PIGMENT STUDIES WITH SPECIAL REFERENCE TO CAROTINOIDS IN FRUITS[1]

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This investigation, concerned primarily with the carotinoids, has been divided into 4 sections, as follows: (1) plastid studies; (2) the use of the colorimeter in the determinations of relative amounts of fruit pigment; (3) determinations of the presence of certain pigments in various fruits; (4) ripening experiments. These sections will be discussed in the order indicated.

Plastid Studies

INTRODUCTION

The problem of the origin and structure of leucoplasts, chloroplasts, and chromoplasts has occupied investigators for many years. The state in which the carotinoid pigments occur in fruits, flowers, and leaves has been much under discussion. Are these carotinoids in crystalline or granular form and more or less free in the cell cytoplasm, or are they necessarily imbedded in lipoid material and usually contained within a stroma or plastid? If, as some workers believe, chloroplastids are the progenitors of chromoplastids in which the carotinoid pigments must be contained, how are we to account for the presence of these pigments in the animal kingdom or in various groups of fungi in which chloroplasts are never present?

In an attempt to throw some light on these problems microscopic studies were made on plastids of green, yellow-orange, and red-ripe fruits. Fruits were chosen because the plastids are large and changes can be very easily noted during the ripening process. In several kinds of fruits the decolorization of the chromoplasts was accomplished, leaving behind a colorless stroma very similar to that of the chloroplast. During the in-

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vestigation evidence was accumulated to show that in some fruits the carotinoids were in granular or crystalline form, while in others these pigments were contained in plastids commonly known as chromoplasts. No attempts were made to show just where the color was located in the plastid.

SURVEY OF LITERATURE

The earliest work on plastids was concerned especially with the study of starch grains. The investigations of von Mohl ('55), Böhm ('57), Nägeli ('58), and Sachs ('62) showed that starch grains appeared in a great many of the chloroplasts. More than 10 years before Schimper's experiments on plastids we have the first work along this line by Kraus ('72) who sought to show that the yellow color of the tomato and the rose was originally green, and that the yellow color originated from the green, also that the green chlorophyll-containing bodies, with or without a continuation of form, acquired a yellow color. He assumed that in this case the blue-green pigment disappeared and the original yellow remained or even increased in amount. Kraus was the first worker to prove that the yellow fruit pigments are identical with those of this class in the chloroplasts of the leaf.

Another of the early workers was Millardet ('76) who believed that the chlorophyll bodies became more or less deformed just prior to the disappearance of the chlorophyll. At the same time a great number of these bodies losing their coherence, became elongated and irregular, appearing separate and viscid. A little later they became swollen and coalesced to form somewhat irregular masses located in vacuoles of the cytoplasm. They assumed a more or less yellow-orange tint, due to the presence of small rods of "solanorubin." This colored substance was finely granulated but coarser than that of the chlorophyll. In a later stage these rods of solanorubin multiplied and increased in size.

Schimper ('83), in his epoch-making studies on the origin of plastids, concluded that all plastids arose from the division of preëxistent plastids, that they never arose *de novo* from the protoplasm. These plastids were then transmitted only in the female line, never through the sperm. He found chloroplasts in the embryos of a great many of the higher plants, such as *Hy-*

pericum, *Tropaeolum*, *Evonymus*, *Helianthus*, *Acer*, etc. Schimper showed that leucoplasts may be transformed into chloroplasts by the chlorophyll becoming photosynthetically active or they may remain as leucoplasts and serve in the building of starch from food already assimilated. Finally, in various parts of the plant, as, for example, in roots, leaves, flowers, and fruits, the chloroplasts may become transformed into chromoplasts of various shapes and diverse colors.

That chloroplasts arise from leucoplasts and chromoplasts from chloroplasts, was Schimper's view. He noted an exception to this in the fruit of *Symphoricarpus racemosus*, which, in the ripe condition, contained leucoplasts which were transformed chloroplasts. The latter phenomenon was found to occur in many embryos. This writer assumed that the chloroplast, or the photosynthetic chromatophore, is the most primitive of the 3 types of plastids. From the phylogenetic point of view the leucoplast and chromoplast represented later development and arose concurrently with an increase in tissue differentiation.

Meyer ('83) substantiated much of this work of Schimper's, so that often the theory of the origin of plastids from preëxistent plastids is known as the Schimper-Meyer theory. This theory of Schimper and Meyer has never been seriously called into question in the higher cryptogams nor in the algae, but in phanerogams it has caused some discussion. Mikosch ('85) and Belzung ('87) could find no chromatophores in the resting seeds which they investigated. After examining ripening seeds, mature seeds, and seedlings of a large number of plants they concluded that growth of starch grains can take place without leucoplasts and that chloroplasts are formed directly by differentiation of the cytoplasm.

Courchet ('88) decided that "chloroleucites" and most of the "chromoleucites" have an analogous structure. They consist of a protein substratum or stroma in which are enclosed granules either of chlorophyll or other pigments. Rarely, these pigments are truly crystalline in nature. He believed with Schimper that the "leucites" always resulted from the division of preëxisting leucites and that they multiplied by direct division. Frequently the chromoleucites arose independently of the colorless leuco-

leucites. He studied many different flowers and fruits, among them the flowers of *Aloe* and the fruits of *Evonymus japonicus* (arils) and *Cucumis Citrullus*.

Another worker to substantiate the Schimper-Meyer theory of the origin of plastids was Chmielevsky ('90), who showed that in *Spirogyra* the chromatophores of the male cell disappeared at the time of cell conjugation, while those of the female cell divided and gave rise to the chromatophores of the new plant. Davis ('99) observed the division of a chloroplast in a spore mother cell of *Anthoceros*. Zimmermann ('94), who worked primarily on algae but also on ferns, discussed the chemical and physical character of the chromatophore pigments. His views regarding the development of plastids were the same as Schimper's.

Lotsy ('07) claimed that the Schimper-Meyer theory was not sustained by sufficient evidence so far as higher plants are concerned. He suggested that if hybrids could be obtained between species with markedly different chromatophores, it might be shown experimentally that chromatophores were transmitted only in the female line. Senn ('08), in his extensive work on the form and change of position of chromatophores, studied a great many different algae, also the leaves and stems of spermatophytes, but no fruits were investigated by him. He found that chromatophores change form and position in response to light, temperature, water content, also chemical and mechanical influences. He observed protoplasmic connections between plastids.

Sapehin ('11) observed that during sporogenesis in mosses each archegonial cell contained 1 or 2 deeply staining bodies lying close to the nucleus; these he considered to be chloroplasts. A similar condition existed in *Anthoceros*. All the other Bryophytes and Pteridophytes as well as the Spermatophytes examined showed a considerable number of plastids in the archegonial cells. He traced the division of the single chloroplast in the spore mother cell of various mosses into 4, one going into each tetrad. The ripe spore was found to contain several chloroplasts, as did all the cells of the gametophyte and of the embryo sporophyte. Sapehin ('13), working with *Lycopodium*, showed that each cell of the young antheridium contained numerous plastids, but the number is reduced to one in the antherozoid mother cells. This work is

very important as a substantiation of the Schimper-Meyer theory, for it has sustained the idea of complete genetic continuity of the plastids throughout the life cycle in various groups.

Rothert ('12), in an extensive study of various vegetable organs from many different plants, examined and described carefully the form and shape of the plastids. He found the pigment, whether yellow, orange, red, or brown, to be in distinct granular form, distributed in the stroma, probably dissolved in drops of oil. In one plant, a saprophytic orchid, the pigment was observed to exist as needle-shaped crystals. He believed that chloroplasts and chromoplasts contained the same pigments but in quantitatively different proportions. The carotin was present in distinct grains but the chlorophyll was distributed evenly in the stroma, the latter often colorless but sometimes pale green. His views on the origin of plastids were very similar to those of Schimper, that is, that they form an unbroken series. Molisch ('13) believed that leucoplasts and chloroplasts may be transformed one into the other, and that chromoplasts may be finally produced from chloroplasts.

No review of literature on the subject of plastids would be complete without some mention of mitochondria or chondriosomes, employing these terms as synonymous. An enormous literature has accumulated on the subject, both from the animal and plant side. Only one phase of it will be given here, namely, that which has to do with the theory that mitochondria are the progenitors of plastids. A very good general review and bibliography of the subject was given by Cavers ('14), and likewise by Sharp ('21). Among those who claim mitochondria to be the progenitors of plastids Lewitsky ('10) stands out as one of the most important of the early workers. He found these bodies present in all essential parts of the cytoplasm, not only in the pollen mother cells but also in the pollen grains of *Asparagus officinalis*. In the stem end of the embryo, the chondriosomes changed to chloroplasts; on the other hand, in the root end they were transformed to leucoplasts.

This work on mitochondria, so contrary to the ideas of Schimper and Meyer, was criticized by the latter (Meyer, '11), on the ground that if such bodies really existed, it was extraordinary

that he and Schimper should have overlooked them. Rudolph ('12) obtained no evidence of any relation between chondriosomes and chloroplasts. He worked with a large number of plants belonging to various groups, using the Benda killing method and iron-haematoxylin stain with best success. He concluded from his results that the value of all previous work was merely to show the existence of such bodies in many different plants. Mottier ('18) was also unable to confirm Schimper's view, in a study of the root-tips of *Pisum sativum* and *Zea Mays*, of the thallus cells of *Marchantia* and of *Anthoceros*, and cells of the stems and leaves of *Elodea canadensis*. In this work he found cells containing both chloroplasts and chondriosomes, and his conclusions were that the primordia of leucoplasts were not the so-called mitochondria. He observed other granular bodies that did not become leucoplasts; these he considered mitochondria whose function could not then be definitely formulated. He suggested that perhaps these last are concerned in some processes of metabolism. Twiss ('19), working with *Zea Mays* and *Preissia*, was able to show an unbroken series from mitochondria to plastids in the root-tips of corn, while in *Preissia* this seriation was not so obvious However, he believed that such definitely staining bodies as mitochondria exist as normal constituents of the protoplasm.

Guilliermond has produced more evidence than any other worker to show that mitochondria are progenitors of plastids. He has published a number of papers, only a few of which can be reviewed here. In an important work (Guilliermond, '13) he described the occurrence of chondriosomes in the young asci of *Pustularia vesiculosa*, in *Penicillium*, *Botrytis*, *Endomyces*, yeasts, and in various *Autobasidiomycetes*. In a later paper of the same year he found chondriosomes in a number of *Pezizeae*. This was especially interesting since these forms contain an abundance of carotinoids. In *Pustularia* he distinguished 3 kinds of reserve substances, glycogen, fat, and the so-called "metachromatic corpuscles" of earlier writers. He claims to show that the latter arise from chondriosomes in much the same way as do the plastids of higher plants. His paper (Guilliermond, '19) records the results of his extensive studies on the epidermal cells of the petals of several different flowers, such as tulip and

iris, and also the mesophyll cells of several fruits including the berry of *Asparagus*. He observed the shape, form, movement, and division of chondriosomes of living material in isotonic solutions, and also studied material fixed by Benda's method and stained with iron-haematoxylin. A detailed explanation was given of just how the different plastids were formed from the chondriosomes. He argued that Schimper's so-called protein crystalloids were only the long-drawn-out ends of the dividing chondriocontes. Upon examination they showed no optical properties. In a later paper (Guilliermond, '21), he described the origin of leucoplasts from their primordia in root-tips of castor bean, bean, pea, corn, and a gourd. He acknowledged the fact already established by Rudolph, Sapehin, and Mottier that, besides the primordia of plastids, there may be other similar granules and rods present in a cell which do not become plastids. Kozlowski ('22), in a criticism, said that the whole subject of mitochondria was too indefinite and needed clearing up. He believed that a worker started with a preconceived idea of what chondriosomes should produce and then fitted them into his particular problem.

There has been a little work devoted especially to the formation of carotinoids. The Toblers ('10) made an extensive study of the nature and appearance of carotin in the fruit of *Momordica balsamina*. They found that after reaching maturity all parts of the pericarp quickly became green and showed rich starch content. In the layers of the mesocarp near to the fibro-vascular bundles and also in the endocarp, starch was especially abundant. As the fruit ripened there was a decrease in the amount of starch, and while the mesocarp was becoming yellow the endocarp was turning a light rose-red color, which constantly increased. In this region the deep red color of the aril finally became apparent. The orange-yellow color characteristic of the mesocarp was due to the presence of lens and needle-shaped crystals. In another piece of work finished in the same year the Toblers ('10a) concluded that, in the absence of more conclusive evidence, the idea of a direct genetic relation between chlorophyll and carotin must be excluded. Using the same fruit, *Momordica balsamina*, they decided that throughout the ripening process the formation of

carotin was connected with the decomposition of chlorophyll. They attempted to show a possible relation between carotin formation and the path of nutrition, since the basal part of the fruit always remained green longest. They showed that if respiration is prevented by covering the fruits with vaseline, the ripening process is retarded.

The same workers (Toblers, '12) made comparative researches on chlorophyll, carotin, starch, and sugar in 3 regions of the carrot, namely, top, middle, and base. They found the amount of carotin increased in indirect proportion to the chlorophyll but directly with the starch and sugar content. Age seems to determine the proportion of starch and sugar present and also the amount of carotin. In old roots both starch and carotin decreased from the top downwards, although in younger ones starch was in excess at the top. The sugar content always increased in all regions with age. The progressive greening marked the decrease of starch, the subsequent products of photosynthesis being stored as sugar. These workers observed that carotin is laid down in medullary rays. They remarked on the presence of carotin, often considered a decomposition product of chlorophyll, in close connection with such storage products as starch, sugar, and oil. They concluded that the relations which govern the appearance of carotin are very complicated.

Duggar ('13) during some microscopic observations on the formation of lycopersicin in tomato found that the development of this carotinoid is preceded by the paling and ultimate disappearance of the chlorophyll, and that following this change there occurred a yellowish or orange cast in the chloroplastids. With the disorganization of the chloroplast there appeared granules of an orange carotin-like pigment. Accompanying these changes typical lycopersicin crystals were formed, and as these increased there seemed to be a decrease in the number of orange granules or crystals.

Lubimenko ('14) believed that lycopersicin appears after the decomposition of the chlorophyll, and especially so in those organs which are characterized physiologically by very energetic oxidations in the tissues. Lycopersicin, however, does not exist within the chloroleucites of the fruit before the decomposition

of the chlorophyll. Later ('16) he found during the conversion of chloroplasts into chromoplasts that (1) chlorophyll and the accompanying pigments were decomposed; (2) yellow pigments accumulated and then (3) undergo alterations.

The decomposition of chlorophyll and the accompanying pigments was brought about by oxidations, with the accumulation of yellow pigments and of peroxidase, at the same time with an increase in acidity.

MATERIAL AND METHODS

Green, partly ripe, and fully ripe fruits of the following plants were used: *Solanum Lycopersicum* L., *Rosa rugosa* Thunb., *Asparagus officinalis* L., *Capsicum annuum* L., *Lycium halimifolium* Mill., *Aglaonema Treubii* Engl. Investigations were also made on the following: yellow and red stages of *Solanum Dulcamara* L. and *Solanum Pseudo-capsicum* L.; green and ripe fruit of *Sorbus sitchensis* (Roem.) Piper, and *Solanum carolinense* L.; red-ripe fruits of *Rhus canadensis* Marsh, *Arisaema triphyllum* (L.) Schott, *Citrullus vulgaris* Schrad., *Celastrus scandens* L. (arils), *Evonymus americana* L., *Evonymus europaea* L., *Lonicera* sp., *Viburnum Opulus* L., *Crataegus Phaenopyrum* (L. f.) Medic., and *Dracaena Godseffiana* Hort.

In studying the green fruits water mounts were made of free-hand sections, or in some cases strips of epidermis were used. The same method was followed with the partly ripe and fully ripe material when possible, but often the ripe fruits were too soft to permit of such treatment. Under these circumstances smears were made either of the mesocarp or of the juice alone. Many of these sections were very satisfactory for study without staining. However, to determine if chromoplasts of most fruits are really plastids, killing and staining were resorted to.

A variety of killing and staining methods was tried, but only some of the more satisfactory will be mentioned here. Free-hand sections were killed by a treatment of 30 minutes in stock chromo-acetic solution made up by Chamberlain's ('15) formula, washed in water for 1 hour, dehydrated in the usual grades of alcohol, stained for 3–10 minutes in 1 per cent erythrosin (made up in 70 per cent alcohol), run through the remaining alcohols to

xylol, and mounted in Canada balsam. A very satisfactory method for staining chromoplasts was to kill in medium chromo-acetic solution for 30 minutes, and then wash in water for 1 hour. The sections were then placed directly into an aqueous solution of 0.5 per cent acid fuchsin for 2–10 minutes, after which they were transferred direct to 95 per cent alcohol, dehydrated in the usual manner, and mounted in Canada balsam. This latter procedure was modified to great advantage by mounting in glycerine directly out of the stain. One of the most satisfactory methods for staining chromoplasts of juicy fruits was to press out on a slide first treated with albumin fixative a drop of the juice, which was almost certain to contain an abundance of the plastids, and these were allowed to dry 15–30 minutes. This material was then killed on the slide for 30 minutes in the stock chromo-acetic solution, washed for the same length of time, stained in acid fuchsin, and mounted in glycerine as indicated above.

Paraffin sections 10 μ thick were cut with a rotary microtome from material killed in stock chromo-acetic solution and stained in Haidenhain's iron-alum haematoxylin.

