smears only of the uterine cornu, uterine fundus and vagina.

Cornified epithelium was found in the normal control vaginal smears of the living. In all these animals after death cornified epithelium was found in smears of the vagina as high as the cervix, and was invariably absent above the cervix. Except in animals that were in oestrus, large non-cornified epithelial cells were found in vaginal smears before and after killing. In no cases did we obtain large non-cornified epithelial cells in the uterus. Small non-cornified epithelial cells were found throughout the genital system in those animals not in heat. Clumps of small non-cornified epithelial cells, such as observers have noticed in normal external vaginal smears, were found in the uteri of all. Polymorphonuclear leucocytes were found abundantly in both uterus and vagina in those animals not in oestrus, whereas those in heat showed none in the vagina and a decreased number in the uterus. Mucus occurred in both uterus and vagina, in several cases being more abundant in the uterus. Some erythrocytes were present in all smears taken after cutting open the tract, and were therefore assumed to be due to unavoidable contamination resulting from the cutting.

Before drawing any conclusions, it is well to keep in mind that we used only eight animals and that only a rough quantitative estimate of the cells was made, so no attempt to point out cyclic variations is justified. Furthermore, the method allowed the accurate determination of the *highest* origin of a constituent only. For instance, we found leucocytes in both the uterus and vagina. We can say with reasonable certainty that they do arise in the uterus, but our only indication that they arise in the vagina is their greater abundance there. Of course from the work of others where sections of the vagina have been made, it has been clearly demonstrated that they do arise in large quantities in the vagina by diapedesis. Then, too, there is a possibility that the oviducts contribute to the débris. The oviducts of the guinea-pig are very slender; their products, if any, must be very slight in amount, so we feel justified in disregarding them.

In the guinea-pig, then, the origin of the large non-cornified and cornified epithelial cells is the vagina. Small non-cornified epithelial cells, often in clumps and sheets, come from the uterus. Leucocytes arise in considerable quantities from the uterus as well as from the vagina. Mucus also arises mainly in the uterus, but of course may possibly also come from the vagina.

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## AVAILABILITY OF VITAMINS IN PLANT TISSUES[1]

AFTER a careful consideration of the natural occurrences of vitamins, it is quite evident that most plants have the ability to produce either vitamins or their precursors. By some means, not yet clearly understood, plants can bring together non-living materials and transform them into the organic compounds of living matter, and undoubtedly during this process, the vitamins or their precursors find their origin.

According to their nature, vitamins are classified as fat-soluble or water-soluble, and within each class are found several individuals. When these vitamins are produced by plants, each species of plant is genetically capable of establishing certain individual vitamins within its tissues. However, the quantity, potency or available amount of a certain vitamin in a plant of a given species is not always uniform. This appears to vary with the variety[2] of the plant, its degree of maturity or the conditions of soil and climate under which it grew, and with seasonal differences.

After the plant produces its vitamins, either for self-defense and protection, or to serve as hormone-like regulators, it stores them in its tissues. They appear to be kept within the plant cell. Carotene, now recognized as a precursor of vitamin A, has already been associated with the chloroplasts of the cell.[3] The occurrence of droplets of fat in the cytoplasm offers a location for fat-soluble vitamins, or they may be connected with the lipoids, that seem to possess great significance in the activity of the cell, by forming thin films at the interfaces between the continuous and disperse phases. As for water-soluble vitamins, they undoubtedly would be found in the watery sap that fills the vacuoles, or in the aqueous part of the cytoplasm of the cell.

Not all the plant cells, however, are equally supplied with vitamins. In some cases, vitamins seem to be stored in that portion of the plant most exposed to sunshine.[4] House[5] and associates have also found the periderm of the carrot root to be a better source of its vitamins than the cortex.

If the cytoplasm of the plant cell is then recognized as the place where the vitamins are located, their

[1] Contribution from Montana State College, Agricultural Experiment Station, Paper No. 18, Journal Series.

[2] M. F. Bracewell, E. Hoyle and S. S. Zilva, *Biochem. Jour.*, 24: 82–90, 1930.

[3] L. S. Palmer, "Carotinoids and Related Pigments," American Chemical Society Monograph No. 9, Chemical Catalog Co., Inc., New York, 1922.

