptoms are noticed. Later the child begins to be sensitive to the touch, especially about the legs. Then the limbs may swell, the tenderness increasing. As in adults there is hemorrhage under the periosteum of the bone, and if the child has teeth the gums will be swollen, and spongy.

Scurvy produces many of the same symptoms in guinea pigs as in man. The onset of the disease is usually marked by a decline in weight. Among the first symptoms noted is a tenderness of the joints especially those of the ankles and wrists. In a few days the joints swell gradually. Younger animals frequently fracture their wrist joints. As the disease develops, the appetite is decreased, the
gums become swollen, and the incisor teeth may come out or break off. The animals seem to become paralyzed in the hind quarters and appear stiff and sore. The body becomes tender, and when touched the animal will cry. While the "face ache" position occurs sometimes, it is frequently absent.

In advanced stages the animal becomes very emaciated, often losing two-fifths of its normal body weight before death. Constipation is also noted then and the animal remains in a humped-up position.

On autopsy other symptoms are noted. The most evident of these, are the enlargements of the costochondral junctions of the ribs, and the extreme weakness and brittleness of the bones. Hemorrhagic conditions are noticed usually at the joints of all the legs, especially the wrist and ankle joints. The kidneys are frequently slightly hemorrhagic. When examined the cecum is nearly always found filled with pasty food masses while the stomach is frequently empty. The heart, lungs and liver are ordinarily normal, when death is due to scurvy alone.

**DISCOVERY:** To Holst and Frømelich (16) of the University of Christianna, we owe the first knowledge of vitamin C. Between 1907 and 1912 they made detailed studies of scurvy in experimental animals, and found that guinea pigs readily develop the di-
sease when fed diets of bread and cereals. They also found something in fresh potatoes, cabbage, dandelions, carrots and lemon juice that was antiscorbutic. Their conclusions were that the cause of scurvy was due to the absence of some factor, yet unknown.

Other investigators were attracted to the problem and in 1916 Jackson and Moore (17) observed that guinea pigs fed a diet of oats and milk at liberty, develop scurvy. This was later observed by McCollum and Pitz (18) and since milk is such a good food they did not believe the disease could be due to the lack of a specific substance. They also reasoned that if scurvy is the result of the lack of a specific essential, it becomes necessary to make the further assumption that the guinea pig and man require this substance while rats, and certain other species do not. Their conclusion was that scurvy was not a deficiency disease, and was based on the following facts (19). "On autopsy of animals found dead with scurvy we usually found the stomach and small intestine empty, or nearly so, the cecum was greatly distended with putrefying feces. There were usually no feces in the lower end of the large intestine." McCollum concluded that the development of scurvy in the guinea pig was due to constipation or retention of feces in the cecum. The observation that paraffin oil and other physics sometimes saved scorbutic animals lent support to their view.
For several years there was considerable doubt as to whether McCollum and Pitz were correct, or whether the conclusions of Holst and Froelich were valid. Gradually the work of McCollum and Pitz was found faulty. No attention had been paid to the amount of milk consumed. Cohen and Wendel (20) autopsied normal guinea pigs and found the cecum was nearly always full of feces. Milk is constipating with guinea pigs, therefore large amounts of milk should increase scurvy if McCollum's view was the real one. Then the fact that orange juice given intravenously, still exerted a curative action was demonstrated. Gradually the presence of an antiscorbutic vitamin was recognized and Drummond suggested the name water-soluble C. In his second edition of his, "Newer Knowledge of Nutrition," McCollum admits that he and Pitz drew faulty deductions from their work.

**OCCURRENCE:** Vitamin C, or the antiscorbutic vitamin as it is frequently called, occurs most abundantly in the citrus fruits, milk contains a small, very variable amount. Fresh vegetables of all kinds contain it in abundance. Meat contains very little if any vitamin C. Lean meat possibly contains a small amount but none is contained by the fat. It probably occurs in hays and roughages in small amounts depending on the time of cutting and method of curing. Honey does not contain it. (21) The
occurrence of vitamin C in heated and cooked products depends upon its stability.

