The Distinction between Mitochondria and Plastids in Living Epidermal Cells
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THE DISTINCTION BETWEEN MITOCHONDRIA AND PLASTIDS IN LIVING EPIDERMAL CELLS [1]

Helen Sorokin

In the living cells of the epidermis of the scale leaves of *Allium Cepa* it has been possible to differentiate mitochondria from morphologically similar forms of plastids by means of the Janus green B reaction (Sorokin, 1938). An extension of this study of mitochondria and plastids is warranted because of the theoretical importance of this problem, and because of skepticism as to the identity of the small bodies which do not stain with Janus green B with plastids (Anderson, 1939), as well as inability on the part of investigators to obtain the Janus green B reaction for mitochondria (Zirkle, 1927; Newcomer, 1940), which requires for its success a combination of very precise conditions. Present investigation substantiates the earlier conclusions that the mitochondria of the living cells react positively toward Hoechst's Janus green B and that the plastids always react negatively. Furthermore, it considers the size, morphology, and distribution in the cells both of mitochondria and of plastids, and, in addition, presents evidence that the small bodies, which are morphologically similar to mitochondria, and which do not color with Janus green B, represent small forms of plastids.

Materials and methods.—Three main requirements were considered in the selection of material: (1) it should yield preparations thin enough for microscopical examinations; (2) cells should survive several hours in the solution of the dye and should not show signs of autolysis during this time; (3) and a rapid penetration of the dye into the cells should be possible. The epidermis from various organs of many commercially grown flowers and market vegetables answers the above requirements. More than fifty representatives of such plants were subjected to investigation, with very consistent results. Space permits a presentation of only a part of the material.

The extreme complexity of the technique of handling living material, in spite of its apparent simplicity, has been fully stressed by Bailey (1930). Consistent results in staining mitochondria were obtained only when the following conditions were observed: (1) Hoechst's Janus green B in a concentration of 1:100,000 was used. (2) As the liquid medium tap water from Winchester, Mass. (pH at 6°C.–7.8), was employed. (3) The isotonic sucrose solution was freshly prepared before each experiment. (4) To permit the easy access of oxygen, the pieces of epidermis were floated with the cuticular surface uppermost upon the liquid medium containing the dye. When these conditions were not fulfilled, negative or erratic staining of mitochondria with Hoechst's Janus green B resulted (Sorokin, 1938). Epidermal tissue was cut with a razor blade into small (2–3 mm.) squares, which were lifted by forceps and floated upon 50 cc. of an 8–10 per cent sucrose solution, to which one drop of a 1 per cent aqueous solution of Janus green B was added. The time necessary for the appearance of the reaction varied in different materials. Comparable experiments were carried on during the fall and winter months of four consecutive years. Only living cells which exhibited normal cyclosis were examined. The appearance of Brownian movement in the cells showing cyclosis was considered as a sign of injury. All examinations were made with Zeiss apochro-

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matic 2 mm., N. A. 1.40 oil immersion objective (×90) and a 15× compensating ocular.

OBSERVATIONS.—*Tulipa Gesneriana L. var. Darwiniana Bailey.*—Because the ornamented cutinized cell wall prevents good visibility, this material had to be mounted on the slide with the cutin side downward. The epidermis from the inner surface of the perianth leaves of lavender, pink, and orchid-colored varieties consists of a few cells with colorless vacuoles, which do not survive in "explants," and of a larger proportion of cells with the vacuoles colored with anthocyanin. The latter survive very well in the dye solution, and frequently do not show any autolysis after twenty-six hours. In cells with gray or light-blue cell sap the mitochondria begin to color after thirty to sixty minutes. In those with pink and rose-colored sap the reaction appears somewhat later. After two hours the reaction is clear in all living cells, and the color of the mitochondria is an intense opaque blue. When the cells containing colored mitochondria are examined under a high-power oil immersion lens, the color disappears after varying intervals of time from all of the mitochondria. The actual process of decolorization proceeds very rapidly in this material, and the reduction of diethylsafraninazodimethylaniline chloride to its leucobase (Cowdry, 1916) can only occasionally be seen in the intermediary stage of the diethylsafranin reaction. When the cells containing decolorized mitochondria are floated upon an isotonic sucrose solution without the dye, oxidation of the leucobase takes place and the mitochondria become colored after one hour. The second decolorization usually proceeds more rapidly. This procedure may be repeated several times, until the cells become injured by mechanical manipulations. Similar experiments are well known for mitochondria of the animal cell (Cowdry, 1924) and serve as one of the indicators of the specificity of the Janus green B reaction.

