HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

VOL. 1 MAY, 1925 NO. 2

THE ANTISCORBUTIC VALUE OF COMMERCIALLY CONCENTRATED ORANGE JUICE

BY

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INTRODUCTION

Concentrated orange juice prepared commercially has only recently been placed on the market. So far as we are aware, all reports on the antiscorbutic property of concentrated juices are based on work with laboratory preparations, except that of Chaney,[1] who reported on the use of a concentrated bottled orange juice as a supplemental lunch for school children and suggested that the favorable results obtained may have been due, in part, to the antiscorbutic principle of the orange juice. It was, therefore, deemed desirable to ascertain whether or not these commercial products retained the antiscorbutic property to as great an extent as did the laboratory preparations.

It was realized as long ago as the sixteenth century that oranges and lemons possessed great value as preventives of scurvy,[2] but at that time little significance was given to this fact and more attention was paid to limes and other fresh fruits. Orange and lemon juice have now been studied more than any other antiscorbutic substance. Limes and lime juice are no longer regarded as excellent sources of vitamin C. Chick, Hume, and Skelton[3] found lime juice to be only one-fourth as potent as lemon juice, while lemon and orange juice are considered equal in value in this respect. Oranges and lemons have the highest known antiscorbutic value and are, therefore, almost always taken as a standard in comparative work.

It has been known for some time that orange juice may be heated to boiling[4] or dried in vacuum under certain conditions and reduced

to a powdered form[5] and still retain significant antiscorbutic power. It has been pointed out by other investigators that oxidation is responsible for the rapid destruction of vitamin C in orange juice; such destruction taking place even in the cold when the juice is sufficiently exposed to air or other oxidizing influences. Neutralization of the citric acid has a similar marked effect.[6, 7] Givens and MacCluggage,[5] in their early work in 1919, pointed out the advantages that would follow if some method of concentration were perfected which would not lower the antiscorbutic value of orange and lemon juice. This would make it possible to prepare and preserve these valuable food products from surplus fruit and put them on the market at a comparatively low price. It has been shown by the results of investigations already carried out that concentration of the juice in vacuum under reduced temperatures is a practical commercial method of accomplishing this object.

Method of Procedure

Using fresh orange juice as a standard antiscorbutic and a basal diet of oats, barley, wheat hay, and water, comparative results were obtained by studying the clinical effects on guinea-pigs of the basal diet plus a measured amount of the product studied. An attempt was thus made to secure approximate quantitative results in terms of fresh orange juice, but as others have often noted[7] the individual variations of the animals and the uncertainty of the border line between protection and failure to protect render exact measurement of the results by this method impossible.

The concentrated juices were, after dilution with suitable known quantities of water, administered orally daily in measured amounts, by means of a 5 cc. hypodermic syringe (fig. 1). Very little difficulty was experienced by this method in administering an exact quantity of the juice. It was found that the animals relished all juices after a few trials with the exception of concentrated lemon. By carefully releasing the liquid from the syringe 5 cc. could be administered without loss.

The desiccated orange juice was dissolved in water and then used with the syringe as described above.

The dried whole orange was given in gelatin capsules each containing 0.2 grams of the product, ground as fine as possible in a food chopper.

In all experiments normal, healthy guinea-pigs were used, ranging in weight from 400 to 800 grams. They were kept, two or three together, in metal cages in a sunlit room, protected from draughts and changes in temperature. The bedding used was covered by a layer three or four inches thick of clear wheat hay, which was replenished every three days when the cages were cleaned. Rolled oats, rolled barley, and water were kept before the animals at all times.

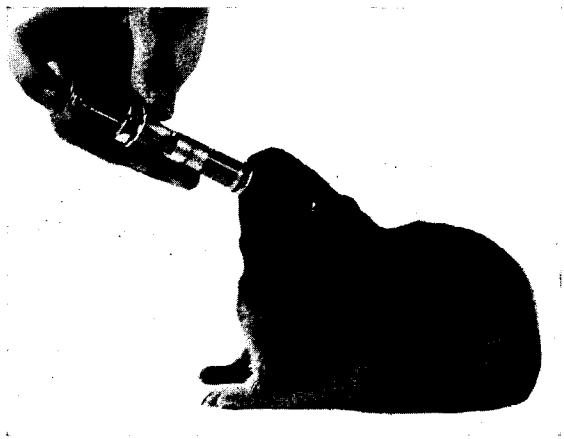

Fig. 1. Method of feeding measured quantity of juice by means of hypodermic pipette

Experimental

Besides the concentrated whole orange juice our study included several other orange and lemon products, prepared recently on a commercial scale. Experimental data is submitted on the following products:

1. Concentrated whole orange juice.
2. Concentrated and clarified orange juice.
3. Desiccated orange juice prepared by a spray process with added cane sugar.
4. Dried whole orange.
5. Concentrated, slightly sweetened lemon juice.