**CHEMISTRY:** Vitamin C is the most unstable of any of the vitamins. It is easily destroyed by heat. Oxidation also results in its destruction. Consequently heating in the presence of oxygen, or oxidizing agents is fatal. It has been found that the amount of heat vitamin C will withstand depends upon the method of heating and also varies with the vegetable or fruit containing it. Hess and Unger (22) obtained good results with canned tomatoes as an antiscorbutic, both in guinea pigs and infants. Usually, drying fruits and vegetables destroys their antiscorbutic potency, although slight traces may be obtained. Air dried roughages probably retain small quantities of vitamin C, the amount depending upon the method of curing. Delf (23) estimates that 70 per cent of the vitamin C content of vegetables is destroyed by heating at 145 degrees F. for one hour, and over 90 per cent by heating to 190 degrees F. for the same time. She suggests that in cooking green vegetables there will be less loss of the antiscorbutic vitamin by cooking at a high temperature for a short time, than at a lower temperature for a longer period. Autoclaving at 15 pounds pressure for 30 minutes absolutely precludes any possibility of any vitamin C remaining in the food.
so heated. The effect of heating (pasteurization) of milk upon its vitamin C content will be considered later.

Vitamin C is water soluble and passes thru a parchment membrane or porcelain filter. It can easily be separated from the water-soluble B. When mixtures of the two are treated with fine precipitates, such as fuller's earth or colloidal iron, the water-soluble vitamin B is absorbed while C is left unchanged.

VITAMINES IN MILK: Milk is one of the most important single article of our diet. Not only does it contain fat, carbohydrates, protein and mineral matter in a form completely digestible, but milk also contains the vitamins A, B, and C. The milk fats are the most important source in American and European diets of the fat soluble vitamin A. Since skimming removes this vitamin and vegetable oils do not contain it, this argument was used effectively in passing the recent legislation prohibiting the sale of filled milk. Filled milk is milk from which the cream has been removed, and cocoanut added to replace the butterfat.

The vitamin content of market milk depends upon two factors: first the diet of the cow, and second the treatment of the milk prior to its consumption. Hughes, Fitch, and Cave (11) fed two cows for over two years on a diet consisting of
pearl hominy, tankage, cottonseed meal and beet pulp, a diet very low in all the vitamins. They found the vitamin A and B content of the milk to be very much decreased. Likewise Kennedy and Dutcher (24) found that the presence of vitamins A and B in milk is entirely dependent upon their occurrence in the ration. Pasture made no influence with the vitamin A and B content of the milk. This was to be expected since vitamins A and B are quite stable and would be present in dried grains and roughages. This was substantiated by Osborne and Mendel (25) who observed no difference in the vitamin B content of milk produced in summer and that in winter.

Vitamin C occurs in milk, but in relatively smaller amounts than does A and B. Its presence in milk is very variable. The University of Wisconsin (26) has maintained a herd of eighteen cows for several years on air dried roughages and grains. Their results show that milk from this herd was distinctly inferior in the antiscorbutic vitamin content, compared to milk from cows which were on summer pasture. They used as a basal ration autoclaved alfalfa hay, rolled oats and salt. Where milk was omitted five parts of casein was added. A summary of their results showed that

<table>
<thead>
<tr>
<th>Amount</th>
<th>Protection Period</th>
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<tbody>
<tr>
<td>15 cc.</td>
<td>6 weeks</td>
</tr>
<tr>
<td>30 cc.</td>
<td>7-8 weeks</td>
</tr>
<tr>
<td>50 cc.</td>
<td>12-14 weeks</td>
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</table>

but all the guinea pigs finally developed scurvy.
75cc. of the dry feed milk gave complete protection from scurvy over a period of eighteen weeks. It proved that winter milk while deficient, was not wholly devoid of vitamin C. When silage and sugar mangels was added to the diet of the cow, Hart and Steenbäck concluded, "Silage made from corn, well matured and partly dried before putting into the silo did not greatly enhance the concentration of the antiscorbutic factor in the milk. The same statement applies to the use of the sugar mangels." They considered milk from silage fed cows to be in a class with dry feed milk in respect to its content of the antiscorbutic vitamin, but admit that their data is too limited to warrant an unqualified statement. This is the only published data relative to the effect of silage upon the antiscorbutic potency of milk. Other investigators including Hess, Dutcher, Kennedy and Unger have obtained results similar to those of Hart, Steenbäck and Ellis of Wisconsin, that milk from cows on winter feed is deficient in vitamin C. This is especially true in late winter as the body of the cow becomes depleted in any vitamin she may have stored while having access to green feed.