The mitochondria of tulip are spherical or nearly spherical in shape, and range from 0.4 μ to 1 μ in diameter (fig. 1–5, m). Diplosomes and figures of division are frequently observed, but rod-shaped forms never occur in the epidermal cells (see also Guilliermond, *et al.*, 1933). Janus green B has no appreciable effect upon the motion of mitochondria, which is not confined to the cytoplasmic streaming but often assumes entirely different directions. Certain forms may move faster than the others, and a number of mitochondria may remain stationary.

The extremely polymorphic plastids of tulip are either colorless or, as in the yellow varieties and in the yellow spots of the petal of other varieties, may acquire xanthophyll. Both the colorless and xanthophyll-colored plastids exhibit amoeboid movement, form protrusions, and occasionally fragment small portions (see also Weiss, 1885; Guilliermond, 1919). The larger plastids usually taper toward their extremities and variations in girth are conspicuous. Transitional forms show gradation in size and pliability between the large plastids and the very small forms of less than one micron in size. None of the plastids (fig. 1–5, cp) stain vitally with Janus green B. After a prolonged staining the oil globules (fig. 2, o) and the oleoplasts acquire a light-blue refractive color which does not disappear after the decolorization of the mitochondria.

The distribution of the cytoplasmic components in the cell is illustrated in a lavender variety (William Copland) (fig. 1–4). Mitochondria stained with Janus green B, as well as colorless plastids, are particularly abundant in the layer of cytoplasm adjacent to the innermost cell wall (fig. 1). The oil globules are located predominantly in a slightly deeper layer of the cytoplasm which moves more rapidly. This is better illustrated at the level of the central part of the nucleus, where the layer nearest the nucleus (fig. 2, arrows) contains numerous oil globules and only a few mitochondria, whereas the peripheral layer contains mitochondria and plastids. At a still deeper focus (fig. 3) thick cytoplasmic strands, containing a large number of oil globules and a smaller number of mitochondria and plastids, can be observed to transverse the large vacuole. Finally, the layer of the cytoplasm which adjoins the cutinized cell wall (fig. 4) contains very few mitochondria, plastids, and oil globules. In other varieties of tulip the number of mitochondria in this layer was still smaller and that of plastids considerably larger. Guilliermond (1919) has also observed a similar distribution of mitochondria in tulip and has expressed the opinion that light is an important factor affecting the distribution.

A definite proof that the small bodies which do not color with Janus green B represent minute forms of plastids is obtained from the study of a white variety of tulip (Kansas). This variety is characterized by the presence of a large deep-yellow spot at the inner surface of the perianth leaves, which gradually becomes fainter and merges with the white portion of the petal. The epidermal cells of the white portion of the petal contain a large number of colorless plastids, whereas in those from the base of the perianth all the plastids are heavily impregnated with pigment. In figure 5 is represented a portion of tissue in which the cells containing colorless plastids alternate with those containing impregnated forms. Material was stained for two hours. In the upper cell colorless plastids (fig. 5, cp) varied widely in size and shape; mitochondria (fig. 5, m) were brightly colored, spherical, and not so numerous as the plastids. In the lower cell all the plastids, including the very small forms, were brilliantly impregnated with xanthophyll (fig. 5, yp), presenting a striking contrast with the deep-blue mitochondria. The decolorization of mitochondria occurred simultaneously in both cells, after which the process of oxidation and reduction of the dye was repeated several times, the material being floated upon a sucrose solution and examined under a lens.