The orange and lemon concentrates were prepared by a standard well-known method of concentration employing glass-lined vacuum pans operated under very high vacuum and low temperature. These

products were furnished by the Exchange Orange Products Company at San Dimas, California, and the Exchange Lemon Products Company at Corona, California, two commercial production plants coöperatively owned and operated by members of the California Fruit Growers' Exchange.

Fig. 2. General condition of scorbutic animal after 30 days on a diet of oats, barley, wheat hay, and water.

The desiccated orange juice was furnished by the Research Laboratory of the Exchange. The dehydrated ground whole orange was submitted by the Laboratory of Fruit and Vegetable Chemistry, U. S. Department of Agriculture, Los Angeles, California.

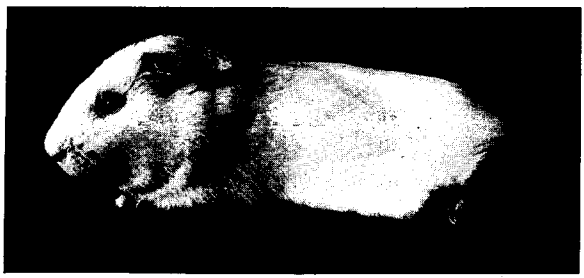

Fig. 3. Guinea-pig suffering from scurvy, showing painful limb extended to relieve pressure.

1. *Basal Diet Alone.*—On the basal diet alone animals usually showed, in about 20 to 25 days, indications of failure of health, usually characterized by weakness, a staring coat (fig. 2), swollen

wrist joints—especially in the young animals—tenderness of the limbs, and signs of pain when handled. Frequently the animal would relieve pain in the affected limb by removing pressure as shown in figure 3. Occasionally the well known "face-ache position" described by Chick, Hume, and Skelton[3] was observed.

Fig. 4. Stomach of a scorbutic guinea-pig distended as a result of gas, showing hemorrhages on the interior, even before being opened (see fig. 5).

Unless fresh orange juice or grass was given when the symptoms were first noticed the animal would invariably die in 10 days more. Post mortem examinations of these animals gave various pictures described by other investigators, but no complete set of symptoms and lesions were observed in all cases.

The most predominating lesion found in our cases were hemorrhages in various tissues of the body, usually in the stomach and intestines (figs. 4, 5, 6, and 7) and occasionally in the muscles of the hind legs. Frequently enlargements of the costochondral junctions of the ribs were well pronounced (see fig. 8 and compare with fig. 9). The bones were always more or less brittle. In two cases, jaw bones

were found fractured. The teeth were usually dull and brittle, the incisors easily yielding to fracture on the ends by the pressure of the finger nail.

Fig. 5. Appearance of inside lining of same stomach shown in figure 4. Note the numerous hemorrhages dotted through the lining.

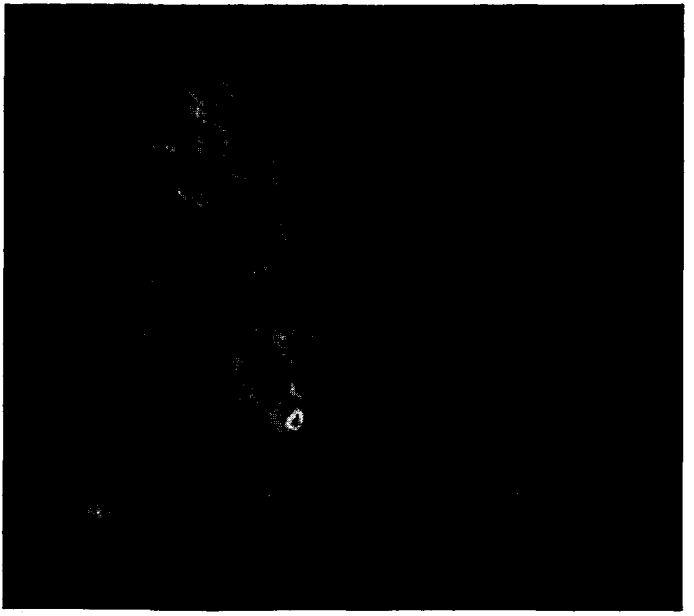

Fig. 6. Severe hemorrhages in the stomach of a guinea-pig which died from scurvy.

The growth curves are not especially significant except to give some idea of the well being of the animals during the test. In nearly all cases symptoms of scurvy were not apparent until after a notable decline in weight was observed.