Since vitamin C is the most unstable of any of the vitamins, it is readily seen that any treatment which the milk undergoes is very liable to affect its content of this factor. We find many statements that pasteurization destroys vitamin C. But there are several methods of pasteurization in
use, using different methods, different temperatures and different times held at the required temperature.

The three common methods are:

1st. Holding method in vat pasteurizer, temperature 142-145 degrees F., time 30 minutes.

2nd. Flash method, in vat or tank pasteurizer, temperature about 160 degrees, F., time one minute.

3rd. Holding method, pasteurizing in bottle or final package, temperature 142-145 degrees F., time 30 minutes.

In the large cities we find the first method in more common use, and this is the method probably used by most investigators. Hess and Unger (27) state that infants require fully one-half pint of unheated raw milk to protect them from scurvy. The following well known incident shows definitely that pasteurization is destructive to vitamin C. In the Hebrew Infant Asylum in New York City (28) it was the custom to feed milk pasteurized at 145 degrees F., to the children with the addition of a small amount of orange juice daily. On this diet there was no scurvy. Later owing to a statement of the American Medical Milk Commission that pasteurized milk was equivalent in every way to raw milk, the orange juice was discontinued. In two to four months there occurred an outbreak of an epidemic of mild scurvy. The scorbutic nature of the disease was proved, by the substitution of raw milk for the pasteurized milk, and the addition of orange juice again, when the symptoms disappeared. Froelich
(29) found that milk heated to 98 degrees C. for ten minutes lost its protective action against scurvy in guinea pigs, yet Dutcher, Harshaw and Hall (30) report that vitamin C is not destroyed by heating at pasteurization temperature, 63 degrees C. for 30 minutes in closed vessels or by boiling, (100 degrees C.) for 30 minutes under reflux condensers. It seems to be universally acknowledged however that pasteurization, at least by the vat method, where oxidization can occur, is quite destructive to the antiscorbutic vitamin.

Since milk is so variable in its antiscorbutic potency, it is natural that milk products, such as powdered, evaporated and condensed milks vary widely in their antiscorbutic properties. In addition to the varying initial amounts of this vitamin, the powders and condensed milks also vary in their potency with the process used in their manufacture. Hess and Unger (31) believe that milk dried on a heated drum as in the Just-Hatmaker process for a few seconds at 116 degrees C., is as efficient as fresh milk in protecting against scurvy. Similarly Hart and others (32) state that milk dried on a drum retains more of its antiscorbutic property than milk dried by the spray process. In the same experiment (32) Hart, Steenbock and Smith found that unsweetened evaporated milk had lost its antiscorbu-
tic power. The nutritive value of milk may be affected, in addition to the loss of vitamin C, in the manufacture of these various products. Daniels and Loughlin (33) conclude from their experiments, "In the process of heating the calcium salts are rendered more or less insoluble depending upon the length of time the milk was heated. In this insoluble form they may be lost owing to the fact that some of the precipitated material adheres to the container."

In conclusion it might be said that in all milk powders and condensed milks the vitamin C content is low, but variable, depending upon the method of manufacture, especially the temperature used and the length of time employed.
EXPERIMENTAL

PURPOSES: The objects of the experiment as conducted were as follows: To determine the antiscorbutic value of different amounts of milk when produced or treated under the following conditions:

1. Milk from cows on winter ration receiving corn silage.
2. Milk from cows on winter ration, receiving no silage.
3. Milk pasteurized in the bottle, (final package) by the holding process, 145 degrees F., for 30 minutes.
4. Fresh raw milk from the college herd.