Thus, in this material the plastids and mitochondria can be differentiated on the following bases:

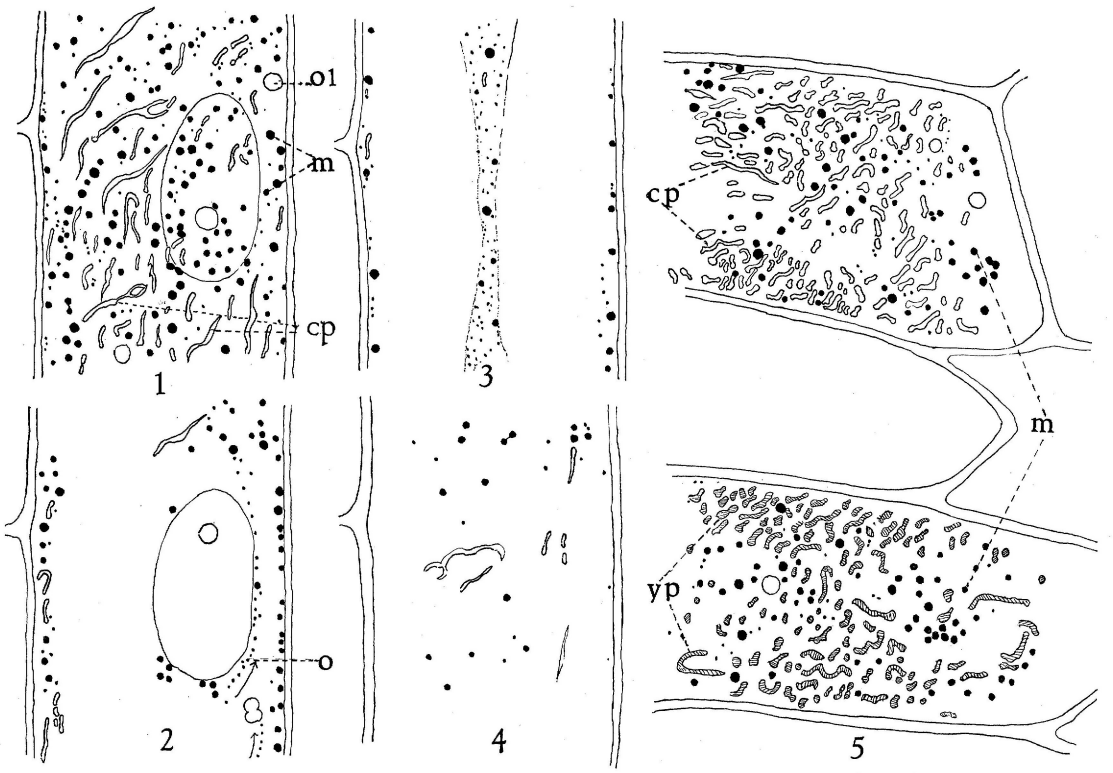

Fig. 1–5. Mitochondria and plastids from living epidermal cells of the perianth of tulip after Janus green B reaction. m, mitochondria; cp, colorless plastids; yp, yellow plastids; o, oil globules; ol, oleoplast.—Fig. 1–4. The same cell at different foci of the microscope.—Fig. 1. Layer of cytoplasm adjacent to the innermost cell wall.—Fig. 2. Through the central part of the nucleus.—Fig. 3. Through the central vacuole.—Fig. 4. Layer adjacent to the cutinized cell wall.—Fig. 5. Two cells from white tulip. The upper cell contains colorless plastids; in the lower cell all the plastids are impregnated with xanthophyll. Camera-lucida drawings at a magnification of *ca.* 2,000, reduced 1/3 in reproduction.

the extreme polymorphism of the plastids, in contrast to the constant shape and size of the mitochondria; the ability of plastids to develop pigments; and, finally, the specificity of the Janus green B reaction toward mitochondria.