Fig. 7. Hemorrhagic erosions in the stomach lining of a scorbutic guinea-pig. The white ring in the upper center is the pylorus.

When any of the above symptoms developed it was assumed that either no protection was being offered by the substance in question or that the protection was not sufficient.

Fig. 8. Section of ribs showing enlarged costochrondral junctions observed in some of the scorbutic animals. Compare with figure 9.

Curative methods are sometimes used in determining the presence of vitamin C but the conditions of the animals were so uncertain that quantitative results were found with difficulty and in many cases not at all. Therefore, more reliance has been placed on the preventive than on the curative method.

2. *Minimum Dose of Fresh Orange Juice.*—When the basal diet was supplemented by 1.5 cc. of fresh orange juice daily, animals were successfully maintained for a period of 93 days, gaining 55 per cent

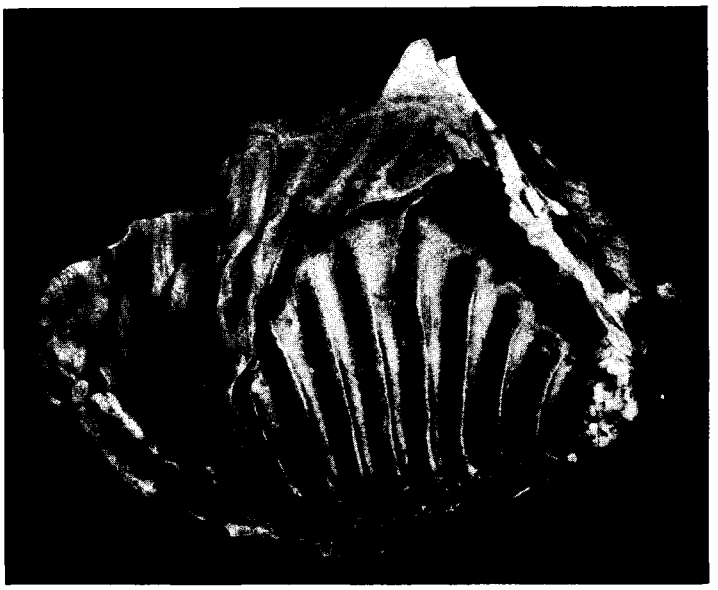

Fig. 9. Section of the ribs showing costochrondral junctions, from guinea-pig fed daily doses of 1 gram of desiccated orange juice. Compare with figure 8.

in weight and showing no signs of scurvy at anytime (fig. 10). Other animals fed similarly, except that alfalfa hay was supplied instead of wheat hay, grew apparently at the same rate, even though the A vitamin factor was probably present in more abundance in the alfalfa. Even when animals were kept on oats, barley, and water, alone, the addition of 1.5 cc. orange juice daily protected them from scurvy for at least 80 days, but they did not gain in weight as did the animals supplied with an abundance of hay.

3. *Concentrated Whole Orange Juice.*—This sample of concentrated orange juice, designated as No. 3, was made from the juice of

very ripe navel oranges. The raw juice was concentrated in a glass enameled vacuum pan under high vacuum, the temperature not exceeding 45° C. at any time, and being for the greater part of the time at or below 40° C. The time required for the processing was about four hours. At the end of the concentration, the sample used in this test was drawn directly from the vacuum pan into one gallon sterilized glass jars stoppered with a sterilized cork and kept moderately cool.

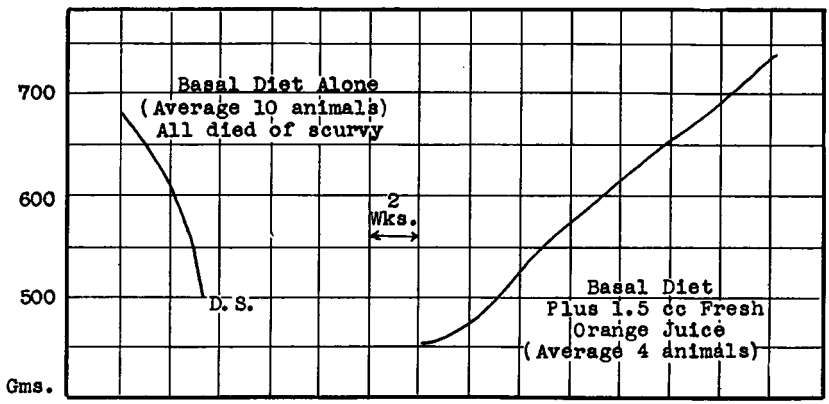

Fig. 10. Growth curves for basal diet alone and for basal diet plus 1.5 cc. fresh orange juice. D.S., died of scurvy.