EXPERIMENTAL DATA AND DISCUSSION

The pepper fruit was studied in some detail. Spaces about 2 cm. in diameter were marked off on each of 3 peppers. These fruits were then placed in an electric oven at about 25° C. to ripen. The marked areas were examined during a period of 15 days while the fruits were ripening, at 5-day intervals. In all 3 peppers the results were approximately the same, so only one set of drawings was made. These were camera-lucida drawings of free-hand sections, mounted in water, showing the changes in shape of the plastids, during the various ripening stages (pl. 10, figs. 1–3). With the gradual change in color from green to yellow to orange, and then to the red-ripe pepper color there was a gradual elongation of the plastid and in some of the red-ripe chromoplasts the ends were actually pointed.

To determine whether the chromoplasts of red-ripe pepper were really plastids they were stained with iron-haematoxylin. Strips of the epicarp containing many chromoplasts were killed and stained in acid fuchsin by the method indicated above. The

results were very satisfactory, another proof of the fact that red pepper chromoplasts are really stainable bodies (pl. 10, figs. 4–5). The chromoplasts stained in this material were mostly of the elongated type. In fig. 4 the chromoplasts appeared to be in various stages of division, and a tendency for them to collect around the nucleus was observed.

Free-hand sections of green and yellow-orange tomatoes, mounted in water, showed up the plastids very well. In the green material the chloroplasts were of the ordinary shape; in the yellow-orange a number of pigment granules beside the plastids were visible, also a few small crystalline bodies. These pigment granules were carotin-like in color, while the crystals had rather a pinkish cast. The red-ripe fruit was very hard to section, and so smears were made. In these, beautiful carmine-red, needle-like crystals were observed. In addition, some granular pigment material was present, but no real plastids of any kind (pl. 11, figs. 1–3). Microtome sections made of the 3 stages of fruit and stained with iron-haematoxylin gave well-stained plastids of the green and yellow-orange fruit, but in the red-ripe fruit no plastids appeared, and the crystals seen in the fresh material had entirely disappeared, or were so distorted that they could not be recognized. Attempts were made to stain these needle crystals with acid fuchsin, but with no success, and indeed in the resulting slides no crystalline forms could be found.

Free-hand sections of all 3 stages of the rose fruit showed up the plastids very distinctly. In this fruit a very marked change in the shape of plastids occurred. In the yellow fruit they were elongated, in the red material much more so; in some cells they looked almost like long irregular streaks of colored oil. To get good results smears had to be made of the red-ripe fruit. Free-hand sections and smears of the green, yellow-orange, and red-ripe material were killed and stained in erythrosin as indicated above. The green and yellow-orange plastids stained very readily, but the red-ripe smears were not so satisfactory. It was very difficult to get slides in which the chromoplasts retained their exact shape during the staining process (pl. 10, figs. 6–8).

Green, yellow-orange, and red-ripe fruits of *Lycium* were studied by the free-hand sections mounted in water; here a

gradual elongation of the plastids was observed, but the red-ripe chromoplasts were not so markedly different as in some other fruits. Smears of the red-ripe fruit juice were very interesting; in these slides plastids of peculiar shape were observed, quite different from those of the mesocarp itself. There is a striking resemblance between these plastids and the chondriosomes of *Asparagus* as pictured by Guilliermond ('19), although the chondriosomes of Guilliermond are much smaller. Smears, also free-hand sections, of the red-ripe *Lycium* fruits killed and stained in acid fuchsin showed plastids of very much the same shape as those in the fresh material (pl. 12, figs. 1–5).

Great difficulties were experienced in attempts to make sections of green *Asparagus* fruits, this being due to a hard, bony epicarp. Smears of the mesocarp of fruits of 3 degrees of ripeness mounted in water showed up plastids very well. Here again the red-ripe chromoplasts were very unlike those of the green and yellow-orange preparations. These plastids also are very much like Guilliermond's chondriosomes of *Asparagus* referred to above, but several times larger. Smears made from the red-ripe mesocarp, also from the juice, were stained in acid fuchsin, and in these preparations much-better stained and more normally shaped chromoplasts were obtained from the juice smears than the mesocarp. There were some peculiarly shaped bodies in the red-ripe juice due, no doubt, to disintegration processes (pl. 12, figs. 6–9).

In green fruits of *Aglaonema* studied by means of free-hand sections mounted in water, very distinct and typical chloroplasts were observed. In the yellow fruit the plastids seemed to have disintegrated, with the formation of carotin-colored crystals and granules. In the red-ripe stage carmine-red crystals were present, these being smaller but very much the same in shape and appearance as those present in red-ripe tomato. No attempts were made to stain any of this material (pl. 11, figs. 4–5).

Yellow-orange and red stages of *Solanum Dulcamara* (pl. 11, figs. 6–7) and *Solanum Pseudo-capsicum* (pl. 13 fig. 9) and green and ripe stages of *Sorbus sitchensis* (pl. 13, figs. 1–2) were studied by free-hand sections and smears mounted in water. Nothing unusual was noted about the plastids of *Solanum Dul-*

camara or of *Sorbus*, but those of red-ripe Jerusalem cherry were very much elongated with long-drawn-out points. In ripe *S. carolinense* fruit no plastids were ever observed; the pigment, although a carotinoid, was present in oily globules. Fair results were obtained by staining smears of *Solanum Dulcamara* and red-ripe fruits of *Sorbus* in acid fuchsin. Smear mounts made from the juice of red-ripe Jerusalem cherry, stained in acid fuchsin, showed very well-stained chromoplasts, exactly like those of the fresh material. In the red-ripe fruit of *Rhus canadensis* nothing like a plastid was ever found, just a mass of oily globules as in *Solanum carolinense*.

In red-ripe fruits of *Arisaema*, *Celastrus*, *Viburnum*, *Crataegus* (pl. 13, figs. 3–8), *Evonymus americana* (pl. 11, fig. 9), *E. europaea* (pl. 14, fig. 2), *Lonicera* (pl. 14, figs. 4–5), and *Dracaena* (pl. 11, fig. 8) definite plastids were found in smears mounted in water. Especially good results were obtained by staining juice smears of *Arisaema* and *Celastrus* in acid fuchsin. The stained chromoplasts of *Celastrus* were of exactly the same shape and size as the unstained (pl. 18, fig. 3). *Evonymus europaea* was very interesting because the plastids were so very small in the red arils and very difficult to find, no doubt due to the fact that they disintegrate very early and the pigment is present in oily globules, much the same as in *Rhus* and *Solanum carolinense* (pl. 14, fig. 2).

The work on watermelon was rather curtailed owing to lack of material, only red-ripe fruit being available. Smears were made of the mesocarp near the epicarp, where it remains green even in the ripe fruit. In slides made from this region of the mesocarp oval plastids were observed, some of which were green and some pink. In smears made from the red-ripe pulp many carmine-red needle-like crystals with uneven ends were observed, and these were very similar to those in tomato and *Aglaonema* (pl. 14, figs. 6–7).

To determine whether the chromoplasts of a number of fruits are true plastids these decolorization experiments were undertaken. At first thought it seemed an easy matter to remove the color from a chromoplast. Carbon bisulphide had long been known as a ready solvent for carotinoids, especially lycopin (lycopersicin)

and carotin. As these two carotinoids were present in larger proportion than xanthophyll in the fruits studied, carbon bisulphide was the first solvent tried. The first thing to be done was to get rid of all possible water from the tissue, as water and carbon bisulphide are miscible only in small proportions. Small pieces of sections of red-ripe tomato, *Lycium* mesocarp, and *Evonymus europaea* arils were placed in 25, 50, 75, and 100 per cent acetone, 30 minutes for each grade. The color came out and appeared in drops over the surface of cells. The acetone was allowed to evaporate, after which, where these sections were placed in CS_2, much color was drawn out over the tissue but nothing could be determined in regard to the presence of plastids. Several smears of red-ripe *Lycium* juice containing plastids were placed in each of the following reagents, acetone, carbon bisulphide, chloroform, benzole, and petrol ether, and the effect of each reagent observed. The slides standing in petrol ether and those in carbon bisulphide were the only ones that gave up any color to the liquid. On none of the slides could there be any changes noticed in the chromoplasts. The pigment went out of the tissue of red-ripe *Lycium* when placed in warm chloroform for 12 hours, but the tissue was too badly broken up to be of any use in a study of plastids. Sections of red-ripe *Lycium* were dehydrated by means of the usual grades of alcohol. The color almost disappeared in absolute alcohol, but the plastid structure was not clear.

After all these unsatisfactory results a method was tried which proved very successful. For the extraction of all the chlorophyll and carotinoid pigments in green leaves Tsweet ('06) recommended petroleum ether and absolute alcohol (1 per cent absolute alcohol in petroleum ether for dry leaves and 10 per cent for fresh leaves). This method was applied to fruit tissue in these experiments. Small pieces of the tissue were placed in vials containing petroleum ether and 1 per cent absolute alcohol and allowed to stand for 48 hours, after which they were mounted in glycerine and examined with the microscope. Red-pepper tissue gave very good results with this treatment. On these slides colorless plastids very much like those of the chloroplasts showed up in the tissue. Fruits with very small chromoplasts gave fair results but not so satisfactory as the pepper.

From these studies the writer feels sure that at least in most ripe fruits studied the amorphous pigment in a lipoid substratum is contained in a stroma or kind of plastid. At least there is some sort of an accumulation of the cytoplasm at these points. In some fruits great difficulty was encountered in staining the chromoplasts. The best success was obtained by staining smears of juice. Mention should be made of the fact that after killing in chromo-acetic acid these chromoplasts in most of the fruits appeared as colorless bodies of the original form and shape. Very good slides were made showing stained chromoplasts of red pepper, *Lycium*, *Solanum Pseudo-capsicum*, *Arisaema*, *Celastrus*, while rose and *Asparagus* gave only fair results. The decolorization experiments seemed to show without a doubt that the so-called chromoplast is a definite body or stroma and very much like the chloroplast. These so-called chromoplasts increase by direct division, which is another proof of their plastid-like nature. In all of these fruits there was a variation in plastid shape from globose to oval in the chloroplast, while the chromoplasts were elongated, irregular forms. *Asparagus* no doubt showed the most remarkable plastids, for, as has been previously mentioned, their similarity to mitochondria was great.

Lycopersicin crystals are present in the natural red-ripe fruit of tomato, watermelon, and *Aglaonema*. Proof of the fact that the pigment here is really in a crystalline state is as follows: (1) These crystals disappeared when any attempt was made to stain them, (2) no re-crystallization could be obtained by means of the alcoholic-potash method of Molisch. These 3 fruits contain a large percentage of lycopersicin, so that a generalization might be assumed to the effect that lycopersicin, at least, is usually found in a natural, crystalline form. *Solanum Dulcamara* might seem to destroy the value of this generalization because the red-ripe fruit in its natural condition shows no crystals, but exhibits plastids of very much the same shape as the chloroplasts. However, it is quite possible that the pigment in *S. Dulcamara* is not lycopersicin. In *Rhus* there is another interesting condition, which is that the pigment of the red-ripe fruit appears in oil globules, and at no time was I able to see plastids or crystals. In the 6 kinds of natural red-ripe fruit in which lycopersicin was

found it appeared in 3 different conditions, namely: amorphous in a lipoid substratum contained in a stroma; crystalline, free in the cytoplasm; and in oily globules.

It is interesting to note that within the genus *Solanum* the fruit carotinoids appear as crystals in tomato, in plastids in *Solanum Dulcamara*, and as oil globules in *Solanum carolinense*. Again, in *Evonymus americana* there are very definite chromoplasts, in *Evonymus europaea* the pigment is in oil globules. Within the genus *Asparagus* we have carotinoid pigments in *A. officinalis*, while in *A. Sprengeri* the fruit pigment is entirely anthocyanin. The same pigment conditions occur in the genus *Rhus*, for the pigment of *R. canadensis* is a carotinoid, while in *R. glabra* the pigment is anthocyanin.

A Colorimetric Method for the Determination of the Relative Amounts of Pigment in Four Varieties of Tomatoes

Materials and Methods

The purpose of this phase of the investigation was to work out a method by which a colorimeter of suitable type might be used as a means for making quantitative determinations of the pigments contained in tomatoes. Since the principal pigment in tomato is lycopersicin, and CS_2 is a ready solvent for this carotinoid, this solvent was used in making the extractions for examination with the colorimeter. It was necessary to devise methods for drying the tomato pulp, since no previous work had been done along this line.

Four varieties of tomatoes were used, Ponderosa, Excelsior, Globe, and Dwarf Champion. These were grown to maturity in the experimental plots at the Missouri Botanical Garden. Three methods were tested for drying the pulp, and the one yielding the most deeply colored extract, as determined by the colorimeter, was considered the best. For these preliminary determinations no special varieties were employed; however, 2 classes of fruits were used: (1) red-ripe fruit; (2) red-orange fruit. Tomatoes from each of these classes were selected, then skinned by plunging into boiling water for a few minutes. From

the central areas of these tomatoes slices were cut transversely and weighed out in 20-gm. portions, each being carefully crushed up in a mortar and spread out on filter-paper. From this point 3 distinct procedures were followed: (1) Using a dry-heat method, the pulped fruit on filter-papers, 2 containing red-ripe tomato pulp and 2 red-orange, were dried for 6 hours by means of an electric fan, then brought to total dryness in a gas oven at a temperature varying from 40 to 50° C. (2) With the alcohol method 2 filter-papers of each of the 2 lots, as before, were successively covered with 30, 50, and 70 per cent alcohol, remaining covered with each grade for an interval of 30 minutes, after which the alcohol was filtered off by means of a filter pump. Then 95 per cent alcohol was poured on and after evaporation the pulp was entirely dry. (3) Using the acetone method, the same number of filter-papers were successively covered with 50, 70, and 85 per cent acetone, remaining covered with each grade for 30 minutes. Then 100 per cent acetone was added and, as in the alcohol method, upon evaporation the pulp was entirely dry. From each filter-paper the pulp was carefully removed and extracted in glass-stoppered bottles with 25 cc. of CS_2. After 4 days these extracts were examined with a Duboscq micro-colorimeter. The method followed will have to be described in some detail, as a colorimeter of this type is not ordinarily used in this manner.

One of the lower cups of the colorimeter was filled about one-third full with the extract from the red-ripe acetone-dried material, and the other was filled with an equal amount of the extract from the red-ripe alcohol-dried material, then the colors were matched and a reading taken on each scale. Two "day-light" globes were used as a source of illumination. A match was then made between the alcohol-dried and heat-dried material and a reading on the scale recorded. By this same method the extracts of the 2 kinds of tomato pulp dried in the 3 different ways were matched, and readings recorded in table 1. Dilutions of 50 per cent and 5 per cent were made of all the extracts matched with the colorimeter, and readings taken. The smaller the scale reading the greater the concentration of the extract, therefore the more of extractable lycopersicin per unit of tomato pulp and

hence the better drying method. The results are recorded in table I.

TABLE I

COLORIMETRIC READINGS IN MM. MADE WITH A DUBOSCQ INSTRUMENT

	No dilution			50 cc. extract, 50 cc. CS_2			5 cc. extract, 95 cc. CS_2		
	Alcohol	Heat	Acetone	Alcohol	Heat	Acetone	Alcohol	Heat	Acetone
Red-ripe tomato	2.5	2.3	1.8	2.3	2.1	1.9	2.0	1.7	1.5
Red-orange "	3.4	1.2	0.8	2.6	0.9	0.8	1.8	0.9	0.5

From the results of this experiment acetone is the best drying agent for tomato pulp, since after drying by this method the most deeply colored extracts were obtained, as indicated by the small readings on the scale. With this problem of the drying determined, so far as the 3 methods described were concerned, the main part of the investigation was undertaken. It was accordingly required to determine the availability of this colorimetric method in estimating the relative amounts of carotinoids in these 4 varieties of tomatoes. In all of these experiments only red-ripe fruits were used.

Experimental Data and Discussion

Experiment 1.—Red-ripe fruits from the 4 varieties of tomato, Globe, Ponderosa, Excelsior, and Dwarf Champion, were skinned, crushed in a mortar, allowed to evaporate 12 hours in evaporating dishes, then placed on filter-papers in Buchner funnels and 5-cc. amounts of acetone poured on at 30-minute intervals until 25 cc. had been used. These filter-papers were then placed in evaporating dishes where in a short time the pulp became entirely dry and ready for extraction after being ground in a coffee mill. One gram of each kind of dried, ground material was placed in a ground-glass-stoppered bottle and extracted with 10 cc. CS_2 for 4 days. Colorimetric determinations were then made by the method indicated above. The results are recorded in table II.

TABLE II

COLORIMETRIC READINGS IN MM.; ACETONE-DRIED RED-RIPE FRUIT, 1 GM. OF MATERIAL EXTRACTED 4 DAYS WITH 10 CC. CS_2

No dilution				50 cc. extract, 50 cc. CS_2			
Excelsior	Ponderosa	Dwarf Champion	Globe	Excelsior	Ponderosa	Dwarf Champion	Globe
0.50	0.70	0.95	1.07	1.40	1.90	3.00	3.10
0.42	0.60	0.80	0.90	0.90	1.20	1.83	1.90
0.42	0.60	0.80	0.90	0.60	0.80	1.22	1.27

Experiment 2.—The same material and methods were used as in experiment 1 (except no Dwarf Champion material was available). The results are recorded in table III.