Iris germanica L., hort. var. Firmament, Jacqueline Guillot.—Shoots 6–8 inches long were collected outdoors in November (temperature about 60°F.). Epidermis from the inner surface of the leaves was used. After the disappearance of traumatic shock cyclosis was usually restored in the uninjured cells, and if the material was floated upon an isotonic sucrose solution, the cells survived easily for two weeks. In the dye solution the mitochondria of the subepidermal cells become colored after thirty to ninety minutes; those from the cells of the uppermost layer stain after two to four hours. The dye has no immediate toxic effects upon the mitochondria. In preparations stained for fifteen hours the typical motion of the mitochondria is often not disturbed, and the process of oxidation of the dye after the mitochondria become decolorized can be repeated several times. However, in the material which had been floating for several days upon a sucrose solution without the dye I was not able to stain the mitochondria, although the cyclosis was quite normal. This might be explained by the general tendencies of a sucrose solution to retard vital staining (Bailey and Zirkle, 1931).

The mitochondria are mostly spherical, 0.7 μ to 1.1 μ in diameter, and diplosomes and figures of division frequently occur (fig. 6, m). After treatment with Janus green B, they may be a uniform intense blue or else segregation of otherwise submicroscopically distributed lipoids may be observed. Both types of coloring may occur in the same cells, and if the material remains alive the mitochondria, after decolorization, may acquire the appearance of unstained mounts. After prolonged staining some other parts of the cell may take up the dye, such as the cell wall, the cell sap, the oil globules, and the elaioplasts, if they are present. The highly refractive and faint coloring of the bodies containing oil is distinctly different from, and cannot be confused with, that of the mitochondria. Furthermore, the color never disappears from all of these parts of the cell when the mitochondria decolorize. Similarly, the color does not disappear from the dark-blue stained globular precipitates formed in the vacuoles of injured overstained cells.