Because of the unusually high percentage of solids in the raw juice (17 per cent) one gallon of concentrate represented only 5½ gallons of raw juice, while in commercial practice the normal volume concentration is about seven to one. On a weight basis, 1 gram of this concentrate is equivalent to about 4.5 grams of raw juice.

Successively reduced amounts of concentrate beginning with 1 gram and ending with 0.25 gram were fed to normal test animals, each day, together with the basal diet described above. The weights of the animals were recorded and observations made daily for the first appearance of the usually noted symptoms of scurvy.

Guinea-pigs nos. 59, 60, and 61 (fig. 11) were fed on the basal diet plus 1 gram concentrate daily diluted with water (period A to B). At the end of 40 days the dose was reduced to 0.5 gram daily. Animal 59 died after 95 days; no scurvy symptoms were found on post mortem. Animals 60 and 61 showed no signs of scurvy after 115

days. It will be recalled here that on the basal diet alone symptoms were apparent after 20 days, followed by death 10 to 15 days later (fig. 10). A second trial with five younger animals (fig. 11) produced no symptoms in 60 days on a 0.5 gram dose.

A third trial using 0.25 gram doses with three animals was started (fig. 11). One guinea--pig, no. 107 (not plotted in figure) died after

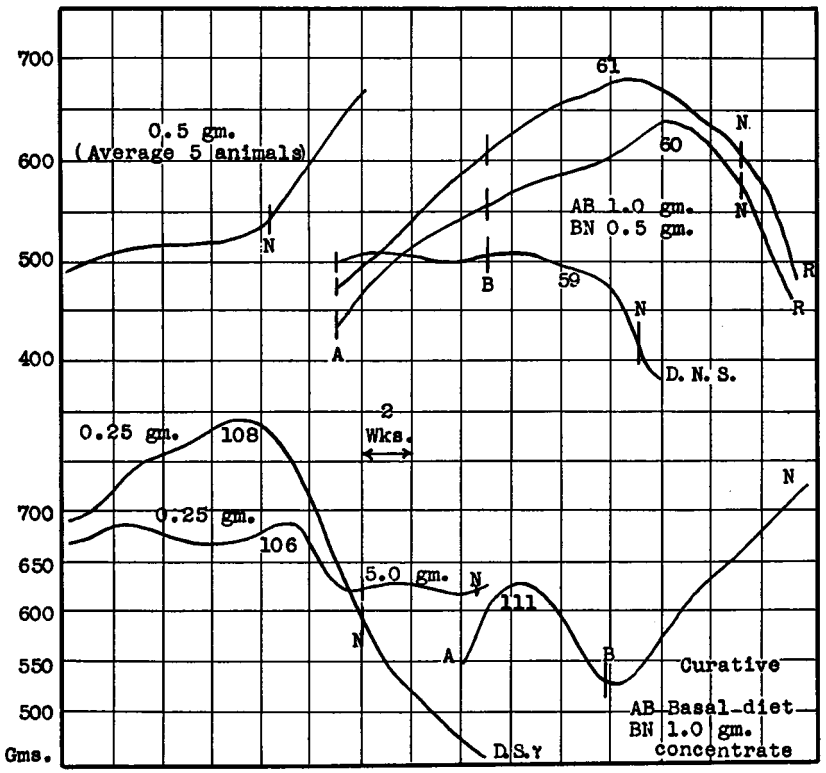

Fig. 11. Growth curves with basal diet plus concentrated whole orange juice (Orange concentrate No. 3). At N fresh grass was given. D.N.S., died, no scurvy found. D.S., died of scurvy. R., recovered.

10 days from unknown causes. Animal no. 106 was continued on this dose for 88 days, when it was doubtful if it would continue to live longer. The dose was then increased to 5 grams of the concentrate, partly neutralized with alkali. This increase caused a rapid recovery to normal, although little increase in weight was noticed. This experiment was discontinued on the 115th day, and the animal was placed on greens to recover. Guinea-pig no. 108 gained weight

for 45 days, then lost weight and vitality and appeared scorbutic on the 68th day. After 87 days the diet was changed to normal. Death occurred later, but no typical lesions of scurvy were noted on post mortem examination.

One curative experiment was carried out, using guinea-pig no. 111. This animal was kept on the basal diet until definite symptoms of scurvy were produced. A curative dose of 1 gram of the concentrated whole orange juice, partly neutralized by sodium hydroxid solution at the time of feeding caused the symptoms to disappear in 24 hours and the animal soon recovered to normal.

The above tests indicate that 0.5 gram of the concentrate representing about 2.2 cc. of fresh orange juice was sufficient to protect against scurvy, while 0.25 gram of concentrate, representing 1.1 cc. of fresh orange juice, although delaying the onset of the disease, was not sufficient to protect. Since 1.5 cc. of the fresh orange juice is considered necessary to protect animals from scurvy for at least 90 days, it was concluded that orange concentrate No. 3 had lost little or none of its antiscorbutic properties during concentration and subsequent storage.

