THE SCURVY OF GUINEA PIGS.*

III. THE EFFECT OF AGE, HEAT, AND REACTION ON ANTISCORBUTIC FOODS.

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(Received for publication, April 17, 1919.)

Recently considerable experimental work has been done on the effect of heat on vitamines, more particularly on the water-soluble vitamine. In a recent article Denton and Kohman (1) come to the conclusion that "ordinary methods of cooking do not perceptibly injure the nutritive value of carrots." These experiments were carried out on rats, which preclude a satisfactory consideration of experimental scurvy.

In the following experiments guinea pigs weighing about 200 to 250 gm. were employed. As will be seen from Chart 1, it was found that in the course of cooking, carrots lose a very large part of their antiscorbutic potency; that whereas 35 gm. of carrots were able to protect a guinea pig from scurvy when added to its ration, the same amount of carrots which had been cooked for about 45 minutes had lost its potency in this respect. An attempt was made to prevent this loss by acidulating the water by the addition of vinegar. As will be noted from Chart 3, however, the animals came down with scurvy within the usual period.

Further experiments demonstrated the fact that from a nutritional standpoint carrots cannot be looked upon as a uniform article of diet; that there is a marked difference in various lots of carrots and probably also of other vegetables, according to whether they are fresh and young, or are old. It was found, for example, that if, instead of employing the carrots which were ordinarily fed

to our laboratory animals, we gave the same amount of fresh young carrots, plucked only a few days previously and cooked, not only did the animals not develop scurvy, but that they gained steadily in weight for a long period (Chart 2). These carrots could be boiled only for about twenty minutes, as they began to disintegrate if cooked longer; they were then allowed to stand in the boiled water for about one-half hour. Old carrots have therefore a twofold disadvantage in that they contain less antiscorbutic vitamin than the young vegetable, and that they require additional boiling, which still further decreases their limited supply of this essential food factor.

The freshness and age of the vegetables sufficed also to enable them to retain their antiscorbutic potency after dehydration. In previous articles (2, 3) it was stated to have been our experience not only in animal scurvy, but in human scurvy as well, that dehydrated vegetables as purchased in the open market or prepared in the household were without noticeable value in the cure of scurvy. Under favorable conditions certain vegetables can withstand dehydration. This has been conclusively shown by Holst (4) and by Cohen and Mendel (5) in connection with cabbage, and in relation to tomatoes by Givens and McClugage (6). After noting the marked difference between old and young carrots in their ability to withstand the cooking process, we decided to make a similar test in regard to drying, and accordingly had some carrots dehydrated the day they were plucked.1 It was found that a per capita daily allowance of 4.5 gm. of the dried carrots added to the ration was sufficient not only to protect pigs against scurvy but to cure them of this disorder (Chart 3). This amount is equivalent to 35 gm. of fresh carrots, the same quantity which failed as an antiscorbutic when the commercially dehydrated carrots were employed. The dehydrated carrots were kept at room temperature in sealed cartons for a month before the experiment was begun, and had been dried for about 3 months at the end of the test.

This factor of the freshness and age of vegetables or fruit has not been considered in relation to their content of the antiscor-

1 These vegetables were furnished and prepared by Mr. C. Eckroth. The dehydration was carried out by the Mrs. Oliver Harriman Food Research Laboratories, the same plant which dehydrated the vegetables referred to in the previous papers.
butic or other essential food factors. From the above experiments it is evident that it plays an important rôle, and that in the course of aging at least the antiscorbutic vitamin is greatly lessened. It will have to be given consideration in judging the results in experimental scurvy. This variable factor renders it very difficult to prepare a table of the comparative antiscorbutic value of various foods. Where this is attempted, as recently by Chick and Hume (7), all the vegetables and fruits should be uniform in their development and freshness. It is evident that it becomes a matter of prime importance for the dehydrating industry to make use of such vegetables. It may come to pass that by observing this precaution manufacturers will attain the goal of furnishing a product comparable to the raw food. It should be mentioned that in our experiments there was an additional factor to be considered; namely, that the vegetables were not only fresh but young, and therefore more cellular than the older vegetables.