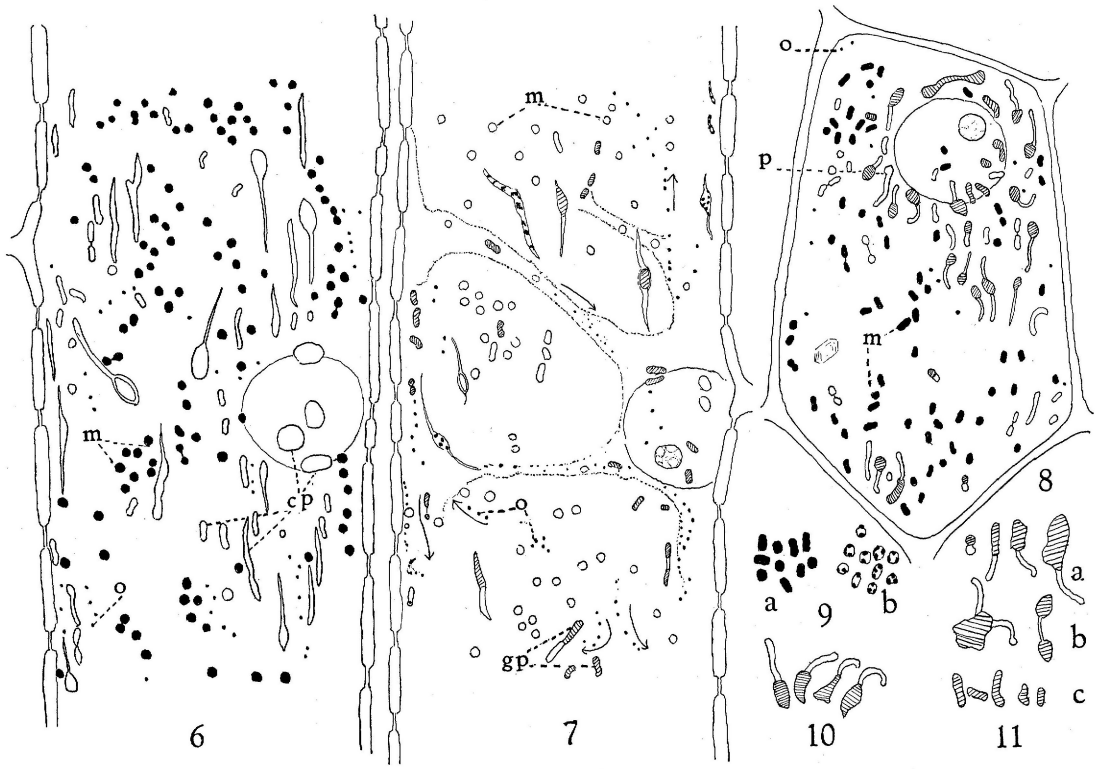

Fig. 6–11.—Fig. 6–7. *Iris germanica* L.—Fig. 6. Epidermal cell from a young leaf after Janus green B reaction. Layer of cytoplasm adjacent to noncutinized cell wall. cp, polymorphic colorless plastids; m, mitochondria, colored blue; o, oil globules.—Fig. 7. Cell, similar to that of figure 6, after a culture upon an isotonic sucrose solution for eleven days. The colorless plastids have acquired chlorophyll, gp.—Fig. 8–11. *Phaseolus vulgaris* L.—Fig. 8. Cell from the inner side of a pod after Janus green B reaction. m, granular mitochondria; p, polymorphic plastids, colorless and partially impregnated with chlorophyll.—Fig. 9. a, mitochondria after Janus green B reaction, colored solidly blue; b, lipophanerose of mitochondria.—Fig. 10. Changes in a single form of plastid during two minutes of observation.—Fig. 11. Plastids: a, with colorless protrusions; b, same as a; c, very small green plastids. Camera-lucida drawings.—Fig. 6–8 × *ca.* 1,333.—Fig. 9–11 × *ca.* 2,000.

The plastids exhibit great variations in their morphology, type, and numbers. In the uppermost layer of epidermis they belong predominantly to the colorless type (fig. 6, cp). They include oval or slightly irregular forms; extremely pliable forms which taper at their extremities, exhibit amoeboid movement, form protrusions, and are often referred to as chondriocontes (Guilliermond, 1919; Faull, 1935); transitional forms; and a varying number of small granular forms of less than one micron in size. In none of the preparations examined have the plastids been observed to become colored with Janus green B. In the subepidermal cells the plastids are represented either by regular chloroplasts or by forms which are similar to the extremely pliable colorless plastids described above, and which differ from them in partial development of chlorophyll in certain portions of the body of the plastid (see also Guilliermond, *et al.*, 1933).

Additional evidence that the small bodies, which do not color with Janus green B, represent small forms of plastids was obtained from the following experiment. Small pieces of epidermis were floated, with the cutinized side uppermost, upon an isotonic sucrose solution without the dye, in containers exposed to sunlight, and the temperature was kept at 60°F. At the beginning of the experiment the cells of the sample of material were examined, and the form, size, and distribution of the colorless bodies, which were negative to the Janus green B test, were recorded. After ten to eleven days a distinct green coloration of the pieces of epidermis was observed even with the unaided eye. Observation under the microscope revealed that most of the cells were alive, with excellent cyclosis (fig. 7, arrows), and that the bodies which had not colored previously with Janus green B had produced chlorophyll and acquired a distinct green color (fig 7, gp). The chlorophyll was observed in all types of plastids, including the very small forms of about one micron in size. The mitochondria of such cells could be recognized as small spherical bodies (fig. 7, m). As the mitochondria lose their ability to stain after floating upon a sucrose solution for several days, they could not be colored with Janus green B in this experiment.

The epidermal cells of iris thus contain two types cytoplasmic inclusions: the first type is morphologi-

cally constant; the second is extremely polymorphic, and, in accordance with functional requirements of the cell, is capable of developing starch, oil, or chlorophyll and other pigments. The Janus green B reaction is specific only for the first type of bodies, namey, mitochondria.