4. *Clarified Orange Syrup.*—This clarified syrup product, Concentrate No. 6, was made from whole ripe fruit of miscellaneous varieties with a small amount of added cane sugar, according to the following method: The raw juice was heated in 30 minutes to a temperature of 185° F., and then transferred from the heating kettle to a large wooden mixing tank. In this operation the temperature dropped from 185° F to 150° F. in two hours. About 4½ hours were consumed in filtering the juice, with the aid of filtercel, the juice cooling somewhat during this time. It then stood overnight in an open tank loosely covered with canvas. The total exposure before concentration was about 18 hours, during the first 2½ hours of which the temperature fell from 185° F. to 150° F., during the next 4½ hours from 150° F. to 120° F., and then for a period of about 11 hours while the juice was exposed to the air it fell from 120° F. to about 90° F.

Sufficient sugar was added to make the ratio of solids to acid 12 to 1, and the filtered, sweetened juice was then concentrated in a glass enameled vacuum pan in about 7 hours. The temperature during this time was about 95° F. and did not exceed 100° F. except possibly through slight local overheating, which the thermometer would not

indicate. However, as this juice was continually under vacuum of about 28 inches of mercury and under violent agitation while boiling under this vacuum, the chances for local overheating were very slight. The final product contained 72 per cent solids. One gallon of the concentrate represents 5 gallons of the raw juice. One gram of this concentrated syrup represents 3.6 cc. of orange juice.

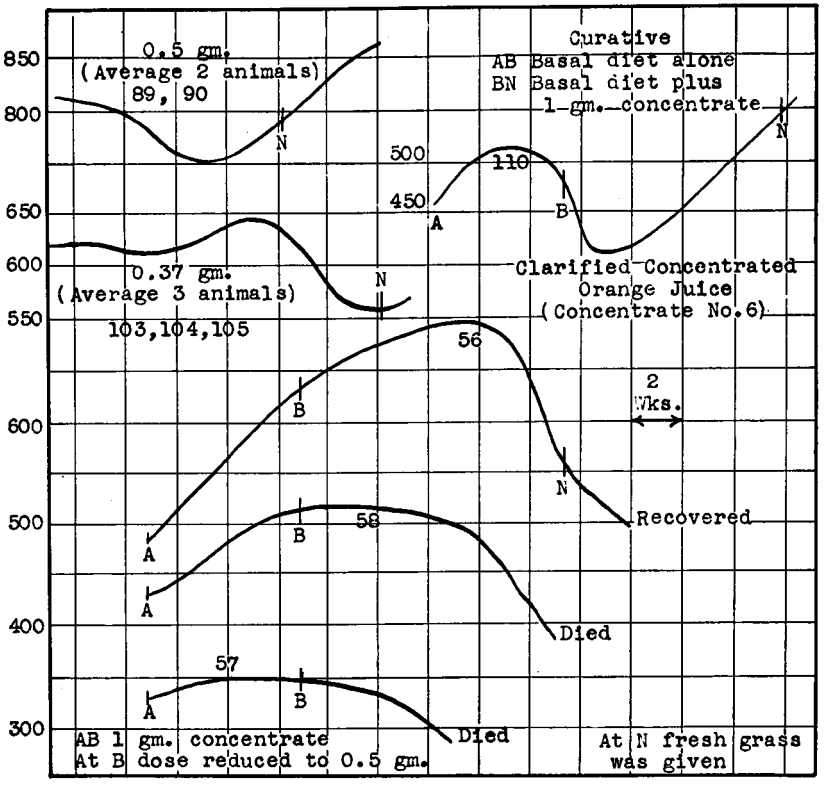

Fig. 12. Clarified concentrated orange juice (Orange concentrate No. 6).

Again, as in the previous trials, successively smaller amounts of the product were administered until the minimum amount which seemed to protect the animals from scurvy was determined.

In a preliminary trial with this concentrate, three guinea-pigs, nos. 56, 57, and 58 (fig. 12), were started on the basal diet plus 1 gram of this product daily. No sign of scurvy appeared in 43 days so the dose was reduced to 0.5 gram of the concentrate, an amount

representing about 1.8 cc. fresh orange juice. One animal, no. 57, soon began to fall off in weight and died at the end of 37 days on the reduced amount of juice or a total time of 80 days on the test. Post mortem did not reveal any significant lesions. The test was continued with the other two animals, nos. 56 and 58, and although both began losing vitality, no recognizable symptoms of scurvy developed. Animal 58, on the dose of 0.5 gram concentrate, died after 70 days, or a total of 113 days. Again, on post mortem examination this animal showed no scurvy lesions. The third guinea-pig, no. 56, was placed on grass after it had been on the reduced dose for 75 days or a total of 118 days. It slowly improved in appearance and the test was discontinued.