TABLE III

COLORIMETRIC READINGS IN MM.; ACETONE-DRIED RED-RIPE FRUIT, .5 GM. OF MATERIAL EXTRACTED 4 DAYS WITH 10 CC. CS_2

No dilution			50 cc. extract, 50 cc. CS_2			30 cc. extract, 70 cc. CS_2		
Excelsior	Globe	Ponderosa	Excelsior	Globe	Ponderosa	Excelsior	Globe	Ponderosa
0.53	0.63	1.32	1.25	1.56	4.32	1.02	1.03	1.42
0.25	0.30	0.63	0.80	1.00	2.13	0.86	0.865	1.20

Experiment 3.—Juice from each of the 4 varieties of tomatoes being tested was pressed out, filtered, and the precipitate dried in a gas oven at 60° C. One-tenth of a gram of each kind of dried material was extracted in ground-glass-stoppered bottles with 10 cc. CS_2 for 4 days. Colorimetric determinations were made and recorded in table IV.

TABLE IV

COLORIMETRIC READINGS IN MM.; JUICE PRESSED OUT, FILTERED, DRIED IN OVEN AT 60° C., .1 GM. OF MATERIAL EXTRACTED WITH 10 CC. CS_2 (EXTRACTED 4 DAYS)

No dilution				50 cc. extract, 50 cc. CS_2			
Excelsior	Ponderosa	Dwarf Champion	Globe	Excelsior	Ponderosa	Dwarf Champion	Globe
1.70	3.50	8.30	13.10	3.50	8.40	29.80	37.80
0.88	1.80	4.30	6.80	0.41	1.00	3.50	4.50
0.84	1.60	4.00	6.20	0.57	1.40	4.90	6.30

Experiment 4.—Juice was pressed out from Excelsior, Globe, and Ponderosa, then centrifuged, and the pigment was dried in a dehydrater. Seven-tenths of a gram of each kind of material was placed in a glass-stoppered bottle and each extracted with 20 cc. of CS_2 for 5 days. Colorimetric determinations were made and recorded in table v.

TABLE V

COLORIMETRIC READINGS IN MM.; JUICE CENTRIFUGED AND DRIED IN DEHYDRATER, .7 GM. OF MATERIAL EXTRACTED WITH 20 CC. CS_2 (EXTRACTED 5 DAYS)

No dilution			50 cc. extract, 50 cc. CS_2			30 cc. extract, 70 cc. CS_2		
Excelsior	Globe	Ponderosa	Excelsior	Globe	Ponderosa	Excelsior	Globe	Ponderosa
0.38	0.73	1.16	0.13	0.83	1.33	1.02	1.16	2.41
0.33	0.63	1.00	0.80	0.66	1.03	0.05	0.06	1.35

From these experiments Excelsior tomatoes seem without a doubt to contain the largest amount of lycopersicin of any of the varieties experimented with. There is too much irregularity of results, as may be seen from the tables, to permit of any very definite statement respecting the relative pigment content of the other varieties. This is perhaps an indication of the fact that these 3 varieties, namely, Ponderosa, Dwarf Champion, and Globe, all contain very nearly the same amounts of pigment; and this colorimetric method is only a rough means of determining relative amounts.

Experiment 5.—In an attempt to make a color standard for use in quantitative determinations the question arose as to the stability, with elapsed time, of the color of dried tomato pulp; accordingly a time-factor test was made. The method followed was to find some dye to match a CS_2 extract of the dry, ground-up tomato pulp. One gram of this material was allowed to extract for 4 days in 10 cc. of CS_2, then 15 cc. of this extract were diluted with 3.5 cc. of CS_2. A very good match was obtained for this extract by using Medalia's ('20) methods for colorimetric determinations of H-ion concentration. Three cc. each of N/10 H_2SO_4 and N/10 KOH were placed in tubes to each of which 9 drops of methyl red were added. These tubes, one in front of the other

with a third one in the line containing CS_2, were matched with another set of 3 tubes. In this second set, the first tube contained the pigment extract, the second pure CS_2, and the third H_2O. These tubes were placed in an ordinary wooden comparator. This comparison was made December 20.

On March 30 an extract of the same material of the same strength was matched by the same method. To get a good match this time 7 drops of methyl red had to be added to the H_2SO_4, and 20 drops to the KOH. The extract now was more of an orange-red tint than a true red.

Again, on May 22, an extract of the same strength was made from the same material, but this time only 6 drops of methyl red were added to the H_2SO_4 and 20 drops to the KOH to give a good match. The ground material from which the samples were taken for testing had been kept in a paper envelope in a drawer away from the light. The conclusion is that dried, ground tomato pulp changes in respect to pigment color very gradually, if kept in the dark, during a period of 5 months. However, the lycopersicin carmine-red shade disappeared much more rapidly than the red-orange.

Determinations of the Presence of Certain Pigments in Various Fruits

INTRODUCTION

Definite scientific knowledge of the kind and quantity of carotinoid pigments found in fruits is rather limited, as may be seen from a survey of the literature. The main reason for this is the great difficulty with which the pigments are obtained in a pure state. There are 2 general methods for quantitative estimations, one the gravimetric, the other the colorimetric. The gravimetric method is unsatisfactory for most workers because, in any case, the amount of pigment is relatively small, hence the necessity for such large amounts of the fruit employed. Again, the large proportions of colorless impurities, which are very difficult to separate from the pigments, and the rapidity with which these pigments oxidize cause trouble. The colorimetric method is better adapted for general use, because smaller amounts of

material can be used, but it doesn't seem entirely satisfactory on account of the difficulty with which the standards of really pure carotin and xanthophyll can be prepared and kept in such a state. Substitutes, such as 25 per cent alizarin in chloroform, or a 0.2 per cent aqueous solution of $K_2Cr_2O_7$, are used to good advantage, because of their stability. Willstätter and Stoll ('13), using $K_2Cr_2O_7$ solutions, established a certain set of relations between the standard 0.2 per cent $K_2Cr_2O_7$ and amounts of carotinoids by means of which quantitative determinations of carotin and xanthophyll might be made on any fruit.

In consideration of these rather unsatisfactory results, no actual quantitative determinations were carried out. However, attempts were made to use the colorimeter determinations in connection with a color code, to estimate the relative quantities of pigments in certain fruits. Spectroscopic determinations were made of a number of fruit extracts, and artificial crystallization of these pigments within the tissues was accomplished.

Throughout this investigation the term lycopersicin suggested by Duggar ('13) has been used in place of the older term lycopin.

SURVEY OF LITERATURE

Citrullus vulgaris.—The first work done on the identification of the watermelon pigment was that of the De Negris ('79) who isolated the pigment from the flesh of the fruit and called it rubidin. Courchet ('88) determined that the natural crystals were the same in form and color as those of tomato. Upon agitation with ether the pulp gave almost instantly a beautiful yellow-orange solution, which deepened rapidly in color in proportion to its concentration. The crystals which formed voluminously upon evaporation were needle-like in form or, more frequently, long, slender, carmine-red sheets, which were often contained in sheaths. Montéverdé and Lubimenko ('13) further confirmed the presence of lycopersicin in watermelon. Lubimenko ('14) gave spectroscopic determinations for petroleum ether and CS_2 extractions of the pulp. Nevertheless, the work on this pigment has been very limited, and about the only definite information is the fact that lycopersicin is present in large amounts.

Capsicum annuum.—Pabst ('92) tried to identify the pigment

in pepper with carotin, but was unsuccessful. Kohl ('02), by means of spectroscopic examinations, was able to find no difference between pepper and carrot pigments. Tschirch ('04) found the following absorption bands for pepper: (1) 517.0–501.0 μμ; (2) 486.0–467.0 μμ; (3) 458.0–439.0 μμ, no end absorption. Duggar ('13) obtained the characteristic, bright red lycopersicin color in a CS_2 extract, which gave the distinctive lycopersicin spectrum, 2 bands in the green and 1 in the blue, though no figures were given for the limits of the bands. The name lycopersicin was suggested by him to take the place of lycopin, because lycopin has long been employed to designate a resinoid compound derived from the bugle-weed. Lubimenko ('14) called the pigments of pepper purified by various washings with 95 per cent alcohol a lycopinoid, because its absorption bands were intermediate between pure lycopersicin, which relation the following data show. *Daucus Carota*, which is considered to contain practically no pigment but carotin, gave a spectrum with 3 bands: (1) 533.0–508.0 μμ, (2) 489.0–472.0 μμ, (3) 455.0–445.0 μμ. Pepper showed 3 bands: (1) 560.0–540.0 μμ, (2) 530.0–500.0 μμ, and (3) 495.0–480.0 μμ. *Areca Alicae*, which he considered to give a very characteristic lycopersicin spectrum, gave the following bands: (1) 565.0–540.0 μμ, (2) 520.0–500.0 μμ, (3) 480.0–470.0 μμ. Van Wisselingh ('15), by means of the alcoholic-potash method of Molisch ('13), obtained the pigment in a crystalline form in the tissue of the fruit. These crystals were mostly in the form of aggregates, but others varying in form were present, and they ranged in color from red (Klincksieck et Valette ('08), Code des Couleurs, pp. 16, 21, also 76, 81, and 106, 127) to orange-red and orange. No definite qualitative or quantitative determinations for the pigments contained in pepper have been made.

Solanum Lycopersicum.—The red tomato pigment has been studied by a great many workers, and from definite chemical determinations we know now that it is identical neither with carotin nor xanthophyll. Millardet ('76), the first worker on tomato pigments, soon recognized that it was not identical with either the red or yellow pigments of other fruits. A. and G. de Negri ('79) considered the pigment identical with "rubidin" which they had isolated from watermelon. Ehring ('96), Ar-

naud ('85), and Passerini ('90) all believed the tomato pigment to be carotin, as did also Tammes ('00) and Kohl ('02). Schunck ('03) suggested the name lycopin for the pigment which he was able to extract from the tomato. By difference in spectroscopic analysis and solubility, he decided this pigment was different from his chrysophyll. Montanari ('04) submitted the pure crystalline lycopersicin to chemical analysis and obtained the first evidence of the true relation between lycopersicin and carotin. He obtained an average composition of C = 84.14 per cent and H = 10.88 per cent. He considered this to correspond sufficiently closely to Arnaud's formula $C_{26}H_{38}$ for carotin, which had not been disproved at that time. Molecular weight determinations in benzene, using the cryoscopic method, gave a value of 698, from which he decided it must be a dicarotin with the formula $C_{52}H_{76}$.

Willstätter and Escher ('10) showed that lycopersicin was identical in general composition and molecular weight with carotin, —differing only in degree of solubility in certain solvents, in the position of the absorption bands, in the form and color of the crystals, and in the melting point. They determined lycopersicin to be a true isomer of carotin. Out of 74 kgms. of tomato conserve (canned tomatoes) they obtained 11 gms. of crystalline lycopersicin. Crystals of carotin were also obtained in small amounts as a by-product. The analyses and molecular weight determinations agreed very closely with the theoretical determinations made by Willstätter and Mieg ('07) for carrot. Montéverdé and Lubimenko ('13) have confirmed these figures. From the above work the principal pigment in tomato has been shown to be lycopersicin, although carotin is present in small amounts. Quantitative determinations for lycopersicin have been made.

Rosa rugosa.—Some variation occurs in the pigmentation of the different species of rose as indicated by the literature. Tammes ('00), the first worker, found the pigment constantly gave a blue color with H_2SO_4, HCI, HNO_3, phenol, and bromine water, thus showing the presence of carotinoids. Kohl ('02) noted the occurrence of carotin in *Rosa* sp. Tschirch ('04), employing the method of capillary analysis with *Rosa canina*, obtained a dark orange zone, and his spectrum analysis gave the following bands:

(1) 492.0–475.0 μμ, (2) 462.0–445.0 μμ, (3) 439.0–418.0 μμ. Montéverdé and Lubimenko ('13) isolated lycopersicin crystals from the dried fruit pulp, but they regarded this as a minor constituent of the pigments. Lubimenko ('14) reported the presence of lycopersicin in the chromoleucites in the form of small needle crystals, and included the rose in his list of fruits containing lycopersicin. Van Wisselingh ('15) by means of the Molisch ('13) alcoholic-potash method was able to obtain crystals of carotinoids from *Rosa rugosa*.

Evonymus.—Courchet ('88) found the arils of *Evonymus* to contain fusiform, orange-red chromoleucites in which the pigment was present in the form of needle-like crystals which he called crystallites. The usual blue color reactions for carotinoids with H_2SO_4 and HNO_3 were obtained. Tschirch ('04), in his capillary colorimetric analysis of the alcoholic extract of *E. europaea*, obtained several yellow to red-orange zones. Spectroscopic analysis gave the following bands: (1) 495.0–472.0 μμ, (2) 467.0–439.0 μμ, (3) 430.0–415.0 μμ, with no end absorption. Tammes ('00) examined the arils of *E. latifolius* and found that the plastids gave the characteristic blue color with H_2SO_4, HNO_3, HCl, and bromine water. Lubimenko ('14) classed *E. japonica* as a fruit containing lycopersicin.

Solanum Dulcamara.—Thudichum ('69) classed the pigment of this fruit as a lutein. Hartsen ('73) found a red substance in granular form, crystallizable in tablets, which was the same substance that he found in *Tamus communis* and *Asparagus officinalis*. Tammes ('00) obtained crystals by the Molisch ('13) alcoholic-potash method. Lubimenko ('14) found the chief pigment to be lycopersicin. Van Wisselingh ('15) also obtained crystals by means of the Molisch alcoholic-potash method.

Sorbus.—The first worker on these fruits was Tammes ('00) who reported the presence of carotinoids, as determined by the characteristic reaction with H_2SO_4, HCL, HNO_3, phenol, and bromine water. By means of the Molisch alcoholic-potash method he was able to obtain yellow-red, pointed crystals, single, or in small bundles. Kohl ('02) reported the presence of carotin in *Sorbus Aucuparia* and *S. Aria*. Van Wisselingh ('15), by various methods, found 3 types of crystals in the pericarp, but he did not undertake to classify them.

Asparagus.—Thudichum ('69) classified the pigments found in this fruit with the luteins. Hartsen ('73) found some red substances in the form of granules that were crystallizable in the form of tablets. These pigments were insoluble in H_2O, slightly soluble in alcohol and ether, but especially so in benzene.

Aglaonema.—Tammes ('00) determined the presence of carotinoids in *A. commutatum,* by means of the characteristic color reactions with H_2SO_4, HCl, HNO_4, phenol, and bromine water. Van Wisselingh ('15) obtained by using the Molisch alcoholic-potash method, characteristic carotinoid crystals. *Crataegus* pigments have been very little experimented with, though Thudichum ('69) classed them as luteins.

Other fruits.—On *Solanum Pseudo-capsicum* very little work has been reported. Kraus ('72) observed red and orange-yellow pigment forms in fruit flesh of Jerusalem cherry, and also made spectroscopic determinations of the alcoholic extract. *Lycium* was found to contain carotinoids by Courchet ('88). In *Lonicera,* Schimper ('83) stated that he found red and orange-yellow crystals in the plastids. Courchet ('88) obtained what he called crystallites in the fruit of *Lonicera Caprifolium.* Molisch ('96) and Kohl ('02) obtained crystals by the alcoholic-potash method of the former worker. *Viburnum Opulus* was worked on by Van Wisselingh ('15), who obtained carotinoid crystals by using the Molisch alkaline method but no classification was made of them. Fritsch ('84), who alone has worked with *Celastrus scandens,* described the shape of the chromatophores and observed that they were colored blue and then dissolved with concentrated H_2SO_4, also that iodine colored them blue-green. In *Cucumis Melo,* Courchet ('88) examined the character of the pigments in the plastids, then extracted the pigment and obtained, upon recrystallization, rhombohedral crystals and a few trapezoidal forms orange-red in color. No work has been reported upon the pigments of *Arisaema, Dracaena* or *Rhus.* In *Momordica charantia* fruit Duggar ('13) obtained spectroscopic and physiological proof that the principal pigment of the carpels is carotin, but that the aril is characterized by lycopersicin. Courchet ('88), from a recrystallized ether extract of the aril of *Momordica balsamina,* obtained carmine-colored needles, which he found to be identical

in form and color with those obtained from tomato and watermelon. The Toblers ('10a) and Duggar ('13) have confirmed Courchet's observation that the red pigment in the aril of *Momordica balsamina* is lycopersicin.

From this review of the literature it is evident that with the exception of a few fruits such as tomato and pepper very little is definitely known concerning just what carotinoids and in what proportions they are present in fruits.

Daucus Carota.—The first yellow plant pigment to be isolated in crystalline form was carotin from carrots. This pigment was first described by Wachenroder (1826) and called carotin by this worker. This investigation serves as the starting point for our knowledge of the properties as well as the nomenclature of the carotinoids. Vauquelin and Bourchadat (1830) were the next workers, but not until the investigations of Zeise ('47) was carotin isolated from carrots in amounts sufficient for analysis. He discovered its ready solubility in CS_2 and made the first analysis of carotin, giving it the formula C_5H_8, with a melting point of 68° C. This formula was not accepted, however, due to the authority of the next experimenter, Husemann ('61), who worked extensively with carrots. His methods were to press the juice from finely grated carrots, and then add weak H_2SO_4, thus throwing down a coagulum, which was partly dried and extracted with 80 per cent CH_3OH, then this residue dried and extracted with CS_2. The carotin crystals were then precipitated by the addition of absolute alcohol, these being purified by washing with hot 80 per cent ethyl alcohol and finally with absolute alcohol. Husemann was the first to show the unsaturated nature of the carotin molecule, although he regarded the chlorine and iodine derivatives which he was able to make as substitution products. From these analyses he proposed the formula $C_{18}H_{24}O$, and his figures were accepted over those of Zeise ('47).