The outline of purposes assumes that there may be differences in the antiscorbutic properties of milk produced by cows on winter ration but receiving different feeds. The purposes further assume that the methods used in pasteurization may also produce differences in the vitamin C content of milk.

PLAN: There are two methods of testing for the presence of any vitamin. One method consists in feeding the material to be investigated to animals that have declined on a diet deficient in this vitamin. Such methods are essentially restorative in character. If the outcome is positive and the animal recovers, the test for the vitamin may be qualitatively successful. However the animals may be so emaciated and undernourished that excessive amounts
of the vitamin bearing food may be necessary to restore them to health.

The other method consists in feeding the supposed source of the vitamin to normal, healthy animals, and observing whether the product offered supplies what is needed to promote normal growth, when the basal diet supplies an adequate amount of all essentials except the one to be investigated.

The second plan was the one followed in this experiment.

ANIMALS: It is obvious that animals of different weight and size will respond differently to certain diets. That is, smaller animals usually respond to a lack of any dietary essential much sooner than larger animals. In all cases growing animals should be used. In the following experiment sixteen guinea pigs were used. Later eight more were added, making twenty-four in all. They were fairly small, varying from 250 to 400 grams in weight. All pigs were kept on a well balanced diet prior to the beginning of the experiment. Weights were taken and all animals found to be normal, healthy and vigorous before beginning the test. The males and females, and the larger and smaller pigs were carefully placed in the pens, so each group on a certain diet should contain some males and some females, and part of the smaller and some of the larger pigs.
QUARTERS: Each pig was kept in a separate compartment of a large cage, with screen wire on both front and rear. Dry, clean sawdust was used for bedding. The cage during the entire experiment was kept in a room at the dairy barn. This room joins the boiler room and was quite moderate in temperature at all times. The health of the pigs during the entire period indicated good ventilation and freedom from drafts. The usual laboratory precautions were taken in regard to the sanitary conditions of the cages and feeding dishes. All dishes were washed in boiling water before each feeding. The dishes were aluminum and were set in wooden blocks to prevent spilling.

FEEDING: The basal ration consisted of rolled oats, good quality alfalfa hay, (autoclaved), water and common salt. All these were consumed ad libitum. The alfalfa was finely cut and autoclaved at 15 pounds pressure for 30 minutes and then dried. Four pigs were placed on this basal diet. The milk was added to the basal ration. Four animals were used in the trial of pasteurized milk. This milk was selected at random from the pasteurized milk at the College Creamery. It was pasteurized in the bottle at 142 to 145 degrees F. for 30 minutes and then cooled rapidly. Two of the pigs received 30 cc. of this milk daily, one 45cc., and one received 60 cc. In all cases the milk was fed in the dishes in two
feeds, morning and evening.

At first eight pigs were placed on the trial of milk from cows fed different rations. Later eight more were added. Milk from the college herd was fed to half of the guinea pigs. Four dairy breeds were represented in the herd, with Holsteins predominating. They received a grain ration of four parts of oats, four parts of corn, two of bran and one part of oil meal. During the early part of the winter and up until about February, a very good quality of alfalfa was fed. Later the hay was rather inferior, containing a little alfalfa, some sweet clover, considerable blue grass and other tame grasses. Corn silage was fed quite liberally. The corn was cut fairly green and the silage was of very good quality. The eight pigs received fresh milk from the dairy herd in the following amounts:

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Amounts</th>
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<tbody>
<tr>
<td>9</td>
<td>15 cc.</td>
</tr>
<tr>
<td>10</td>
<td>15 cc.</td>
</tr>
<tr>
<td>11</td>
<td>20 cc.</td>
</tr>
<tr>
<td>12</td>
<td>20 cc.</td>
</tr>
<tr>
<td>1</td>
<td>30 cc.</td>
</tr>
<tr>
<td>2</td>
<td>30 cc.</td>
</tr>
<tr>
<td>3</td>
<td>45 cc.</td>
</tr>
<tr>
<td>4</td>
<td>60 cc.</td>
</tr>
</tbody>
</table>