The experiment using 0.5 gram dose of this concentrate was repeated, using guinea-pigs nos. 89 and 90. No symptoms developed in 60 days.

In a third series of tests the dose of this concentrate was reduced to 0.37 gram, an amount equivalent to about 1.4 cc. of fresh orange juice. Three guinea-pigs, nos. 103, 104, and 105, received this diet.

These animals maintained their weight for 60 days, then began to lose until the 90th day of the experiment, when they were placed on grass to recover. No symptoms of scurvy were noticed at any time. Two of the animals recovered their weight while the third died some time later, revealing no lesions of scurvy at autopsy.

One curative experiment was carried out, using guinea-pig no. 110. The animal was kept on the basal diet until definite symptoms of scurvy appeared. A dose of 1 gram of the concentrated clarified juice administered daily caused the symptoms to disappear rapidly and the animal soon recovered to normal.

In this case the minimum for protection appeared to be between 0.37 and 0.50 gram representing, respectively, 1.3 to 1.8 cc. of fresh orange juice. Although this juice was exposed to much higher temperatures for a longer time, at these temperatures there does not seem to be an appreciable destruction of the vitamin C factor.

5. *Desiccated Orange Juice.*—This product was made in New York in 1922 by a spray drying process and when the experiments began was over two years old. The Exchange Research Laboratory, which furnished the product, reported that cane sugar had been added before the drying, for otherwise a sticky product would have been obtained.

Because of this addition of sugar, 1 gram of the dried product represented only about 3 cc. of fresh orange juice. Accordingly, 0.5 gram of this product, representing 1.5 cc. of fresh orange juice, should protect guinea-pigs from scurvy, provided the C factor was not lowered in drying and subsequent storage.

The product, which was very hygroscopic, was kept in an airtight container and a small amount was taken each day, diluted with water, and the required quantity administered by mouth, just as were the orange juices.

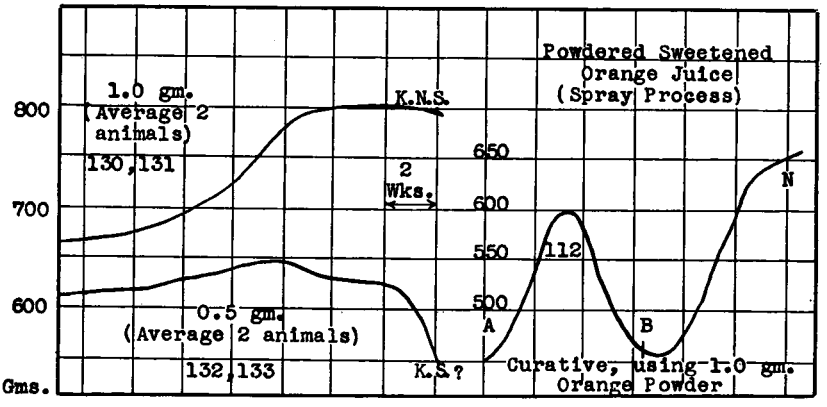

Fig. 13. Experiments with powdered, sweetened orange juice, prepared by a spray process. This desiccated orange juice was two years old at the beginning of the experiment. At N, fresh grass was given. A to B, basal diet alone. B to N, 1.0 gm. powdered orange juice given daily. K.N.S., killed, no scurvy found. K.S.?, killed, scurvy doubtful.

A curative experiment was first tried, using guinea-pig no. 112 (fig. 13). This animal was rendered scorbutic on the basal diet alone and when typical symptoms of scurvy appeared, characterized in this case by painful limbs and face-on-one-side position, 1 gram of the desiccated orange juice was given. The outward symptoms disappeared in less than 12 hours. This animal slowly gained weight and on the 84th day seemed to be fully recovered and was placed on the normal diet.

A second series of tests were carried out, using 1 gram and 0.5 gram of the dry orange juice as a preventive of scurvy. On a 1 gram dose guinea-pigs nos. 130 and 131 (fig. 13) gained weight and appeared to be in perfect health even at the end of the 103rd day. These two animals were killed after the 107th day and a very careful

post mortem examination was made to determine if any symptoms of scurvy could be detected. Both animals appeared to be normal in every respect.