Arnaud ('85), the next investigator, pressed the juice from grated carrots and precipitated the pigment by the addition of lead acetate, then dried this precipitate *in vacuo* and extracted with CS_2. The residue was washed with cold petroleum ether, and the pigment purified by crystallization from CS_2, with absolute alcohol, and it was then allowed to recrystallize spontane-

ously from cold petroleum ether. About 3 gms. of crystals per 100 kgms. of carrots were obtained in this way. His analysis of freshly prepared crystals showed an average composition of 88.67 per cent carbon and 10.69 per cent hydrogen, thus definitely proving the correctness of Zeise's statement that carotin is a hydrocarbon. This worker for the first time prepared the crystalline iodine derivative of carotin. The elementary composition of this product and the composition of pure carotin led Arnaud to give the formula $C_{26}H_{38}$ to carotin and $C_{26}H_{38}I_2$ to the iodine derivative. Kohl ('02) did a great deal of detailed work on the chemical and physical properties of carotin. His own analyses of the crystalline pigment in carrot gave unsatisfactory results, so he accepted Arnaud's formula as correct. His publication contains an extensive and valuable list of plants and animals containing carotin.

Schunck ('03) obtained a very good yield of crystalline pigment by drying the juice of grated carrot and dissolving in ether. It crystallized in a form very much like the chrysophyll of Hartsen ('73) and Schunck ('01), which was no doubt identical with carotin. He made spectroscopic examinations in which the bands corresponded very closely with those of chrysophyll. Tschirch ('04) cut into pieces carrot which had been soaked in water for a day to remove all sugar, then cooked in water, covered with alcohol for a day, and finally dried by gentle heating. These dried pieces were then ground in a powder mill and extracted in a Soxhlet with petroleum ether of boiling point 60° C. After evaporation of the petroleum ether the residue was crystallized out of alcohol. Later he found it unnecessary to cook the carrot slices, and the soaked pieces were treated immediately with petroleum ether in a Soxhlet. This ethereal extract was allowed to evaporate spontaneously. After a few days the walls of the glass container were covered with the characteristic, dark red to orange, metallic carotin crystals. These were washed with ethyl alcohol and dried under reduced pressure. The alcoholic solutions gave a spectrum with three absorption bands: (1) 487.0–470.0 μμ, (2) 457.0–439.0 μμ, (3) 429.0–417.0 μμ. Solutions in chloroform gave two bands: (1) 507.0–486.0 μμ, (2) 473.0–454.0 μμ. Willstätter and Mieg ('07) definitely settled the composition of

carotin and proved its identity with the pigments in leaf chloroplasts. They prepared the crystalline material in large amounts, making several extractions with CS_2, which were in alcohol and crystallized from petroleum ether. Their data show a mean ratio of C:H of 1:1.406 for which the simplest formula is C_5H_7. Molecular weight determinations in chloroform and CS_2, using the ebulloscopic method, showed an average of 536, which corresponds exactly with the formula $(C_5H_7)_8$ or $C_{40}H_{56}$—which is now accepted as the empirical formula for carotin. Euler and Nordenson ('08) also isolated carotin from carrots in quantities sufficiently large for analysis. Their results confirmed the Willstätter and Mieg ('07) formula. The purified crystals were also found to contain xanthophyll, which was identified by the color of the crystals and their differences in solubility. Escher ('09), in a very comprehensive paper, gave minute directions for making quantitative determinations of carotin in carrot, methods in which he used both large and small quantities. He confirmed the work of Willstätter and Mieg, but was unable to determine the structural formula, because of impurities in the crystalline material, which he was unable to eliminate. Palmer and Eckles ('14) have shown the presence of xanthophyll in the carrot root by the Tsweet ('06) chromatograph method. Van Wisselingh ('15), using the microchemical methods, was unable to show the presence of xanthophyll crystals in the carrot root. From a survey of this work it is evident that carotin and xanthophyll are the carotinoids in carrot, also that the empirical formula for carotin has been definitely established, and that some quantitative determinations have been made.

MATERIALS AND METHODS

The fruits used were *Lonicera, Lycium, Citrullus vulgaris, Cucumis Melo, Aglaonema, Arisaema, Solanum Dulcamara, Viburnum, Celastrus* (carpels and arils), *Solanum Lycopersicum, Rosa, Asparagus, Evonymus americana,* and *E. europaea, Sorbus, Rhus, Capsicum, Solanum Pseudo-capsicum,* and *Crataegus.* These were all fully ripe fruits.

For drying the fruit there was used a vegetable dehydrater constructed by Dr. G. T. Moore after one designed by the U. S.

Department of Agriculture (Pugsley, '17). For the colorimetric determinations, the same colorimeter was used and the same methods followed as in a previous part of the investigation. All extractions were made with CS_2, the material being first ground in a coffee-mill or mortar. Extractions were then made in ground-glass-stoppered bottles. Klincksieck and Valette's ('08) 'Code des Couleurs' was used for color comparisons.

The instrument used for spectroscopic analysis was an A. Krüss, Hamburg, spectroscope. Two 115-volt, 75-watt Mazda lamps were used, one to illuminate the scale and the other as a source of light for the collimator. All readings were taken with a very narrow slit in the collimator tube. This type of instrument has a movable mm. scale reflected into one of the tubes. The 70th mm. division was adjusted to the sodium flame spectrum, and with this adjustment the flame spectrum of lithium fell at 51 mm., that of calcium at 61 mm., and of rubidium at 160.5 mm. The conversion from mm. on the scale to wave lengths, the standard unit for expression of the width of absorption bands was accomplished by means of an interpolation curve. The method for plotting this curve was taken from Landauer ('07). The divisions of the scale were plotted along the abscissae on coordinate paper; wave lengths from 400 to 750 μμ formed the ordinates. The divisions on the scale where the flame spectra appeared for sodium, calcium, rubidium, and lithium, 60, 61, 160.5, and 51 mm., respectively, were converted into wave lengths by using Rowland's Table, Landauer ('07). A smooth curve was then constructed through these points. Measurements were then made and these scale measurements were then converted into wave lengths by means of the interpolation curve mentioned.

The methods used for crystallization of pigments in tissues were 2: (1) The Molisch ('13) alcoholic-KOH method, and (2) a modification of this. In the first method small strips of the fruit tissue were placed in vials containing alcoholic-potash (40 per cent C_2H_5OH by volume and 20 per cent KOH by weight). These were then put in the dark at room temperature, 20° C., and for a short period out of every 24 hours the temperature was raised to 75° C. They were kept for varying lengths of time until crystallization appeared in the tissues, upon microscopic exam-

ination. The second method, a modification, was proposed by Van Wisselingh ('15) to hasten crystallization. The same process was followed as before, but 10 per cent by weight of KOH and 100 per cent glycerine was used and a constant temperature of 140° C. In this work a constant temperature of 95° C. was found satisfactory. In both methods, after crystallization had taken place, the crystals showed up more clearly if the pieces of tissue were thoroughly washed in distilled H_2O and mounted in glycerine.

EXPERIMENTAL DATA AND DISCUSSION

One gram of each kind of dried, ground fruit tissue was placed in separate ground-glass-stoppered bottles, and 5 cc. of CS_2 added to each and allowed to extract in the dark for 4 days. Then spectroscopic and colorimetric determinations were made, also comparisons with the color code. The results of the spectroscopic analysis are given in table VI, those for colorimetric determinations in table VII, and for the comparison with the color code in table VIII.

Table VI shows that the pigment of tomato, watermelon, *Rhus*, *S. Dulcamara*, *Arisaema*, and *Aglaonema* is largely lycopersicin. These readings approximate those made by Willstätter and Escher ('10) for tomato. They show the limits of the 3 characteristic absorption bands of lycopersicin. The typical lycopersicin absorption bands are 2 in the green and 1 in the blue region of the spectrum. The first band is never so distinct as the second, which is a fact pointed out by Lubimenko ('14) and found to be true in this investigation. Pigments of *Rhus* and *Arisaema* have never been shown to contain lycopersicin before, so far as I was able to ascertain. Other carotinoids are also present in these fruits, as demonstrated in the crystallization experiments. When the CS_2 extracts from these fruits were mixed with equal amounts of petroleum ether, in every case only a very little yellow to orange color appeared in the petroleum ether layer, thus showing the portion of carotin and xanthophyll to be rather small.

The pepper pigment requires some discussion. It has attracted the attention of several workers because of contradictory results. Palmer ('22) calls attention to the fact that the absorption bands given by Tschirch ('04) for the pepper pigment, namely, (1)

TABLE VI

SPECTROSCOPIC ANALYSIS OF VARIOUS FRUITS. EQUAL WEIGHTS OF MATERIAL DRIED IN DEHYDRATER, POWDERED IN MORTAR, AND EXTRACTED 4 DAYS WITH EQUAL AMOUNTS OF CS_2. ABSORPTION BAND LIMITS ARE GIVEN IN μμ

Material	Band I	Band II	Band III
Aglaonema Treubii	562.0–548.5	524.0–513.2	495.2–483.2
Solanum Lycopersicum	557.0–536.5	520.0–503.0	485.0–477.5
Rhus canadensis	557.0–536.5	520.0–503.0	485.0–477.5
Solanum Dulcamara	557.0–541.0	522.5–505.0	485.0–476.5
Arisaema triphyllum	557.0–541.0	520.0–503.0	485.0–476.5
Citrullus vulgaris	557.0–541.0	522.5–505.0	485.0–476.5
Celastrus scandens (arils)		531.3–509.2	498.0–485.0
Solanum Pseudo-capsicum		528.5–509.2	494.5–480.0
Celastrus scandens (carpels)		513.2–503.0	480.0–470.0
Evonymus americana		528.2–513.2	494.5–483,2
Lonicera sp.		522.5–509.2	494.5–480.0
Rosa rugosa		522.0 over, shaded no good bands.	
Evonymus europaea		531.3–513.2	483.5–490.0
Asparagus officinalis		531.3–511.3	501.3–488.8
Capsicum annuum		522.5–503.0	494.5–483.2
Lycium halimifolium		522.5–503.0	496.5–480.0
Cucumis Melo		531.3–513.2	501.3–490.0
Viburnum Opulus		513.2–503.0	480.0–476.5
Sorbus sitchensis		513.3–517.5	500.0–485.0

TABLE VII

COLORIMETRIC DETERMINATIONS MADE WITH A DUBOSCQ INSTRUMENT. READINGS GIVEN IN MM. NO DILUTIONS

Rhus	.05	.01	Capsicum annuum	2.9	.7	Citrullus vulgaris	10.5	2.8
S. Dulcamara	1.90	.47	E. americana	5.4	1.5	Lonicera	11.88	3.03
C. scandens (arils)	2.15	.54	Asparagus	6.8	1.9	E. europaea	13.4	3.7
S. Lycopersicum	2.30	.55	Lycium	8.2	2.2	S. Pseudo-capsicum	13.9	3.9
Arisaema	2.30	.57	Rosa	9.1	2.3	Viburnum	16.04	4.5
C. scandens (carpels)	2.35	.60	Cucumis Melo	9.9	2.5	Sorbus	42.4	11.9

517.0 μμ–501.0 μμ, (2) 486.0 μμ–467.0 μμ, and (3) 458.0 μμ–439.0 μμ, and those by Willstätter and Escher do not correspond exactly, but that Tschirch's ('04) bands coincide more nearly with the measurements made by Willstätter and Escher ('10) for carotin, which are 525.0 μμ–511.5 μμ and 488.5 μμ–474.0 μμ. In a true lycopersicin spectrum the band furthest towards the red end of the spectrum should lie at least as far over in the green as 550.0 μμ. Lubimenko ('14) does not class the pepper pigment with the fruits he considers to contain lycopersicin, because its absorption bands lie intermediate between pure lycopersicin and pure carotin, and also because he was never able to crystallize this pigment. In my work a good pure lycopersicin spectrum

was never obtained. At times a very faint band appeared in the characteristic position, but never with any degree of certainty. The spectrum obtained always indicated the presence of carotin and xanthophyll rather than lycopersicin. This may be due to the presence of a mixture of lycopersicin and carotin, in which the proportion of carotin is considerably greater than that of lycopersicin, under which condition there is much difficulty in getting distinct absorption bands. Even when greatly diluted with CS_2, the CS_2 extracts of these 6 previously listed lycopersicin-containing fruits exhibit a decided pink color. At equal dilutions the other fruit extracts were orange in color. This interesting phenomenon, due perhaps to dichromatism, is striking in a comparison of the pepper and tomato. From these 2 plants CS_2 solutions of equal strengths are about the same color, but upon dilution with CS_2 the tomato remains red, pink in great dilution, while with corresponding strengths the pepper turns orange in slight dilutions and yellow in greater dilution.

The spectrum analysis of the other fruit extracts require no particular comment. They showed 2 bands, one in the green and one in the blue region. This is a characteristic carotin spectrum.

TABLE VIII

COMPARISON WITH KLINCKSIECK ET VALETTE "CODE DES COULEURS." SAME EXTRACT AS USED IN TABLE VI. FIGURES TAKEN FROM TABLE IN KLINCKSIECK & VALETTE'S "CODE"

Material	Code Readings		
	1	2	3
Solanum Lycopersicum	31	56	56
Rhus canadensis	66	66	66
Solanum Dulcamara	36	51	56
Arisaema triphyllum	51	61	56
Citrullus vulgaris	46	46	46
Solanum Pseudo-capsicum	126	131	126
Celastrus scandens (arils)	56	56	56
Celastrus scandens (carpels)	101	101	101
Evonymus americana	101	106	106
Lonicera sp.	91	96	96
Rosa rugosa	81	81	81
Evonymus europaea	136	131	136
Asparagus officinalis	111	111	111
Capsicum annuum	76	76	76
Lycium halimifolium	106	126	126
Cucumis Melo	91	096	91
Viburnum Opulus	96	0121	0121
Sorbus sitchensis	096	096	096

Table VIII is of little value without the Klincksieck and Valette 'Code des Couleurs.' The smaller numbers represent the true pinks and reds, the lycopersicin shades. Beginning with 76 the red-orange tints come in, which are characteristic of carotin and xanthophyll colors. Again, evidence is obtained from this table that the above-mentioned 6 fruits contain large proportions of lycopersicin. An interesting point is the reading 76 for pepper, which places it nearer the true lycopersicin color than any of the other fruits which are known definitely to contain only carotin and xanthophyll. There is an exception to this in the extract from the arils of *Celastrus* with a reading 56, which I cannot explain.

In table VII, the determinations made with the colorimetre show the relative order of intensity of the pigments contained in the various fruits. According to their strength they have been arranged in descending order in table VII, *Rhus* containing the most concentrated pigment, and *Sorbus* the most dilute.

Crystallization within the fruit tissues was attempted in order to gain more knowledge of the kinds of pigments found within certain fruits. Identification of pigments by means of crystals is not very satisfactory, because of differences of opinion among the various investigators as to the color, shape, and form in which these pigments crystallize. Xanthophyll often crystallizes in quadratic, often trapezoidal tables, frequently showing indentation, or it may crystallize in lance or wedge-shaped prisms. Sometimes the crystals are rhombic, almost hexahedral, with a color variation from greenish yellow to rose. They are not infrequently similar to carotin, but usually contain less color, and are often more pleochromatic.

Carotin crystals are rhomboidal in outline, tabloid or leaf-like in form, and the color is frequently orange to orange-red, but sometimes vermillion, with a steel-blue luster.

Lycopersicin yields brownish rose to carmine-red crystals, usually in the form of needles or minute elongated prisms, and often fissured at the ends. Sometimes crystal aggregates are formed, consisting of elongated prisms, at the center of which the color is a bluish red. Often lycopersicin appears in long, fine, hair crystals.

Certain microchemical tests have been definitely established for these 3 kinds of crystals. Those given here have been taken from Molisch ('13), Willstätter and Escher ('10), Wilstätter and Mieg ('07), Van Wisselingh ('15), and Palmer ('22). All 3 kinds of crystals turn blue when treated with H_2SO_4, HNO_3, or HCl. Lycopersicin crystals give more of an indigo-blue than carotin or xanthophyll. IKI usually turns carotinoid crystals green, although Van Wisselingh found an exception to this in some of the crystals of the *S. Dulcamara* pigment. All 3 give a blue color with chlorozinciodide solution, and the same reaction with bromine water. Xanthophyll crystals turn blue immediately, and are not dissolved by 65–75 per cent H_2SO_4, while carotin crystals turn blue only after the treatment with the acid has continued for some time, or when the acid is stronger. Lycopersicin is dissolved in concentrated H_2SO_4. Carotin and lycopersicin are insoluble in phenol-glycerine (3 parts by weight of phenol and 1 part by weight of glycerine), but xanthophyll is readily soluble.

Xanthophyll crystals are entirely insoluble in low boiling petroleum ether. The solubility in cold methyl alcohol is low, greater in ethyl alcohol, a little greater in CS_2, and still greater in ether. The solubility in acetone and chloroform is quite rapid. Carotin crystals are almost insoluble in cold ethyl alcohol and even less soluble in methyl alcohol, and they dissolve with difficulty in hot alcohols. Their solubility in low boiling petroleum ether is poor, but somewhat greater in the higher boiling ones. Acetone dissolves them with difficulty, even when hot. Benzole dissolves them more easily, chloroform and CS_2 with great ease. Lycopersicin crystals are less soluble than carotin in all the carotinoid solvents. Ethyl and especially methyl alcohol are exceptionally poor solvents. Petroleum ether of low boiling point dissolves only a small amount, while ether is a better solvent, but CS_2 is much better. In CS_2 a 2 per cent solution may be obtained. These crystals are insoluble in acetone and glacial acetic acid.