The remaining eight guinea pigs were fed similar amounts of milk from cows receiving no silage. The cows producing this milk were kept on a farm near the city. There were six cows milking in the herd and they were mostly grade Jerseys. Their grain ration consisted of ear corn and oats. For roughage,
they received an excellent quality of alfalfa up to the first of February. This hay was very green and had been cured without exposing it to rain. Red clover was then fed for about two months. After April first the roughage was a rather poor quality of alfalfa, considerably bleached and discolored, having been exposed to several showers of rain. The eight pigs received the milk as follows:

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>13</th>
<th>15</th>
<th>16</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
</table>

A composite sample of the autoclaved alfalfa was analyzed showing the following percentages:

- moisture: 6.38%
- crude protein: 19.4%
- ether extract: 1.4%
- nitrogen free extract: 42.4%
- crude fiber: 21.2%
- ash: 8.9%

Composites of the milk were taken over a period of several weeks, and tested for butterfat. Milk from the college herd:

<table>
<thead>
<tr>
<th>tests</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.0 per cent</td>
<td>3.3 &quot; &quot;</td>
<td>3.6 &quot; &quot;</td>
<td>3.4 &quot; &quot;</td>
<td>4.0 &quot; &quot;</td>
<td>3.6 &quot; &quot;</td>
</tr>
<tr>
<td>Average</td>
<td>3.65 per cent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Milk from the farm herd where no silage was fed:

<table>
<thead>
<tr>
<th>Tests</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.1%</td>
</tr>
<tr>
<td>2</td>
<td>5.4%</td>
</tr>
<tr>
<td>3</td>
<td>5.5%</td>
</tr>
<tr>
<td>4</td>
<td>5.0%</td>
</tr>
<tr>
<td>5</td>
<td>5.1%</td>
</tr>
<tr>
<td>6</td>
<td>5.3%</td>
</tr>
</tbody>
</table>

Average = 5.26% per cent

A complete analysis of a sample of this milk at the beginning of the experiment showed:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids</td>
<td>14.2%</td>
</tr>
<tr>
<td>Fat</td>
<td>5.3%</td>
</tr>
<tr>
<td>Total protein</td>
<td>4.0%</td>
</tr>
<tr>
<td>Sugar (lactose)</td>
<td>4.2%</td>
</tr>
<tr>
<td>Ash</td>
<td>.75%</td>
</tr>
</tbody>
</table>

At the close of the trial another analysis was made of the dry-feed milk:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids</td>
<td>13.11%</td>
</tr>
<tr>
<td>Fat</td>
<td>4.6%</td>
</tr>
<tr>
<td>Total protein</td>
<td>3.2%</td>
</tr>
<tr>
<td>(lactose) sugar</td>
<td>5.1%</td>
</tr>
<tr>
<td>Ash</td>
<td>.73%</td>
</tr>
</tbody>
</table>

An analysis of the milk from the cows of the college herd receiving silage was as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids</td>
<td>12.61%</td>
</tr>
<tr>
<td>Fat</td>
<td>3.7%</td>
</tr>
<tr>
<td>Total protein</td>
<td>3.3%</td>
</tr>
<tr>
<td>Lactose (sugar)</td>
<td>5.0%</td>
</tr>
<tr>
<td>Ash</td>
<td>.74%</td>
</tr>
</tbody>
</table>

The milk from both sources at all times appeared perfectly normal.

**CARE:** All animals were fed twice daily; a graduated pipette being used to measure the milk. The milk was entirely consumed in all cases, except in several instances just prior to death. In no case was the milk force fed, but was consumed readily from the dishes. Every three days the pigs were carefully
weighed on a gram scale and all weights recorded. A loose-leaf record book was used in which the weights, deaths, autopsies, and all observations were noted. Carbon copies were made, the originals being removed and kept in a permanent book in the dairy building. All animals were autopsied as soon as possible after death. In one case where death was evident within a few hours, the pig was chloroformed and posted immediately. In cases in which the cause of death appeared doubtful, the autopsies were made by Dr. J. B. Taylor of the Veterinary Department of the college.
Preliminary Growth Period

Baseline ration +
Posteurized milk

No. 13: 30 cc. milk daily
No. 14: 30 cc. daily
No. 15: 45 cc. daily
No. 16: 60 cc. daily