[4] V. C. Heller and R. R. St. John, *Jour. Nutr.*, 4: 227–33, 1931.

[5] M. C. House, P. M. Nelson and E. S. Haber, Research Bulletin No. 120, 1930, Iowa Agricultural Experiment Station, Ames.

availability to the consuming animal will depend to a considerable extent upon the condition of the cell itself. Sharp[6] believes that the protoplasm of the cell is a polyphase, film-partitioned organization and that a great variety of chemical substances coexist in protoplasm without interacting until certain conditions prevail. It is thought that films separate the different phases of the system, and under appropriate circumstances the properties of these films are altered.

When the plant material is subjected to varied treatment, such as long storage during winter months, or the heat of cooking, changes are recognized as occurring in the organization of the plant cells. In the former case, enzymic activity, and in the latter, coagulation, may cause alterations in viscosity, permeability and rate of oxidation.

It has seemed to the authors that the above conception of the placement of vitamins in the plant cell, together with a variation in their degree of availability, depending upon the condition of the cell, would greatly assist in the interpretation of results obtained in vitamin studies, not only of those conducted in our own laboratory but those reported by other workers.

For five years, the home economics department of the Montana Experiment Station has been testing the Netted Gem variety of potato for its vitamin B (complex) and C potency. The tests have been made under the following conditions: Mature potatoes in the fall (a) raw, (b) boiled twenty-five minutes; potatoes stored six months in cool, damp cellar (c) raw, (d) boiled as above; potatoes stored six months in warm, dry cellar, (e) raw, (f) boiled as above.

Similarly, Chantenay carrots have been tested during four years for their vitamin A, $B_1$ and C potency, under the same conditions as noted for potatoes. In addition, the carrots have been subjected to home canning, by the hot-pack method, in both the oven and the steam pressure cooker.

When the results of these feeding experiments (to be published later) are summarized, the general changes in the vitamin potency of carrots and potatoes may be presented by means of a system of numerical grading shown in the following chart. As tests for vitamin C potency consisted not only in observations of protection from scurvy but also of gains in weight, and as these did not always show parallel agreement, we have separated these observations under two headings $C_s$ (vitamin C as a protector from scurvy) and $C_g$ (vitamin C as effective in growth).

It will be noted at once that the same method of treatment may result in no change, a gain or a loss

[6] L. W. Sharp, "Introduction to Cytology," Ed. 2, 214–222, 1926.

CHANGES IN POTENCY OF VITAMINS IN PLANT TISSUES, AS DETERMINED BY ANIMAL-FEEDING EXPERIMENTS, AND EXPRESSED BY NUMERICAL GRADING.

| Conditions when tested | Carrots A | Carrots $B_1$ | Carrots $C_s$ | Carrots $C_g$ | Potatoes B | Potatoes $C_s$ | Potatoes $C_g$ |
|---|---|---|---|---|---|---|---|
| Fall | | | | | | | |
| Raw | 1.0* | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Boiled | 0.8 | 0.8 | 1.05 | 1.0 | 1.0 | 1.0 | 1.0 |
| Spring | | | | | | | |
| 1. Cool, damp storage | 4 mos. | | | | 6 mos. | | |
| Raw | 1.0 | 1.0 | 1.2 | 1.1 | 1.0 | 1.0 | 1.1 |
| Boiled | 1.1 | 0.8 | 0.9 | 1.2 | 0.8 | 0.9 | 1.1 |
| 2. Warm, dry storage | 4 mos. | | | | 6 mos. | | |
| Raw | 1.0 | 1.0 | 1.3 | 1.2 | 1.0 | 1.2 | 1.2 |
| Boiled | 1.1 | 0.8 | 0.8 | 0.8 | 0.8 | 0.9 | 1.1 |
| Fall | | | | | | | |
| Canned | | | | | | | |
| in Oven | 1.0 | 1.0 | 0.5 | 0.4 | | | |
| in Pr. Cooker | 1.0 | 0.9 | 0.6 | 0.6 | | | |
| Spring | | | | | | | |
| Canned and kept 6 mos. | | | | | | | |
| in Oven | 0.8 | 0.5 | 0.3 | 0.3 | | | |
| in Pr. Cooker | 0.8 | 0.5 | 0.4 | 0.4 | | | |

1.0* = initial potency.

of potency in the several factors here considered. Due to this lack of uniformity of effect upon the vitamins, each will be discussed individually in an attempt to interpret the above trends.