According to the methods described above attempts were made to crystallize the pigments in the tissue of each kind of fruit used in this section of the work. In some fruits no crystals could be obtained.

In tissue of Jerusalem cherry placed in alcoholic potash solution, long, bright red needle-like crystals and a few aggregates were observed after 16 days (pl. 18, fig. 2). Oftentimes small needle crystals were seen within the cells. No success was obtained with the Jerusalem cherry material placed in glycerine potash. In tissue of *Arisaema* there was no crystallization after 21 days in either alcoholic or glycerine potash. Tissue of *Celastrus* after 16 days showed rather short orange and yellow needle crystals (pl. 15, fig. 5). Tissue of *Sorbus* placed in alcoholic potash solution yielded many very fine pale yellow-orange crystals (pl. 15, fig. 7). Tissue of *Lonicera* under the same conditions showed orange to yellow crystals (pl. 15, fig. 1), but broader than those of *Sorbus*, and more like the xanthophyll crystals pictured by Willstätter and Stoll ('13).

The crystals formed in the pepper tissue were of special interest, due to the uncertainty of the presence of lycopersicin. Strips of tissue were placed in both potash solutions, but the best results were obtained after 16 days in the alcoholic potash. The crystals were of 2 types, the typical rhomboidal tabloid or leaf-like forms, which were orange to orange-red, with a metallic luster so characteristic of carotin, as described by Willstätter and Escher ('10); and rose-red rosette-like crystals, which is one of the forms ascribed to lycopersicin and one of the types found in tomato (pl. 15, fig. 6).

A great variety of crystals was produced in the *Asparagus* tissue by the glycerine-potash treatment after 10 days. Some indications of carotin, xanthophyll, and lycopersicin were obtained (pl. 15, fig. 11). In *Evonymus europaea* crystals formed more rapidly than in any other tissue, which is no doubt due to the fact that the pigment is already present in an oily substrate distributed through the entire cell. Van Wisselingh ('15) gives a very interesting explanation of how these crystals are formed by the application of the Molisch method. He says the pigments are dissolved within the plastids in an oily substance, then through treatment with alcoholic potash the plastid or stroma is destroyed and the pigment rounds up into colored globules; the KOH then saponifies the oily substance and the pigment is set free, crystallizing in the alcohol, in which carotinoids are not very soluble. Heat no doubt

increases the saponification process. When fruit tissues are placed in either of these KOH solutions the first change to be noted is the formation of colored globules. Since the plastids in *E. europaea*, at the point of highest coloration of the aril, are already broken down and the pigment is distributed in oily globules, the addition of the alkali effects at once the saponification of the lipoid material and the precipitation of the pigment in a crystalline form, thus accounting for the rapid crystallization in this tissue. The crystals appeared to originate from the colored globules mentioned (pl. 14, fig. 1, pl. 17, fig. 4). After 5 days in glycerine potash *Crataegus* showed very distinct carotin crystals of a deep vermilion red with the characteristic metallic luster (pl. 15, fig. 3). After 10 days in alcoholic potash there were formed in the fruit of *S. Dulcamara* beautiful carmine crystals of lycopersicin similar to those shown by Willstätter and Mieg ('07) (pl. 15, fig. 8, pl. 17, fig. 2). Good lycopersicin crystals were obtained in the tissue of *Rhus* after treatment of 30 days with alcoholic potash (pl. 15, fig. 4). With *Evonymus americana* an interval of only 7 days in the solution was required in order to produce many fine needle crystals, orange-yellow in color (pl. 17, fig. 3). In *Lycium* the crystals observed in the cells after a treatment of 7 days with alcoholic potash were rather broad needles (pl. 15, fig. 2).

Some of the tests described earlier were applied to certain of these crystals. *Lycium*, *Celastrus*, *Sorbus*, *Aglaonema*, *Asparagus*, and *Crataegus* all gave a green color with IKI solution. With the exception of *Sorbus*, crystals from the same fruits gave a blue reaction after a treatment of 1 hour with 85 per cent H_2SO_4. In *Sorbus* the crystals changed color almost immediately, thus indicating the presence of xanthophyll rather than lycopersicin or carotin. No results were obtained with bromine water nor acetic acid. Phenol-glycerine dissolved some of the crystals in the *Sorbus* tissue, but this reagent had no effect upon most of the crystals.

From the results of this work it is difficult to draw conclusions as to just which carotinoids are present in all of these fruits. All the workers on fruit pigments have agreed that lycopersicin is the principal carotinoid in tomato, watermelon, *Aglaonema*

(several species) and *Solanum Dulcamara;* while by some workers pepper and rose are added to this list. The results of this work confirm the presence of lycopersicin in tomato, watermelon, *Aglaonema Treubii* and *Solanum Dulcamara,* adding to the list *Arisaema triphyllum* and *Rhus canadensis.* The presence of lycopersicin crystals in pepper is certainly an evidence of the presence of the pigment, the failure to get good characteristic lycopersicin absorption bands no doubt being due to the small proportion of the pigment in pepper tissue. I have found no indication of lycopersicin in rose, although Lubimenko ('14) lists the rose among fruits that do contain it. In some fruits the spectroscopic work and the crystallization are rather contradictory. In Jerusalem cherry the long, pinkish red, needle crystals characteristic of lycopersicin were found, while in *Asparagus* tissue rosette crystals, also characteristic of lycopersicin, were observed. From the spectroscopic determinations there was no indication of lycopersicin in either of these tissues.

Some fruits were found to contain both anthocyanin and carotinoid pigments. These were *Crataegus, Rosa, Sorbus, Lonicera,* and *Viburnum.* In each fruit the anthocyanin pigments were located in the cells of the epicarp. As these pigments are soluble in the sap and not contained in plastids, these cells appeared as if filled with a colored liquid. The mesocarp tissue when examined under the microscope contained many plastids. All of these fruits when boiled in water lost their bright external colors, but the carotin-colored plastids remained without any change.

Since the anthocyanin pigments when in combination with carotinoids in the same fruit were always in superficial cells, the writer accepts the view that perhaps light is a strong factor in effecting this type of distribution. It would seem that light is more necessary for the production of anthocyanin than for carotinoid pigments. No satisfactory experimental work has been carried out during this investigation to substantiate this theory. Observational evidence is both for and against the idea. The most highly colored apples are raised in the regions of most intense light, also the brightest colors of most fruits such as peaches, plums, grapes, pears, strawberries, etc., are in the epicarp

or close to the surface of the fruit, where the light is greatest. Among roots we find certain ones that are highly colored with anthocyanin, such as red beets, radishes, and turnips; in other cases the pigments are carotinoids, as in carrot. According to Wheldale ('16) few definite experimental data have been accumulated and the evidence is very conflicting. Some flowers and fruits grown in the dark develop color while some do not. A general survey of the distribution of anthocyanin shows without a doubt that it occurs most often in the external tissue.

The beet root is a good illustration of the development of an anthocyanin pigment practically in darkness. A few other instances are recorded of pigment formation in the dark. Wiesner ('77) observed that potato sprouts, which formed in the light, showed little, if any, yellow pigment, while those formed in the dark developed from 30 to 150 per cent more pigment. Elfving ('82) and Immendorff ('89) found that carotinoids increased greatly in leaves under conditions which depressed chlorophyll formation, that is, low temperature and very diffuse light. Lubimenko ('14) maintained that light was not necessary for the formation of lycopin. Duggar ('13) proved that light is absolutely unnecessary for the normal pigmentation of red tomatoes.

Evonymus europaea offers an interesting condition, for here the arils colored with a carotinoid become bright red before the colorless carpels have burst open. The carpels are later colored by an anthocyanin. After the carpels break open they assume a rosy red color. An experiment was tried in which some green fruits of *Evonymus europaea* were placed in the dark and some in the light at the same temperature and at the same humidity. No satisfactory results were obtained as the small fruits dried up without any decided appearance of color in the carpels, under either set of conditions.

Gaultheria ovatifolia Gray, *Symphoricarpus orbiculatus* Moench, *Fragaria* sp. (cultivated), *Rubus spectabilis* Pursh, *Sambucus callicarpa* Greene, *Vaccinium parvifolium* Smith were found to contain only anthocyanin pigments.

Two tests were made to determine what kind of pigments are present in these fruits. (1) Each kind of fruit was boiled and in every case a colored liquid was obtained and a decrease or entire

loss of color in the fruit itself. (2) Air-dried and powdered material of each fruit was treated with CS_2 and allowed to stand for 2 weeks. No colored extract was ever obtained with any of the fruits tested.

CARROT PIGMENTS

Experiment 1.—To determine the state in which carotin is found naturally occurring in the carrot root, thin, free-hand sections of mature carrot containing an abundance of crystals were cut and placed in vials containing petroleum ether and absolute alcohol (10 cc. of petroleum ether and 1 cc. of absolute alcohol) maintained in darkness (pl. 15, figs. 9–10). Upon examination with the microscope after 48 hours no evidence of any crystals or granules could be found in the tissue, and no trace of color remained.

For the same purpose microtome sections, 10 μ in thickness, of mature carrot material, killed in chromo-acetic and imbedded in paraffin were stained in Delafield's haemotoxylin as described by Chamberlain ('15). Upon examination under the microscope no crystals or granules were observed in these slides.

Free-hand sections from the same region of the carrot were placed in iodine and 70 per cent alcohol and left in darkness for 24 hours. Upon examination with the microscope after this treatment no color bodies were observable in the tissue. From these experiments the conclusion was drawn that in the mature carrot root the pigment is in a crystalline or granular form free in the cytoplasm and not contained in any plastid or stroma, otherwise some evidence of a stroma would have appeared in the sections treated by the above 3 methods. The size, shape, and general form of most of these color bodies under the microscope indicated the presence of crystals rather than granules.

Experiment 2.—To study the question of where and how the carotinoids in carrot are formed, very young carrot roots were examined both by means of free-hand and paraffin sections. The microtome sections proved to be of little interest as the pigment itself was dissolved by all of the methods used. Free-hand sections were cut from very young carrots from 3 regions: (1) very close to the root tip, (2) half way between the root tip and the

top, and (3) at the top of the root just where the stem joins the root. Sections from these 3 regions were all tested for starch with IKI. Sections from region (1) gave no test for starch, from region (2) very good starch tests. An abundance of leucoplasts could be seen before the IKI was added, and in some sections a peculiar arrangement was noted between the carotin-colored bodies, which were sparingly present and the starch (pl. 16, fig. 9). There was a close connection between these starch grains and masses of pigment, as indicated by the blue coloration and the orange pigment. Leucoplasts containing starch inclusions were also observed (pl. 16, fig. 12). Sections cut from region (3) gave practically no starch test, but an abundance of chloroplasts were present and in addition many tiny carotin crystals in the fibro-vascular bundles. Upon the addition of H_2SO_4 these crystals gradually turned blue, and green with IKI, two decisive tests for carotin (pl. 16, fig. 11).

Experiment 3.—Mature carrots often show a green coloration near the top of the root. Sometimes this extends down into the center of the vascular cylinder. Tests were made for starch and sugar by means of IKI and Fehling's solution respectively. An abundance of starch was present but no sugar, and more starch was found to be present in the top region than in the bottom. Sections cut about .5 mm. in thickness from mature carrots and examined under the low power of the microscope showed very distinctly that the arrangement of the great masses of pigment followed the medullary rays (pl. 16, fig. 10).

Experiment 4.—To show the distribution relation between the chloroplasts and carotin crystals, a mature carrot was divided into 4 regions, each 1 cm. wide. From each region free-hand sections were cut, mounted in water, and examined under the microscope. Beginning at the top these regions were numbered 1, 2, 3, and 4. In each region sections were cut from the vascular cylinder and the cortex. In region (1) the sections from the cortex showed only chloroplasts, while those from the vascular cylinder showed chloroplasts and some carotin crystals. In region (2) the cortex sections showed only chloroplasts, while the vascular cylinder only greenish yellow and orange crystals. In region (3) the cortex sections contained both chloroplasts and carotin crystals, some

of the latter being of good size. In the vascular cylinder sections many carotin crystals and a few chloroplasts were observed. In region (4) the vascular cylinder sections contained many large carotin crystals, while the cortex contained many very large carotin crystals and also an abundance of smaller ones and granules but no chloroplasts. These crystals were of various shapes and forms with some variation as to color. They varied in size from 2.1 to 3 μ by 10.0 to 42.7 μ. Color variations were from pale orange to red (pl. 16, figs. 1–8).

Experiment 5.—This part of the work was undertaken to determine whether mitochondria are present or not in carrot. Both mature and various stages of young carrot roots were killed by Renand's IV B method, imbedded in paraffin, and stained in Haidenhain's iron-haematoxylin (Cowdry, '18). No definitely staining bodies resembling mitochondria could be found. Many chloroplasts stained well in the region where root and stem join (pl. 16, fig. 13).

From these experiments the following conclusions seem warranted: (1) The carrot carotin is typically in a crystalline or granular state, and not contained in a stroma. This is opposed to the conception of Schimper ('83) who considered the carrot carotin to be contained in chromoplasts. (2) Carotin is laid down as a storage product in the medullary rays. (3) The amount of carotin crystals increases from the top of the root towards the bottom, while the chloroplasts decrease. (4) No evidence could be found of mitochondria in young carrot roots.

Ripening Experiments

Experimental Data and Discussion

These experiments were undertaken in an attempt to determine the most favorable temperature for rapid fruit ripening, that is, to bring the fruit to the point of red ripeness. Very little work has been done on temperature relations. Duggar ('13) in some experiments with tomatoes showed that light was unnecessary for normal ripening, and that the most favorable temperature range was 18–25° C. In another experiment he found that red peppers ripen normally at higher temperatures.

Experiment 1.—Green fruits of *Lycium halimifolium* Mill. were placed under 4 different conditions, 9 fruits in each lot. These conditions were as follows: (1) wrapped in black paper in a drawer at 17–20° C.; (2) in front of west window at 18–22° C.; (3) in an incubator at 26–28° C.; (4) in an incubator at 32–35° C. The results are given in table IX.

From these data the rapid factor in ripening fruits is shown to be heat and not light, a temperature of from 26–28° C. being the most favorable. These results agree with those found by Duggar ('13), although he was working with pepper.

Colorimetric determinations were made of the red-ripe fruit from each lot. These fruits were dried in a gas-drying oven at 60° C., ground up in a mortar, and extracted with CS_2 (0.3 gm. of dried material in 4 cc. of CS_2 extracted for 4 days). The methods were the same as those already indicated.

TABLE IX

COLOR CHANGES IN GREEN LYCIUM FRUITS UNDER 4 DIFFERENT RIPENING CONDITIONS DURING A PERIOD OF 5 DAYS. NOTES ON 9 FRUITS IN EACH LOT.

Days	Lot 1 17–20° C. Wrapped in black paper put in desk	Lot 2 18–22° C. On table in front of west window	Lot 3 26–28° C. In electric oven	Lot 4 32–35° C. In electric oven
1	5 green 2 yellow 1 almost orange 1 orange	6 green 2 yellowish green 1 orange	1 green 1 yellow 2 nearly orange 5 orange	3 green 3 yellow 3 orange
2	1 green 3 yellow 2 orange 3 red-ripe	2 green 4 yellow 3 orange	1 green 1 yellow 1 orange 6 red-ripe	1 green 2 yellow 2 orange 4 red-ripe
3	1 green 3 yellow 2 orange 3 red-ripe	1 green 1 pale yellow 4 yellow 2 orange 1 red-ripe	1 green 1 orange 7 red-ripe	1 green 2 yellow 6 red-ripe
4	1 greenish yellow 3 yellow 1 orange 4 red-ripe	4 yellow 4 orange 1 red-ripe	1 yellow 1 orange 7 red-ripe	2 green 1 yellow 6 red-ripe
5	1 greenish yellow 3 yellow 1 orange 4 red-ripe	4 yellow 3 orange 2 red-ripe	1 yellow 1 orange 7 red-ripe	2 green 1 yellow 6 red-ripe

TABLE X

COLOR CHANGES IN GREEN PEPPERS UNDER 5 DIFFERENT RIPENING CONDITIONS DURING A PERIOD OF 42 DAYS, 20 FRUITS IN EACH LOT*

	Lot 1 26–28° C. Wrapped	Lot 2 22–24° C. Unwrapped	Lot 3 20–22° C. Unwrapped	Lot 4 16–18° C. Unwrapped	Lot 5 14–16° C. Wrapped
6 days	2 red-ripe 8 almost red-ripe 7 red-orange 3 green, few red-orange spots	4 one-half orange to red-spotted 10 orange to red-spotted 6 green	3 red-ripe 5 almost red-ripe 5 two-thirds red-ripe 2 green, red spots 5 green	1 red-ripe 3 almost red-ripe 1 one-half orange-red 11 one-fourth orange-red 1 three-fourths green 3 green	4 half orange to red-spotted 10 orange to red-spotted 6 green
12 days	4 almost red-ripe 11 three-fourths red-ripe 2 few orange-red spots 1 spoiled	5 red-ripe 3 red-ripe beginning to spoil 9 almost red-ripe 2 three-fourths red-ripe 1 one-fourth red-ripe	4 red-ripe 5 almost red-ripe 1 three-fourths red-ripe 5 one-fourth red-ripe 1 few yellow spots 1 green	4 red-ripe 11 three-fourths red-ripe 1 yellow 1 green-yellow striped 2 green	2 almost red-ripe 9 three-fourths red-ripe 5 green, orange-red spots 3 green 1 spoiling
18 days	2 red-ripe 4 almost red-ripe 9 three-fourths red-ripe 2 few orange-red spots	3 red-ripe 6 almost red-ripe 1 three-fourths red-ripe 5 spoiled	1 red-ripe 3 almost red-ripe 5 three-fourths red-ripe 4 spoiled	2 red-ripe 7 almost red-ripe 3 three-fourths red-ripe 3 spoiling	4 almost red-ripe 8 three-fourths red-ripe 2 one-third red-ripe 3 few red spots 1 yellow 2 spoiled
24 days	4 red-ripe 9 almost red-ripe 2 three-fourths red-ripe	5 red-ripe 2 almost red-ripe	6 almost red-ripe 2 three-fourths red-ripe	8 almost red-ripe 2 three-fourths red-ripe 3 spoiled	8 almost red-ripe 3 three-fourths red-ripe 3 one-half red-ripe 1 yellow 3 spoiling
30 days	2 red-ripe 8 almost red-ripe 1 three-fourths red-ripe	1 red-ripe 1 almost red-ripe	3 red-ripe 3 almost red-ripe 2 three-fourths red-ripe	2 red-ripe 6 almost red-ripe 2 three-fourths red-ripe	4 red-ripe 4 almost red-ripe 3 three-fourths red-ripe 1 yellow 6 spoiled

* As soon as a pepper became red-ripe or spoiled a record was no longer kept of it.