S - scurvy symptoms
+ - death

30 days 60 days 90 days 120 days
24 day preliminary growth period

Basal ration
+ milk from cows
Getting no silage

No. 1 - 30 cc. milk daily

No. 2 - 30 cc. milk daily

No. 3 - 45 cc. milk daily

No. 4 - 60 cc. milk daily

S - scurvy symptoms
+ - death
B - porphyria

< 30 days

Periods

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
24-day preliminary growth period

Basal ration + milk from cows being fed silage

No. 3 - 30 cc. milk daily

No. 6 - 30 cc. milk daily

No. 7 - 45 cc. milk daily

No. 8 - 60 cc. milk daily

S - scurvy symptoms
+ - death
No. 13 - 15 cc. milk daily

No. 14 - 15 cc. milk daily

No. 15 - 20 cc. milk daily

No. 16 - 20 cc. milk daily

S - Scurvy symptoms
+= death
DISCUSSION OF EXPERIMENTAL DATA

The basal ration of autoclaved alfalfa hay, rolled oats, water and salt was found to be free from antiscorbutic vitamin. This was demonstrated by the fact that pigs 9, 10, 11 and 12 on this diet all succumbed. Pigs, number 11 and 12, both died on the nineteenth day after being placed on the check trial. When posted they revealed the following conditions: The costochondral junctions were enlarged and inflamed. The knee joints were swollen and hemorrhagic. There were marked hemorrhagic areas in the thighs. On examination the kidneys were found to be inflamed. Brittleness of the bones, especially the leg bones was very noticeable, the bones breaking extremely easy. The contents of the cecum and intestines were soft and pasty and no impaction or constipation was noted. Scours were in evidence in pig number 11.

Of this group pig number 9 lived the longest, dying on the thirteenth day. Two days previous pig number 10 died. These pigs also showed symptoms similar to those of 11 and 12. Diarrhea was apparent in pig number 9 for several days previous to death.

The results of the trial with milk pasteurized in the bottle were variable. Pig number 14 lived 54 days on 30 cc. of this milk daily, while pig number 13, receiving the same amount of milk did not die till the ninety-seventh day. This would in-
dicate that there is considerable difference in the susceptibility of different animals to a lack of vitamin C. Altho making no gain in weight, pig number 13 showed unusual resistance. Pig number 15, receiving 45 cc. of this milk daily, lived 72 days. In this trial occurred the only death that could not be attributed directly to scurvy. Pig number 16, on 60 cc. of pasteurized milk, died after 65 days on this ration. On post mortem, scurvy symptoms were evident, however, the immediate cause of death was attributed to pneumonia. Since none of the other pigs showed symptoms of pneumonia it is possible that the onset of scurvy lowered the vitality of the pig and rendered it especially susceptible. Except in the case of pig number 16, the results indicate that considerable of the vitamin C content of milk is destroyed by pasteurization in closed bottles. This is evident if the growth curves of these animals are compared to those of pigs 5, 6 and 7, receiving similar quantities of raw milk. Results of other investigators, with milk pasteurized in the vat show even more destruction of the vitamin C. This is attributed to the oxidization which occurs in vat pasteurization.

The principal part of the experiment dealt with the feeding of milk from cows on winter feed; one herd receiving silage and the other herd receiving dry feed only. Hart, Steenbock and Ellis (26) in 1920, reported that 30 cc. of milk from cows
on dry feed protected only seven to eight weeks, and 50 cc. of such milk daily, prevented the onset of scurvy for twelve to fourteen weeks. Similarly, Chick, Hume and Skelton (34) observed that 50 cc. of raw milk was not sufficient to prevent scurvy in guinea pigs.