Two other guinea-pigs, nos. 132 and 133, fed on 0.5 gram of the powdered orange juice and the basal diet failed to gain in weight, but no symptoms of scurvy were noticeable in 90 days. After 105 days the animals appeared to be weakening and it was noticed that they hopped around the cages as scorbutic animals were previously noted to do. Both animals were chloroformed and only a mild case of scurvy found in one animal at autopsy.

Unfortunately our remaining supply of the dried orange juice accidentally absorbed water and no further experiments were carried on.

Cavanaugh, Dutcher, and Hall have just published a paper[8] in which they include the results of tests with powdered orange and lemon juices made by a spray process similar to that used in making powdered milk. They have concluded also that the antiscorbutic potency of these powdered fruit juices was retained.

Our above limited number of experiments are in agreement with this report and suggest that it is feasible to prepare a desiccated orange juice which retains an appreciable proportion of its antiscorbutic value. However, from a practical standpoint, not much is gained if so much sugar must be added to the juice that the final product represents a concentration of only one to three. A much higher concentration prevails in both of the concentrated orange juices discussed above.

6. *Dried Whole Orange.*—Another product of considerable interest studied was the dry ground whole orange, furnished by the Laboratory of Fruit and Vegetable Chemistry, U. S. Department of Agriculture.

This product was made from cull oranges from which all unsound fruit had been removed. After quartering, the fruit was coarsely ground in a power mill and allowed to stand overnight so that the juice which might have been squeezed out was reabsorbed in the pulp. The ground fruit was then dried on trays in a dehydrator at a temperature of 155°–175° F. for about 8 hours at a fairly low humidity. The moisture was thus reduced from about 80 per cent to about 8 per cent. One part of the dried product represents four to five parts of the original fresh orange. The product has been used as an ingredient in the manufacture of marmalade and mince meat.

In preliminary experiments, 1 and 2 gram doses were found to be sufficiently large to protect the animal from scurvy for at least 90 days (fig. 14, animals nos. 40, 65).

Considerable difficulty was experienced in feeding with a definite small amount of this product which could be considered representative, as it was not found possible to grind the material to a fine

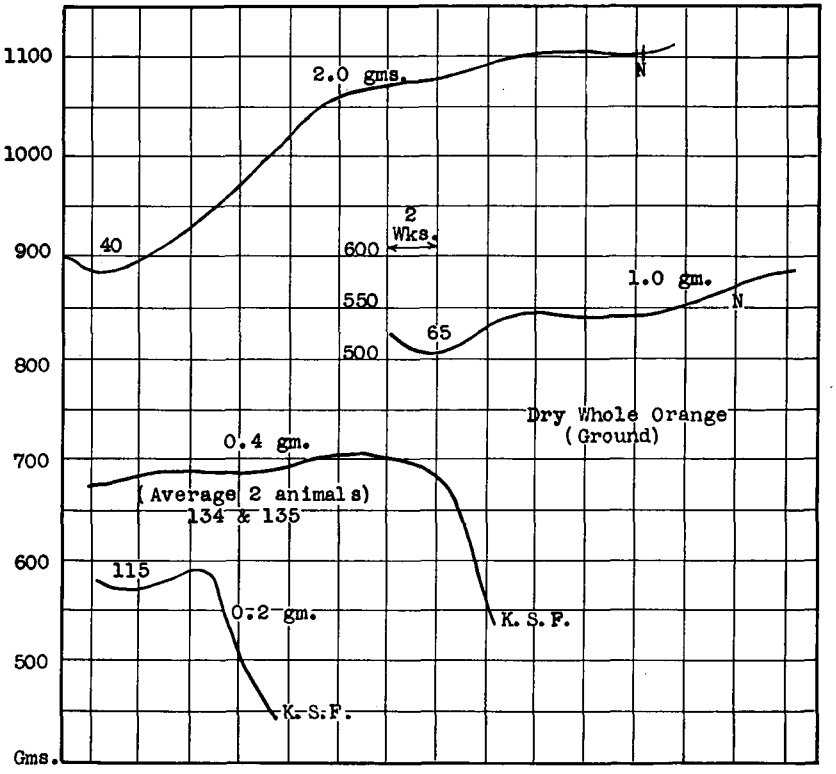

Fig 14. Growth curves with basal diet plus dry whole orange. At N fresh grass was given. K.S.F., killed, scurvy found on post mortem.

powder. The method finally adopted was to crumble the coarsely ground product in a food chopper and stuff the crumbs into small gelatine capsules. Each capsule held approximately 0.2 gram. A definite quantity could thus be administered by mouth each day.