TABLE X (Cont.)

	Lot 1 26–28° C. Wrapped	Lot 2 22–24° C. Unwrapped	Lot 3 20–22° C. Unwrapped	Lot 4 16–18° C. Unwrapped	Lot 5 14–16° C. Wrapped
36 days	7 red-ripe 2 almost red-ripe	1 red-ripe	3 almost red-ripe 2 three-fourths red-ripe	3 red-ripe 4 almost red-ripe 1 three-fourths red-ripe	4 almost red-ripe 3 three-fourths red-ripe 1 yellow
42 days	2 red-ripe		3 red-ripe 2 almost red-ripe	4 almost red-ripe 1 three-fourths red-ripe	5 almost red-ripe 2 three-fourths red-ripe 1 yellow

The results showed that fruits ripened under the condition of lot 3 give a more deeply colored extract than any of the others. From this it was concluded that 26–28° C. is the most favorable temperature for ripening fruits of *Lycium*. The temperature range doesn't agree with that of Duggar ('13) for tomato, but the presence of a large percentage of lycopersicin in tomato and none in *Lycium* may account for this difference.

Experiment 2.—Bell peppers of uniform size and greenness, 20 in each lot, were placed under 5 different conditions as to temperature and light. Lot 1 was placed in an electric incubator (26–28° C.), each fruit being first wrapped in brown paper to prevent too much drying out; lot 2 on a table in front of a south window (22–24° C.) unwrapped; lot 3 on the floor (20–22° C.) unwrapped; lot 4 on the floor (16–18° C.) unwrapped; lot 5 on the floor (14–16° C.) wrapped. Detailed color notes taken at 6-day intervals are given in table x. From these results 22–24° C. is the best temperature for ripening peppers, the higher temperatures being more favorable, however, than the lower. Light is not a controlling factor.

Spectroscopic determinations were made on samples from peppers ripened under conditions of experiment 2. The methods followed in this work have been previously described. The pulp of the red-ripe fruits from each lot, after removal of the seeds, was placed in the dehydrater until perfectly dry, then ground as fine as possible with a coffee mill. Weighed amounts of this material were then extracted with relatively equal portions of CS_2 for 2 days. The extract in all cases was too dense to show absorption

bands. The whole spectrum was blotted out from 652.2 μμ over to 412.5 μμ, so that dilutions had to be made before the absorption bands could be observed. The amount of dilution varied with the different lots of peppers. The absorption bands in all lots lay approximately in the region of the carotin and xanthophyll spectrum. These spectroscopic analyses are given in table XI. The most concentrated extract was believed to indicate the best ripening condition.

TABLE XI

SPECTROSCOPIC ANALYSES OF PEPPER EXTRACTS FROM EXPERIMENT 2. ALL MEASUREMENTS ARE IN μμ.

Lot	1	2	3	4	5
Dilutions	2 cc. extract 10 cc. CS_2	2 cc. extract 11 cc. CS_2	2 cc. extract 8 cc. CS_2	2 cc. extract 5 cc. CS_2	2 cc. extract 3 cc. CS_2
Absorption bands	(1) 522.5–505.0 (2) 494.2–483.0	(1) 523.5–507.3 (2) 497.5–482.0	(1) 521.5–506.5 (2) 496.5–482.0	(1) 523.2–507.5 (2) 497.5–483.0	(1) 522.6–507.5 (2) 497.5–483.0

The same conclusion was drawn from these results as from experiment 2. Lot 2, with a temperature range of 22–24° C., offered the most favorable temperature for fast ripening. Lot 1 ripened at 26–28° C., the next most favorable. Lot 5, ripened at 14–16° C., gave a very weak extract needing only about one-fourth the dilution of lot 2.

Experiment 3.—These extracts from experiment 2 were further tested by comparison with Klincksieck et Valette's 'Code des Couleurs.' The method followed was to dip 3 equal-sized pieces of filter-paper in each of the 5 extracts obtained from experiment 2. These were allowed to dry for 5 seconds and then a comparison made with the charts. Three readings were made for each extract. In most cases these triplicate readings were exactly the same. They are recorded in table XII.

TABLE XII

PEPPER EXTRACT FROM FRUITS RIPENED UNDER CONDITIONS OF EXPERIMENT 2. COLOR FIGURES TAKEN FROM KLINCKSIECK & VALETTE'S "CODE"

Lot	1 26–28° C.	2 22–24° C.	3 20–22° C.	4 16–18° C.	5 14–16° C.
Readings					
1	61	66	66	71	71
2	61	66	66	71	71
3	61	61	66	71	71

Since the smaller the figures the deeper the color this experiment is further proof that higher temperatures (22–24° C.) are necessary for deep pigmentation of fruits during the ripening process.

Experiment 4.—Color changes on the individual pepper were observed with green fruits placed in the dark at a temperature of 22–25° C. The first changes were a few yellow spots, then darkening of these spots to an orange, then to an orange-red. Finally this orange-red color spread gradually over the entire fruit until red-ripeness was reached. These same color changes were observed in 12 individual fruits.

Summary

1. During the ripening process of fruits a change occurred in the general shape of the plastids from oval or subglobose to a very much elongated spindle form. In some of the red-ripe fruits the pigment was crystalline in form.

2. In most of the ripe fruits studied the pigment was found to occur in a stroma or in a definite body. This pigment was in an amorphous state contained in a lipoid substratum.

3. Lycopersicin was present in a crystalline form in the red-ripe fruits of *Solanum Lycopersicum*, *Cucumis Citrullus*, and *Aglaonema Treubii*.

4. A Duboscq micro-colorimeter was used with good results as a means of determining relative amounts of pigments in tomatoes. Of the 4 varieties studied, Ponderosa, Excelsior, Dwarf Champion, and Globe, Excelsior was found by this method to contain the most pigment.

5. Dried tomato pulp kept in the dark for a period of 5 months lost very little color. This was proved by comparisons of the CS_2 extract of the dried material, at certain intervals, with a methyl red solution.

6. A characteristic lycopersicin spectrum was obtained with a CS_2 extract of the pigments in the ripe fruits of the following: *Aglaonema Treubii*, *Solanum Lycopersicum*, *Rhus canadensis*, *Solanum Dulcamara*, *Arisaema triphyllum*, and *Citrullus vulgaris*.

7. A characteristic carotin spectrum was obtained with a CS_2 extract of the following fruits: *Celastrus scandens*, *Solanum Pseudo-capsicum*, *Evonymus americana*, *Lonicera* sp. *Rosa rugosa*,

Evonymus europaea, Asparagus officinalis, Capsicum annuum, Lycium halimifolium, Cucumis Melo, Viburnum Opulus, and *Sorbus sitchensis.*

8. Only anthocyanin pigments were found in the fruits of *Gaultheria ovatifolia, Symphoricarpus orbiculatus, Fragaria* sp., *Rubus spectabilis, Sambucus callicarpa,* and *Vaccinium parvifolium.* Both anthocyanin and carotinoid pigments were found in the fruits of *Crataegus phaenopyrum, Rosa rugosa, Sorbus sitchensis, Lonicera* sp., and *Viburnum Opulus.*

9. The carrot carotin occurred in both crystalline and granular forms, but free in the cytoplasm. It appeared to be laid down as a storage product in the medullary rays.

10. The optimum temperature for the rapid ripening of *Lycium halimifolium* fruits was 26–28° C. The optimum temperature for the rapid ripening of pepper fruits was 22–24° C.

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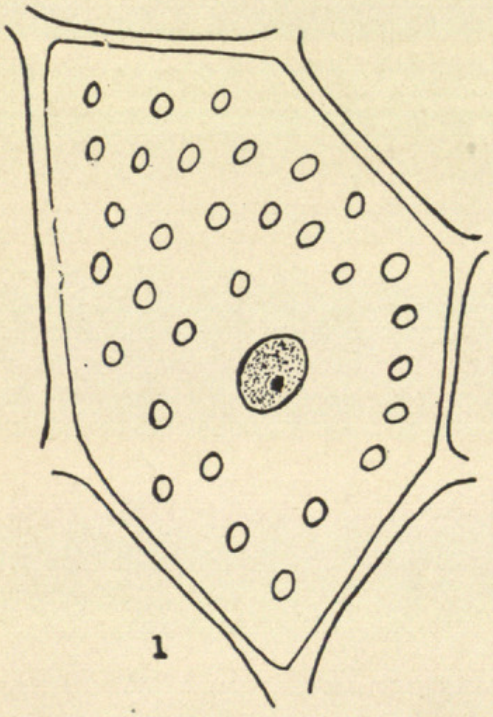
Explanation of Plate

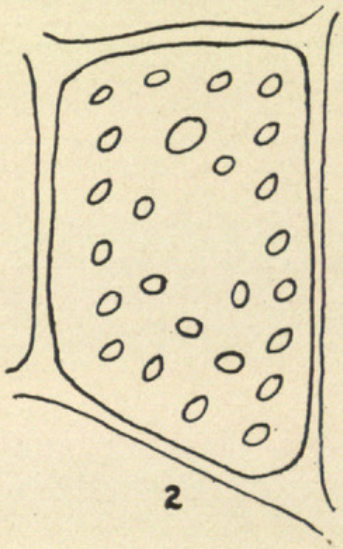
PLATE 10

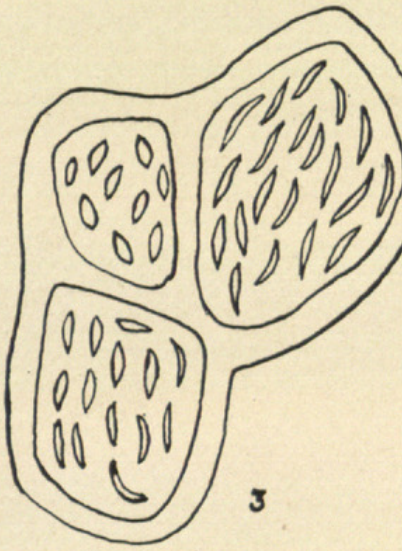
(Camera-lucida drawings, × 880)

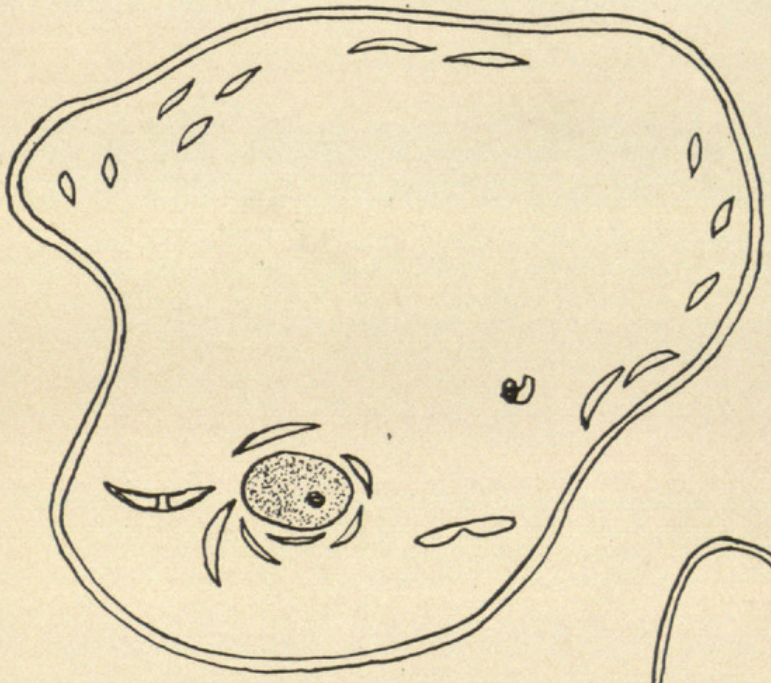
Figs. 1–5. *Capsicum annuum:* figs. 1–3, successive changes in plastid shapes during ripening; fig. 1, from green pulp; fig. 2, from yellow-orange pulp; fig. 3, from epicarp of ripe fruit; fig. 4, from microtome sections of ripe pepper stained with iron-haematoxylin; fig. 5, from free-hand sections, stained in acid fuchsin.

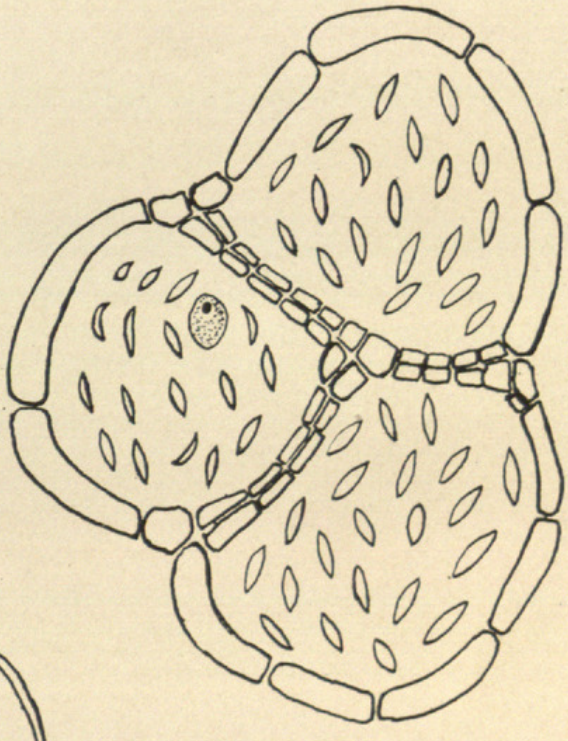
Figs. 6–8. *Rosa rugosa.* Successive changes in plastid shapes during ripening: fig. 6, from green pulp; fig. 7, from yellow-orange pulp; fig. 8, from ripe pulp.

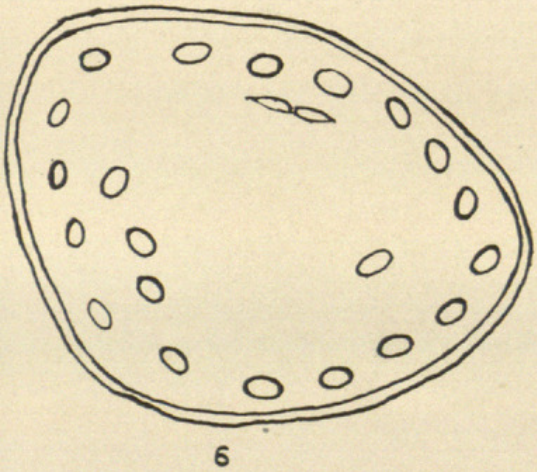












HOWARD—CAROTINOIDS IN FRUITS

Explanation of Plate

PLATE 11

(Camera-lucida drawings, × 880)

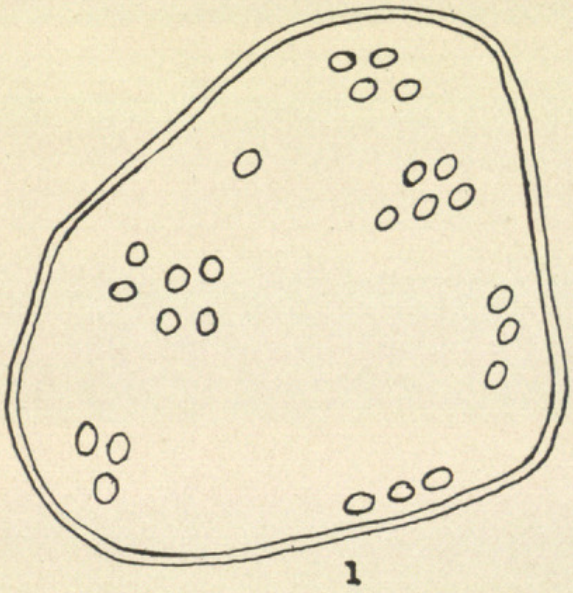
Figs. 1–3. *Solanum Lycopersicum:* figs. 1 and 2, successive changes in plastid shapes during ripening; fig. 1, from green pulp; fig. 2, from yellow-orange pulp; fig. 3, from ripe pulp, showing lycopersicin crystals.

Figs. 4–5. *Aglaonema Treubii:* fig. 4, from green pulp, unstained free-hand sections, mounted in water; fig. 5, ripe juice showing natural lycopersicin crystals.