### VITAMIN A

This vitamin is now recognized as occurring in the form of its precursor, carotene, when in plant tissue. In the carrot root, carotene is associated with fatty substances in the chromoplasts of the cell. When equivalent amounts of carrot are ingested in a raw form, apparently equal amounts of vitamin A are available to the consuming animal, irrespective of whether the carrot has just reached fall maturity or has been stored in various ways for four months. A comparison of the vitamin A potency of cooked carrots, in the fall and after storage, presents a different picture. While the cooked carrots, tested in the fall, appear to have suffered a loss in potency as compared with raw carrots, the authors believe that may be attributed to some change in the condition of the chromoplasts that has rendered the vitamin A less available. This conclusion was reached after finding that cooked carrots from cool or warm storage showed a gain in potency. This latter phenomenon is explainable on the basis of greater availability of vitamin A from the chromoplasts of the cells. Cold

and warm storage are known to bring about transformations in the cell structure of plant tissue that might result in changes at the interfaces concerned, thereby increasing the permeability of the chromoplasts when exposed to short cooking.

Canning, on the other hand, subjected the carrots to higher temperatures for a longer period of time, which might readily have caused a weakening in the boundary films of the chromoplasts. When tested for vitamin A in the fall immediately after canning, its availability was comparable to raw carrot but was greater than that of carrots cooked for only a short period. When these canned carrots were kept for six months and then tested, they showed a decided loss in vitamin A potency, that might be due to greater susceptibility to oxidation resulting from greater availability after canning.

### Vitamin B (Complex) and $B_1$

Vitamin $B_1$, in its simple or complex form, is water-soluble and, as previously mentioned, undoubtedly resides in the aqueous part of the cytoplasm of the cells. When either potato or carrot is ingested in a raw form, at the point of maturity in the fall or after warm and cool storage, vitamins $B_1$ or B (complex) appear to be available to a maximum degree. After boiling for 25 minutes, however, there is a distinct loss in the potency of these vitamins, with the exception of potatoes in the fall. This change in potency might again be explained as due to changes in cell conditions that permit the release of the solution of vitamin B and its ready oxidation before being ingested. During the canning process, vitamin $B_1$ in carrots undergoes a slight loss in potency in the pressure cooker, but no loss when canned in the oven. When the canned carrots were again tested in the spring, they showed a pronounced loss in potency. This might be attributed to oxidation of the vitamin after it is made more available by the canning process.

### Vitamin C

This vitamin is also water-soluble, and for this reason may be regarded as occurring in the aqueous part of the cell. Results from our animal feeding experiments indicate that the vitamin C potency of raw carrot increases during either cool or warm storage. This increase is greater, however, in carrots from warm storage. Because of this evident increase, the authors believe that the total amount of vitamin C present in the raw fall carrot is not available to the consuming animal. It is again assumed that during storage cellular changes occur, which render the vitamin C in the carrot available to a greater degree, especially when stored in a warm, dry cellar. The fall carrot, when cooked for a short period, offers slightly more protection from scurvy than it did in the raw form. It would seem then as if cooking produced some changes that also made vitamin C somewhat more available. Tests made on stored carrots when cooked show, on the other hand, some loss of vitamin C in the case of cool storage, and definite loss in warm storage. As the tests of raw carrot from these storages indicate a greater availability of vitamin C, it is apparent that this vitamin has become more susceptible to oxidation during cooking. This point is more strongly emphasized in the pronounced progressive loss of vitamin C potency in canned carrots that were kept for six months.

The preceding summary of a five-year study of the vitamin C potency of potatoes, before and after storage, shows some agreement with the results of the carrot investigations. Again there is an increase in vitamin C potency in the raw potatoes from warm storage, which suggests greater availability. Also, as in carrot, there is greater loss of vitamin C potency in stored potatoes after cooking than in the fall, indicating susceptibility to oxidation.

It is interesting to note the data on animal growth ($C_6$) that were observed while testing the anti-scorbutic potency of potatoes and carrots. Their variations do not always coincide, but again there appears to be an increase in the growth tendency after storage, which only strengthens the theory of greater availability.

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