Experiments were carried out to determine whether the water in which the carrots were cooked contained the antiscorbutic factor. It has been reported by Daniels and McClurg (8) that the liquor from cooked beans contains water-soluble vitamin. Our experiments showed that the water in which the carrots were cooked was of little or no value in protecting guinea pigs from scurvy. The water was given, as will be seen from Charts 4 and 5, in the amount of 40 cc. per capita daily, but did not delay the onset of the disease. This addition to the dietary seemed to prevent the marked loss of weight, probably due in part to its salt content. No appreciable difference was noted between the liquor in which old or young carrots had been cooked (Charts 4 and 5).

In the experiments carried out at the Lister Institute by Chick and Hume and other workers, dried milk has been made use of as part of the standard diet in bringing about scurvy in guinea pigs. This food was added to the dietary in order to render it complete and because it was found that this milk was devoid of antiscorbutic power. The inference should not be drawn from these experiments, however, that all dried milk is the same in this regard. It would be an error to infer that milk necessarily loses its antiscorbutic potency when it is reduced to a dry state. In mak-
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After an experimental test of this question we employed a brand of milk which had been dried at a temperature of 116°C. for a period of a few seconds. This milk powder was diluted with eight volumes of water and given to the equivalent of 80 cc. of fresh milk for each guinea pig. This amount was selected as it had been found in previous experiments that 80 cc. of fresh raw milk sufficed to protect a guinea pig from scurvy.

Numerous experiments of this kind were carried out. One of them is shown in Chart 5. It will be seen that five guinea pigs which developed scurvy on a diet of hay and oats and 40 cc. of carrot water were rapidly cured, and gained in weight when the dry milk was added to their dietary. This and similar experiments led to the conclusion that milk which has been dried in this manner (the Just-Hatmaker process) loses little of its antiscorbutic factor. This inference was confirmed by finding that infantile scurvy could be cured by giving dried milk of this variety (2).

In a preliminary communication we reported that canned tomatoes are an excellent antiscorbutic (9). This opinion has been borne out by tests on animals as well as clinical experience. In a recent publication on infantile scurvy it was shown that one ounce of canned tomatoes is sufficient to protect an infant from scurvy and that this food is an excellent antiscorbutic for use in infant feeding and can well replace orange juice, which is so much more expensive. We have carried out an extended series of experiments to ascertain the amount of strained canned tomatoes which it is necessary to add to our ration to protect the guinea pig. It will be seen (Chart 6) that when 1.5 cc. of this food is added to the dietary of each pig scurvy supervenes, but that when this amount is increased to 5 cc. daily, the animals recover and continue to gain in weight for a period of months. Further experiments showed that when only 3 cc. per capita are used some of the animals developed scurvy, whereas others escaped this disorder. If the tomato is boiled it loses somewhat in efficacy, although even under these conditions 5 cc. were found to be sufficient to protect the pigs (Chart 7). It may be added that the tomatoes which were made use of had been canned almost one year previously.
In Chart 7 graphs are reproduced which are composites of three series of experiments; one in which 10 cc. of canned tomato were added to the dietary, another in which 30 cc. were added, and a third in which 60 cc. were given. These experiments were prolonged for 4 to 6 months in order to ascertain whether any symptoms of scurvy would eventually become manifest. All animals, however, remained entirely free from this disorder and gained steadily in weight. The group which received only 10 cc. of tomato thrived just as well as that which received 60 cc., showing that the antiscorbutic quota was entirely satisfied by the smaller amount. It may be added, in this connection, that it was found by tests on pigeons that canned tomato contained considerable of the "antineuritic vitamine," and that pigeons suffering from polyneuritis could be cured by giving them 5 cc. daily of this foodstuff.