Figs. 6 and 7. *Solanum Dulcamara.* Unstained, free-hand sections, mounted in water: fig. 6, from yellow-orange pulp; fig. 7, from epicarp of ripe fruit.

Fig. 8. *Dracaena Godseffiana.* Unstained, free-hand sections, mounted in water, from ripe fruit.

Fig. 9. *Evonymus americana.* From unstained smears of pulp of ripe fruit.



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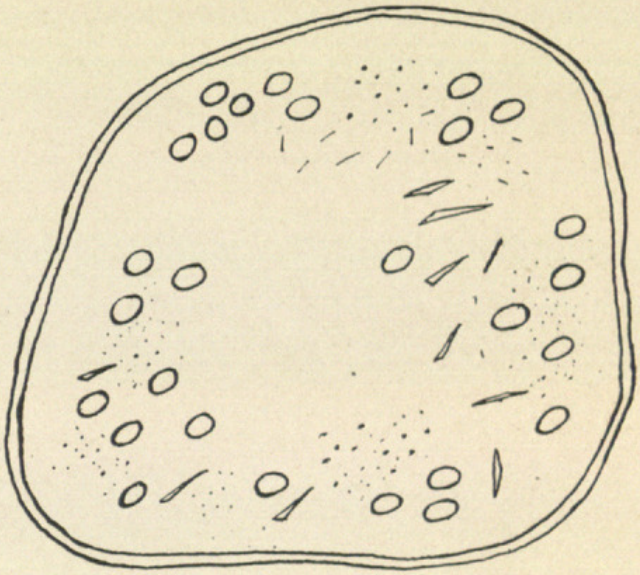

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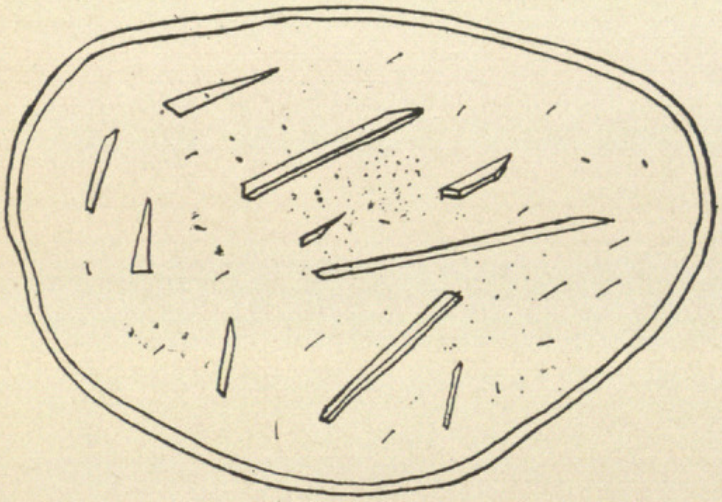

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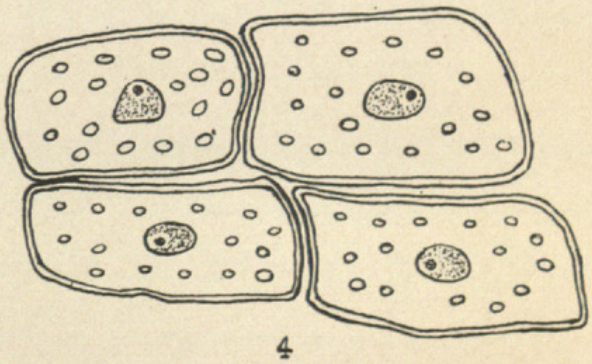

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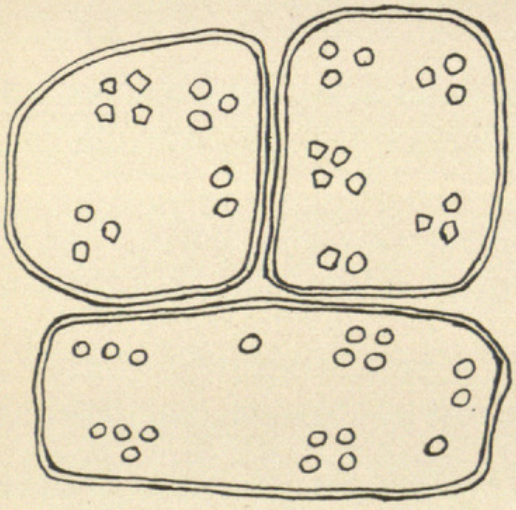

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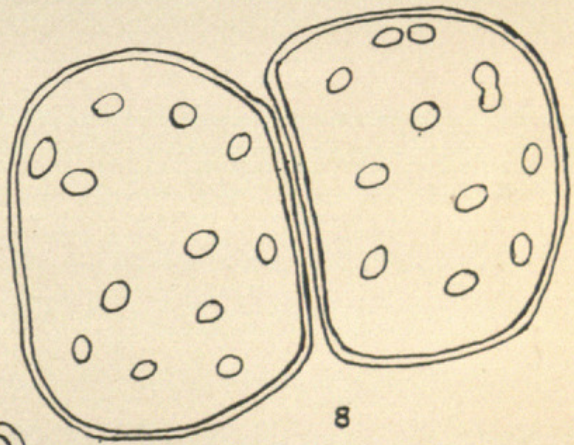

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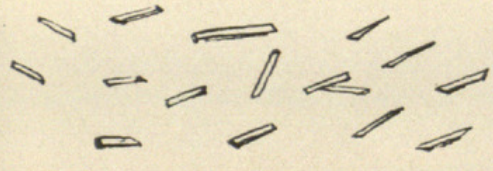

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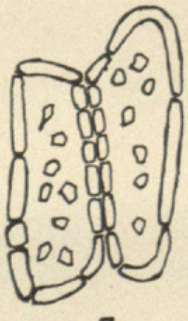

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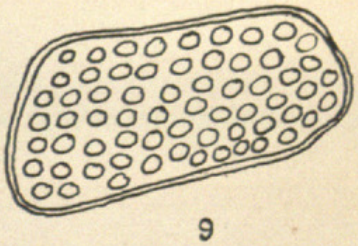

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HOWARD—CAROTINOIDS IN FRUITS

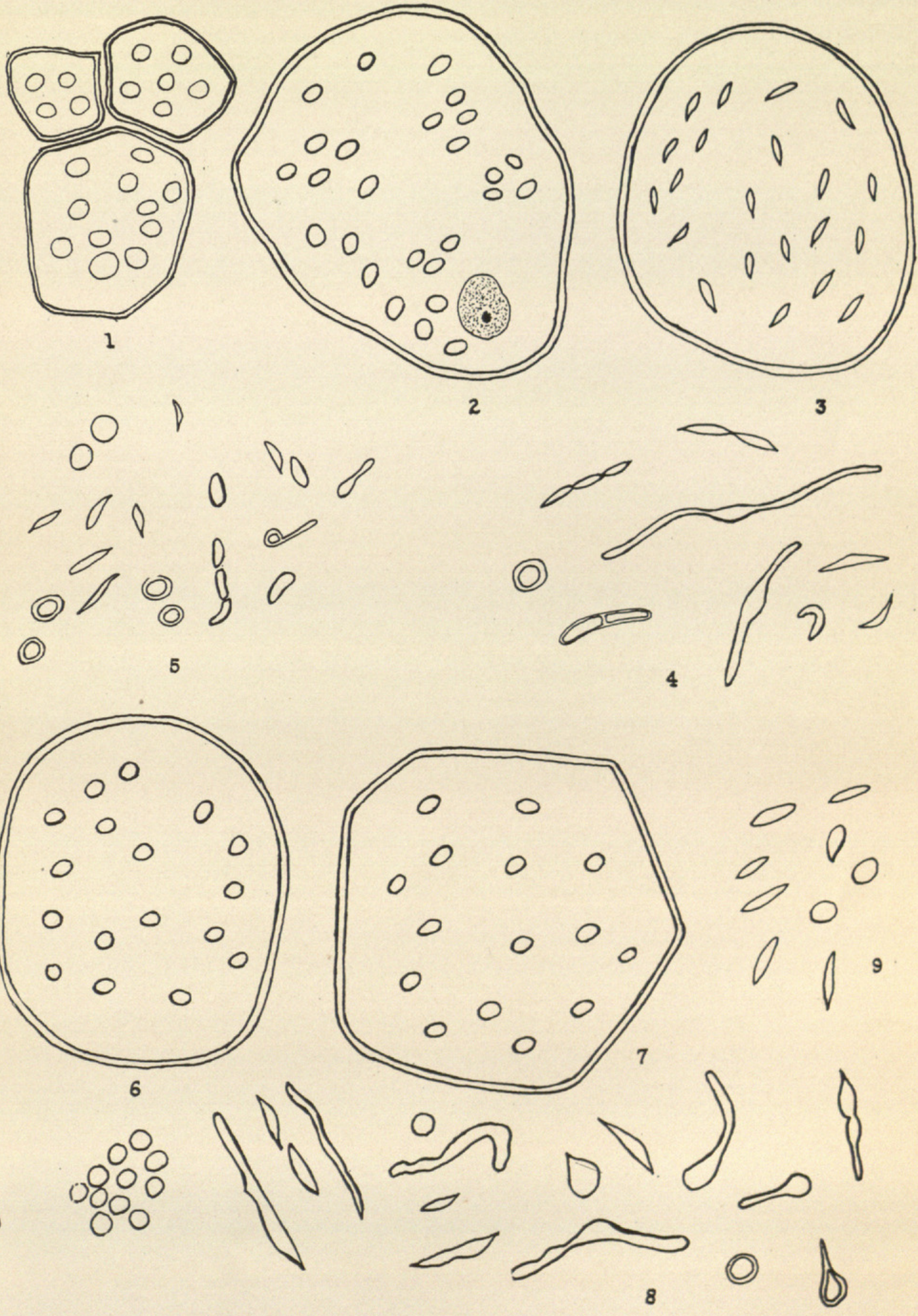
Explanation of Plate

PLATE 12

(Camera-lucida drawings, × 880)

Figs. 1–5. *Lycium halimifolium:* figs. 1–3, successive changes in plastid shapes during ripening; fig. 1, from green pulp; fig. 2, from yellow-orange pulp; fig. 3, from red-ripe pulp; fig. 4, unstained chromoplasts from ripe juice; fig. 5, chromoplasts stained with acid fuchsin from ripe juice.

Figs. 6–9. *Asparagus officinalis:* figs. 6–8, successive changes in plastid shapes during ripening; fig. 6, from green pulp; fig. 7, from yellow-orange pulp; fig. 8, chromoplasts stained with acid fuchsin from ripe juice; fig. 9, from ripe juice.



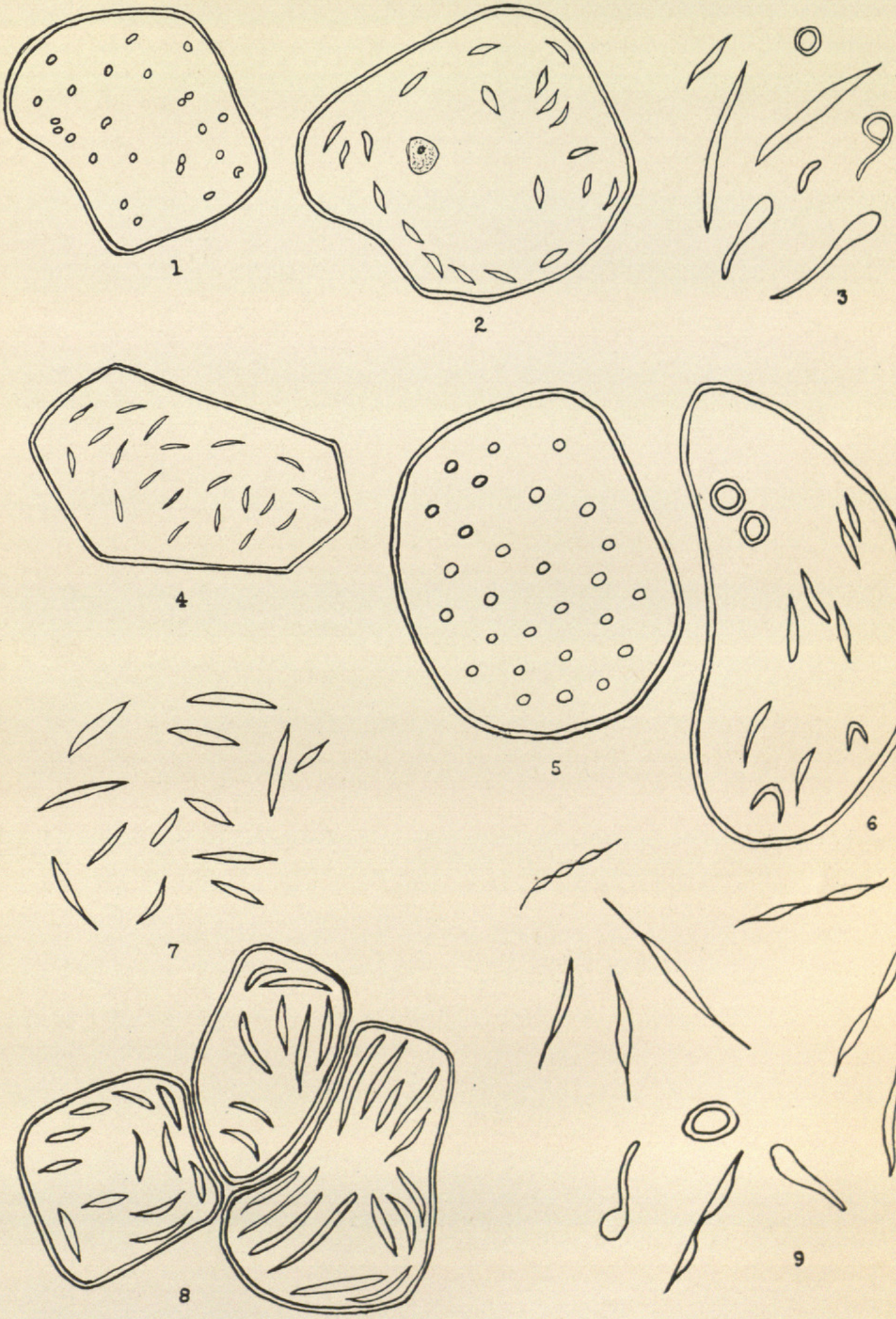
HOWARD—CAROTINOIDS IN FRUITS

Explanation of Plate

PLATE 13

(Camera-lucida drawings, × 880)

Figs. 1 and 2. *Sorbus sitchensis.* From green and ripe pulp respectively.
Figs. 3 and 4. *Arisaema triphyllum.* From ripe juice and pulp respectively.
Fig. 5. *Viburnum Opulus.* From ripe pulp.
Fig. 6. *Crataegus phaenopyrum.* From ripe pulp.
Figs. 7 and 8. *Celastrus scandens:* fig. 7, from ripe juice stained with acid fuchsin; fig. 8, from ripe pulp.
Fig. 9. *Solanum Pseudo-capsicum.* From juice of ripe fruit.



HOWARD—CAROTINOIDS IN FRUITS

Explanation of Plate

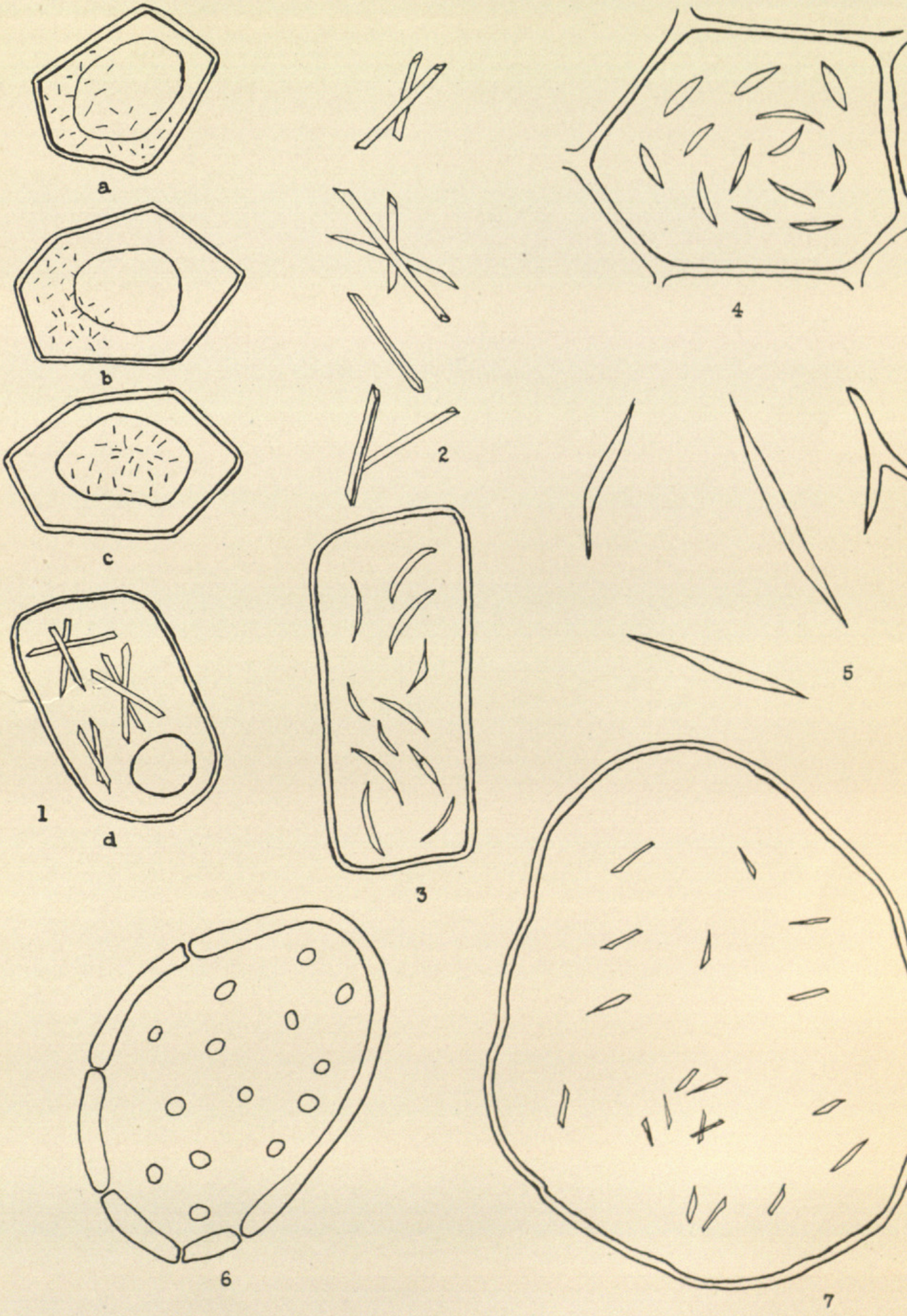
PLATE 14

(Camera-lucida drawings, × 880)

Fig. 1–3. *Evonymus europaea:* fig. 1, a, b, c, and d, successive changes in the formation of crystals by the addition of alcoholic potash in ripe tissue; fig. 2, from ripe fruit showing chromoplasts; fig. 3, crystals obtained by the addition of alcoholic potash.