In a second series of tests the dose was reduced to 0.4 gram daily. Guinea-pigs nos. 134 and 135 were maintained for 118 days on this amount but after chloroforming the animals it was found on post mortem that definite scurvy symptoms were present. In one case,

no. 134, the stomach contained numerous well developed hemorrhages throughout, 2 to 3 mm. in cross-section. On post mortem of no. 135 similar well marked lesions were revealed. There were numerous small hemorrhages in the costochondral muscles. Both fore and hind legs showed unmistakable lesions of scurvy. Beginning about one-third way upon the humerus and extending down to the digits, there was a diffuse hemorrhagic area underneath the facia and between the muscles. The muscles of the right hind leg were spotted with many fair sized diffused hemorrhages extending from the hip joint all the way down to the digits. In this case the muscle proper was apparently involved.

On a 0.2 gram dose one animal, no. 115, lived 52 days but at the end of this time was in a very serious condition, having lost a great deal of its weight. It was then chloroformed and a post mortem examination made. Well developed hemorrhages were found in the large intestine and also underneath the skin in the region of all four legs.

So far as we are aware no information is available which would tell us what is the minimum dose of fresh whole orange which would protect the guinea-pig from scurvy. The therapeutic value of this fruit has generally been believed to be contained in the juice and there is little evidence, we believe, to show that the peel and pulp contain the antiscorbutic factor. However, it is apparent from the above test with 0.4 gram of the dried product, representing about 2 grams of fresh whole orange, that the minimum must be near 2 grams. These few tests indicate that a dry whole orange product may be prepared which furnishes a concentrated source of the antiscorbutic factor.

The question of stability of this factor is next to be considered. The above tests were carried out on material which was over a year old, kept sealed in two-quart fruit jars. Further experiments will be carried out on this material when it has been stored for a much longer time.

7. *Concentrated Lemon Juice.*—This product was produced in the glass enameled vacuum pan, just as was the orange juice No. 3. However, the raw lemon juice was held for 24 hours preserved with 6 ounces of potassium metabisulphite to 100 gallons before being concentrated. Sufficient cane sugar was added to the raw juice to make the ratio of total solids to acid three to one.

Concentration required about four hours, temperature conditions being the same as those prevailing during the preparation of the orange concentrate No. 3. One gallon of the concentrated lemon juice represents 5.9 gallons of the raw juice. One gram of the concentrated lemon juice was equivalent to 4.53 grams of the raw juice.

Considerable difficulty was experienced in finding guinea-pigs that would readily submit to feeding with the concentrated lemon juice, even when considerably diluted with water. Two guinea-pigs, however, were maintained for two months on a 0.5 gram dose, an amount equivalent to about 2.2 cc., without visible signs of scurvy.

The high concentration of citric acid in this product undoubtedly is responsible for the aversion of the animals to the lemon juice. An early attempt to prepare a de-citrated lemon juice from the concentrate by removal of the citric acid with calcium carbonate resulted in a product which failed to protect three guinea-pigs from scurvy. This was to be expected since no precautions were taken to exclude air. It has been shown by Zilva[9] that destruction of the antiscorbutic factor is favored by alkalinity and exposure to air. In this connection Zilva[10] has prepared a concentrated de-citrated lemon juice which was active after three months, having been acidified and stored under anaerobic conditions.

Experiments are now in progress in which an attempt will be made, with the knowledge gained from Zilva's work, to determine the antiscorbutic value of this same concentrated lemon juice, which is now over a year old.

The difficulty of feeding the concentrated lemon juice unaltered has greatly handicapped the experimental work on this product. However, the results given indicate that a considerable portion of the antiscorbutic value of the juice has been retained.

Summary

Feeding experiments with guinea-pigs were made to determine whether the antiscorbutic value of commercially concentrated orange juice was lowered during concentration. Fresh orange juice was used as a standard antiscorbutic for control. Similar experiments were made to determine the antiscorbutic value of dried whole orange and desiccated whole orange juice, and a few experiments were made with a commercially prepared concentrated lemon juice. The data obtained show:

1. That commercial orange juice concentrated in vacuum at a low temperature retains practically all of the antiscorbutic value of the original orange juice.

2. That a clarified concentrated orange juice retained the antiscorbutic factor to a great extent although there is a probability that the longer exposure to the air at higher temperatures lowered this value.

3. That a desiccated orange juice prepared by a spray drying process retained a good proportion of the antiscorbutic value of the fresh orange juice even after two years' storage.

4. That a dried whole orange product prepared in a commercial dehydrator was a very concentrated source of the antiscorbutic factor.

5. That concentrated lemon juice, commercially prepared by evaporation in vacuum at a low temperature, could be considered a concentrated source of the antiscorbutic substance.

Acknowledgments

The writer wishes to thank C. M. Carpenter, formerly of the Division of Veterinary Science, and J. Traum, a member of the same division, for assistance with autopsies, and also to acknowledge the advice and encouragement of Professor M. E. Jaffa.

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