Pigs 1, 2, 3 and 4 were fed milk from the cows on dry feed during the entire experiment of 150 days. Three of these pigs developed scurvy, pig number 3 dying after 108 days. Death was hastened by the loss of all four incisor teeth, which had become brittle and had broken off. Neither of the pigs fed 30 cc. of this milk died although their growth curves were not normal. One of these, pig number 1, showed scorbutic symptoms after 100 days, and shortly before the end of the trial, pig number 2 began to decline in weight. Paralysis in the rear limbs was evident. The death of pig number 3 also indicates variations in the resistance and susceptibility of guinea pigs to scurvy. An explanation of the growth curve of pig number 4 is necessary. She was pregnant when put on experiment, and after about 42 days on the diet gave birth to one offspring. The young pig showed no scurvy symptoms when born and grew rapidly for two weeks. It was left with its mother. It was used later in the experiment being pig number 12'. Shortly after her parturition, pig number 3 fell
and from indications broke her back. The incidents delayed her growth, but she made very good gains during the last sixty days of the trial. The fact that the offspring showed no symptoms of scurvy while sucking, would indicate that the mother was getting sufficient vitamin C.

Contrasting the growth and development of these animals with pigs number 5, 6, 7 and 8, a marked difference is noted. The latter received similar quantities of milk, from the college herd where silage was fed. All four pigs showed a good growth curve. At the end of 150 days, all were vigorous and healthy, with glossy coats and no scurvy symptoms were in evidence. Three of these pigs, number 6, on 30 cc. of milk, number 7, on 45 cc., and number 8 receiving 60 cc. daily, doubled their initial weight during the experiment.

On February 28, eight more pigs were secured. Four of these, 13', 14', 15' and 16', received milk from the cows on dry feed; while numbers 9', 10', 11' and 12' were fed milk from the cows receiving silage. The graphs of the development of these pigs are significant. All four of the pigs on the dry-feed milk developed scurvy within 30 days and two died within that time. Pig number 13' lived 48 days and the death of pig number 15' did not occur before the termination of the experiment. The other four pigs, numbers 9', 10',
11' and 12' were fed similar amounts of milk from the college herd, two receiving 15 cc. daily and two receiving 20 cc. daily. None of these pigs died and only one, pig number 9' on 15 cc. of milk, developed symptoms of scurvy during the 54 days they were on experiment. Two of these, pig number 10' and pig number 12' showed good growth curves. This seems to be unusual for it is universally believed that the milk from cows on winter feed becomes progressively poorer in vitamin C.

The data seems to indicate that the cows receiving corn silage were maintaining to a considerable degree the vitamin C content of their milk. This conclusion is substantiated by the fact that seven of the eight pigs on the dry-feed milk developed marked scurvy symptoms and four of these died of the disease while only one of the pigs on the silage-milk developed scurvy and none of the eight died.

**CONCLUSIONS**

The results of the experiment indicate the following conclusions:

1. Autoclaving at 15 pounds pressure for 30 minutes results in the total destruction of vitamin C in alfalfa hay.

2. Pasteurization of milk in closed bottles at 142 degrees F. for 30 minutes diminishes the vitamin C content of milk considerably. This
is in accordance with the observation of Miller (35) that since milk contains only a small amount of the unstable antiscorbutic vitamin, just within the margin of safety, any manipulation is dangerous.

3. Silage made from corn, cut fairly green, and of good quality, increased to a considerable degree the antiscorbutic potency of cows milk.

ACKNOWLEDGMENTS

I wish to acknowledge the assistance of Dr. J. B. Taylor for his aid in some of the autopsies.

Special acknowledgment is due to the Board of Regents, who made it possible for me to pursue graduate work, and to Professor Thomas M. Olson under whose direction and guidance the experimental work was conducted.
Experimental Animals on Silage milk.

No. 5 - 6 - 7 after 150 days
30 cc. 30 cc. 45 cc. 60 cc.

No. 10 - 11 - 12 after 57 days
15 cc. 20 cc. 20 cc.

No. 5 on 30 cc. daily
No. 6 on 30 cc. daily
Both taken after 150 days.

No. 9 - 15 cc. milk daily after 57 days
Slight scurvy symptoms.
Cage in which pigs were kept.

Animals on dry-feed milk

No. 1 on 30 cc.  
No. 2 on 30 cc.  
Both after 150 days

No. 1 was paralyzed in the rear legs.

No. 15% on 20 cc. after 5-7 days

marked scurvy
BIBLIOGRAPHY