It has been stated by Harden and Zilva (10) that 'when orange juice is rendered slightly alkaline it loses its antiscorbutic potency. This proved to be the case under certain conditions. This question was investigated in relation to canned tomato and to orange juice, and it was found that shortly after having been rendered 0.06 N alkaline to phenolphthalein neither of these foods had lost an appreciable amount of its antiscorbutic power. 5 cc. of alkalized tomato were still able to protect a guinea pig from scurvy (Chart 8), and 5 cc. per capita daily of freshly alkalinized orange juice were able to cure a group of pigs which had developed scurvy on another diet (Chart 9). This is in conformity with the efficacious results obtained in human scurvy by the intravenous injection of boiled orange juice which has been rendered faintly alkaline (11). If, however, 24 hours were allowed to elapse between the alkalinization and the feeding, instead of only \( \frac{1}{2} \) to 1 hour, then a considerable amount of the antiscorbutic factor was lost. This is well illustrated in Chart 9, where, after the scurvy had been cured by means of freshly alkalinized orange juice, it redeveloped when we gave an equal amount of orange juice which had been alkalinized 24 hours previously. The same rule seems to hold for alkalinization as for heating; i.e., the length of time the antiscorbutic food is subjected to the deleterious influence is fully as important as the intensity of the process.
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**Chart 1.** Showing the destruction of the antiscorbutic vitamin by cooking. The raw and the cooked carrots were of the same lot. The vegetables were cooked in an open vessel for about 45 minutes. (Scurvy developed in all 4 animals between the 14th and 16th days.)
CHART 2. Guinea pigs were given the same amount per capita of cooked carrots as in the previous experiment (Chart 1), the only difference being that in the first test the carrots were old, whereas in the second they were young and fresh, having been plucked only a few days before being fed. No scurvy developed.
Period I
Hay, oats, water, ad lib.
Cooked acidulated old carrots 35gm daily.

Period II
Raw dehydrated fresh, young carrots 45gm daily equivalent to 35gm fresh carrots.

Chart 3. An attempt was made to retain the antiscorbutic vitamin by means of adding 10 per cent of vinegar to the water in which the old carrots were boiled. This failed, however, the animals developing scurvy.

The second or curative part of this experiment shows that if the carrots are young and fresh they will withstand dehydration. In a previous report it has been shown that this amount of commercially dehydrated carrots failed to protect guinea pigs. The carrots were dehydrated the same day they were plucked, at a temperature of about 71°C.
Chart 4. Period I of this experiment shows that the water in which the carrots have been cooked contains little if any antiscorbutic vitamin.

Period II demonstrates that 10 gm. daily of raw old carrots, such as are used ordinarily for feeding animals, supply an insufficient amount of this vitamin.

Period III shows that the same amount (10 gm.) daily of carrots which were young and freshly picked was able to cure scurvy.
Chart 5. These guinea pigs developed scurvy in spite of receiving a large quantity of the water in which young carrots had been cooked for only 20 minutes. It will be noted, however, that although the pigs developed scurvy they did not lose weight, as is usually the case.

After they had developed definite scurvy they were given in addition the equivalent of 80 cc. of a dried milk prepared by being heated to about 116°C. for a few seconds. The addition of this milk to the diet cured the scurvy, showing that it had largely retained its antiscorbutic vitamine.

Chart 6. Guinea pigs were fed 1.5 cc. daily of canned tomato, in addition to their diet of hay, oats, and water. This was insufficient to protect them from scurvy. When the amount of tomato was increased to 5 cc. all symptoms of scurvy disappeared and they gained steadily in weight.
Chart 7. Each of these four curves is a composite representing an average of a group of guinea pigs, each containing 3 to 5 animals. Guinea pigs A received an addition to their diet of 5 cc. of canned tomato which had been boiled for 5 minutes. It will be seen that although scurvy did not develop, growth was not satisfactory.

Animals in groups B, C, and D received respectively additions of 60, 10, and 30 cc. of canned tomato to their diets. In all cases continuous growth was maintained for several months. It will be noted that the growth was about the same in the animals which received only 10 cc. as in those fed 60 cc. of tomato.
CHART 8. This chart shows that alkalinization does not destroy the antiscorbutic vitamin in canned tomato if it is fed soon after the sodium hydroxide is added. In two of the animals, indeed, it seemed to bring about temporary gain in weight. The tomato was rendered 0.05 N alkali to phenolphthalein.

CHART 9. Animals which developed scurvy on a diet of hay, oats, and water plus lactose were cured by means of adding freshly alkalinized orange juice.

Period III of this experiment shows that when this alkalinized orange juice was allowed to stand in the refrigerator for 24 hours it lost its antiscorbutic potency and was unable to protect the guinea pigs from scurvy.
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