Figs. 4 and 5. *Lonicera* sp. From pulp and juice respectively of ripe fruit.

Figs. 6 and 7. *Citrullus vulgaris:* fig. 6, from ripe pulp showing lycopersicin crystals; fig. 7, from green portion of pulp, showing chloroplasts.



HOWARD—CAROTINOIDS IN FRUITS

Explanation of Plate

PLATE 15

(Camera-lucida drawings, × 880)

Fig. 1. *Lonicera* sp. Yellow crystals obtained by the addition of alcoholic potash.

Fig. 2. *Lycium halimifolium.* Orange crystals obtained by the addition of glycerine potash.

Fig. 3. *Crataegus phaenopyrum.* Deep red crystals obtained by the addition of glycerine potash.

Fig. 4. *Rhus canadensis.* Carmine-red crystals obtained by the addition of alcoholic potash.

Fig. 5. *Celastrus scandens.* Orange and yellow crystals obtained by the addition of alcoholic potash.

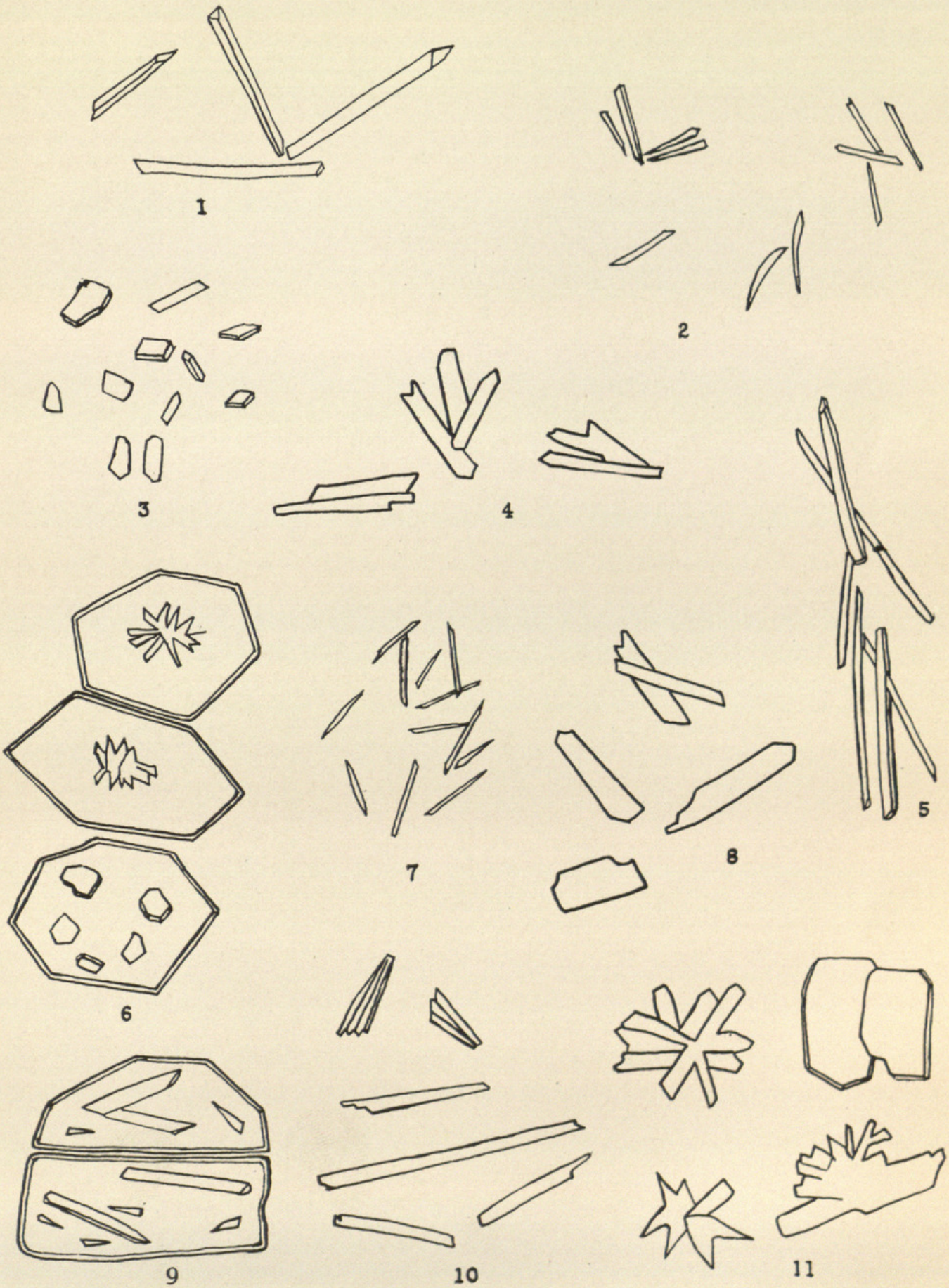
Fig. 6. *Capsicum annuum:* (a) carmine-red crystals obtained by the addition of alcoholic potash; (b) orange crystals obtained by the addition of alcoholic potash.

Fig. 7. *Sorbus sitchensis.* Pale orange crystals obtained by the addition of alcoholic potash.

Fig. 8. *Solanum Dulcamara.* Carmine-red crystals obtained by the addition of alcoholic potash..

Figs. 9 and 10. *Daucus Carota.* Natural carotin crystals.

Fig. 11. *Asparagus officinalis.* Red-orange crystals obtained by the addition of alcoholic potash.



HOWARD—CAROTINOIDS IN FRUITS

Explanation of Plate

PLATE 16

(No camera-lucida drawings)

Daucus Carota

Figs. 1–8. Free-hand sections, unstained, mounted in water from mature root: figs. 1 and 2, from cortex and vascular cylinder respectively, near top of carrot; figs. 3 and 4, from cortex and vascular cylinder respectively, from region just below preceding figures; figs. 5 and 6, from cortex and vascular cylinder respectively, from region just below preceding figures; figs. 8 and 7, from cortex and vascular cylinder respectively, from region nearest tip of root.

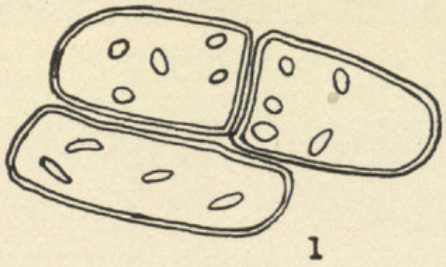
Fig. 9. From young roots, irregularly shaped orange bodies with starch grain inclusions, treated with IKI.

Fig. 10. Cross-section .5 mm. thick, under low power, showing distribution of carotin crystals.

Fig. 11. Cross-section of fibro-vascular bundle in young root, showing plastids and carotin crystals.

Fig. 12. From young root, showing leucoplasts and starch inclusions.

Fig. 13. From young root, showing chloroplasts stained with iron-haematoxylin.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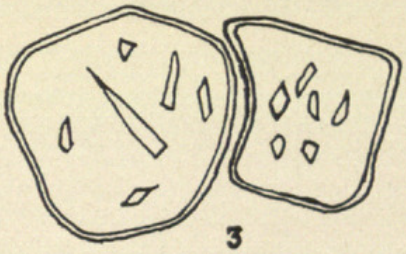
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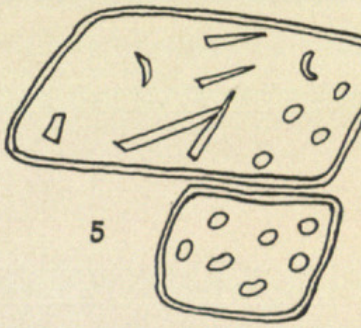
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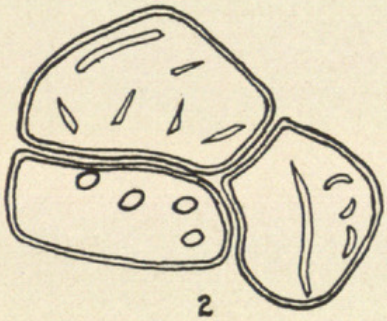
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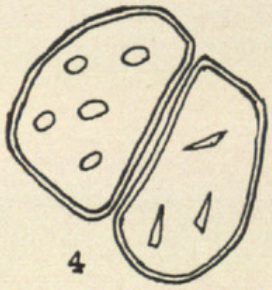
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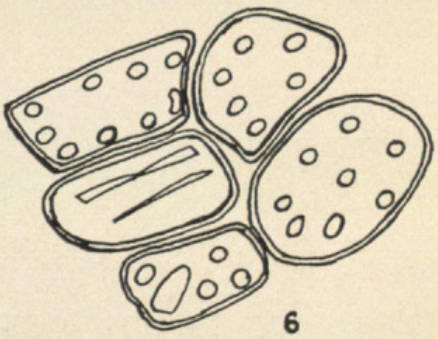
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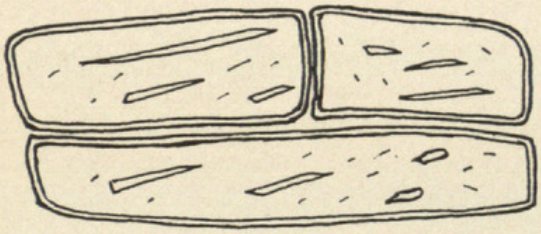
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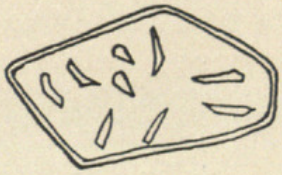
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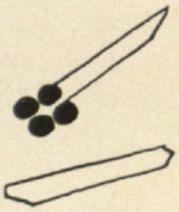
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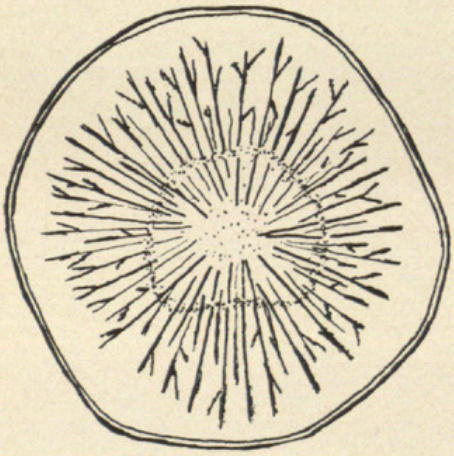
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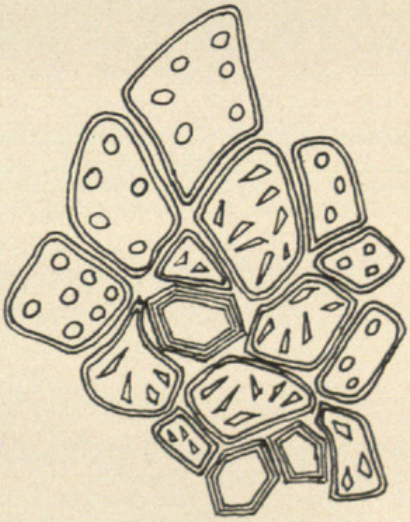
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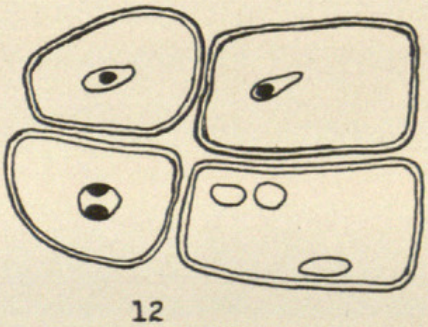
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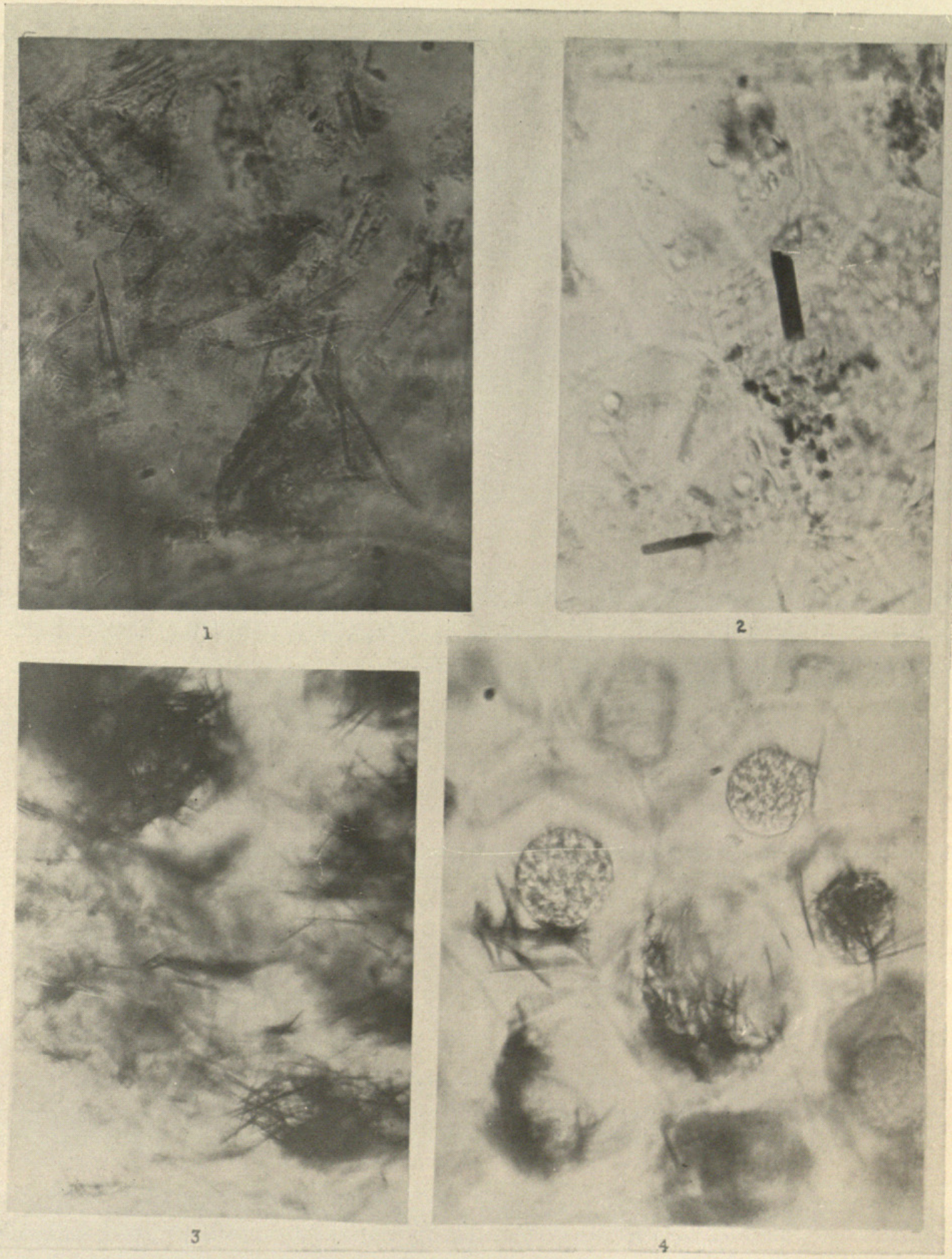
HOWARD—CAROTINOIDS IN FRUITS

Explanation of Plate

PLATE 17

(All photomicrographs, × 800)

Fig. 1. *Solanum Lycopersicum.* Natural lycopersicin crystals in ripe fruit.
Fig. 2. *Solanum Dulcamara.* Lycopersicin crystals obtained by the addition of alcoholic potash.
Fig. 3. *Evonymus americana.* Crystals obtained by the addition of alcoholic potash.
Fig. 4. *Evonymus europaea.* Crystals obtained by the addition of alcoholic potash.



HOWARD—CAROTINOIDS IN FRUITS