Phaseolus vulgaris L.—Epidermal tissue from the inner side of the green pods, containing immature seeds, was used in the experiments. Only the uppermost layer of epidermis showed satisfactory survival of the cells; therefore, the material was always mounted on the slide with the cutinized cell wall uppermost. Penetration of the dye into the cells is fairly slow, and the mitochondrial reaction appears in uninjured cells next to the margin of the piece after sixty to ninety minutes. The mitochondria are predominantly granular, ranging from 0.6 μ to 1 μ in dimension (fig. 8, m), although diplosomes and figures of divisions are frequently noted. The mitochondria may be stained solidly blue (fig. 9, a) or (more often) the lipoids may segregate into dark-blue granules at the periphery of the mitochondria, and the bodies themselves remain colorless (fig. 9, b). The latter type of staining at first gives the impression of a slight vesiculation. Careful measurements reveal, however, that the size of the mitochondria thus stained is exactly the same as that of the mitochondria colored solidly, except that the granular forms assume a more spherical shape. Furthermore, after fifteen to sixty minutes of observation, decolorization takes place equally in the solidly stained forms and in those in which lipophanerose takes place. In both cases, if the cell remains alive, the mitochondria resume the normal appearance of unstained mounts. In living cells, therefore, lipophanerose may be a reversible process, and does not necessarily imply degeneration. In the cells with definite signs of autolysis, such as Brownian movement of the particles (which is an indication of pronounced changes in the viscosity of the cytoplasm), or formation of sarcode, vesiculation of the mitochondria may take place and is usually followed by coagulation of the protoplast.

The mitochondria are equally distributed throughout the cytoplasm and appear in great numbers in the layer adjacent to the cutinized cell wall (fig. 8). As this tissue was taken from the inner side of the pod and was not exposed to light, these observations support the theory of the negative heliotropism of mitochondria.

The plastids are extremely polymorphic and occur both as colorless pliable bodies, which vary widely in size (fig. 8, p), and as forms with chlorophyll developed in a part of the stroma. In the latter type both the chlorophyll-containing part and the colorless portion are continuously changing shape, and a single form may exhibit changes during two minutes of observation, as recorded in figure 10. The portion with chlorophyll may be of the same size as the colorless part (fig. 11, a; forms to the left) or (more often) it is slightly wider (fig. 11, a; forms to the right). Still other forms are represented in figure 11, b. Finally, there are always present very small green plastids, 0.5 μ in width and 1 to 3 μ in length (fig. 11, c).

Phenomena of clarity and opacity of the cytoplasm are likely to occur frequently in this material, and autolytic processes begin to be apparent after two hours of experimentation. If the observations are continued on definitely injured cells, the mitochondria begin to aggregate into irregular clumps and form networks and other bizarre figures.

Lactuca sativa L. Iceberg lettuce.—Epidermis from the outer surface of the stout midrib of the leaves was used in these experiments. When floated upon a dye solution, the mitochondria of subepidermal cells become colored after ten to twenty minutes. They are granular, spherical or rod-shaped, and either 0.7 μ in diameter or 0.7 μ in width and 1 to 4 microns in length. The number of mitochondria in this material is small as compared with that of the tulip and iris. The plastids of the subepidermal cells are predominantly oval-shaped forms with or without attenuated protrusions. They often exhibit a slight green coloration. A large number of cells very soon show definite signs of injury, as is manifest from the conspicuous Brownian movement of the particles. In such cells a copious blue precipitate is rapidly formed upon the surface of the vacuole, and coagulation of the cytoplasm is noted. In the cells with cyclosis the mitochondria decolorize after ten to thirty minutes, whereas the precipitate of the dying cells remains brightly colored.

The cells of the upper layer exhibit better survival, and, after being stained for sixty minutes and mounted on the slide with the cutin side uppermost, show mitochondria in the layer of cytoplasm adjacent to the innermost cell wall (fig. 12, m). They become uniformly colored, and in size and shape are similar to the mitochondria of subepidermal cells. At first, Janus green B has no perceptible effects upon mitochondria, and their motion is not disturbed. After observation for ten to fifteen minutes the colored mitochondria begin to aggregate into smaller or larger clumps, which may either appear stationary or be carried, as whole units, by cytoplasmic streams. The aggregates of stained mitochondria often resemble beaded chains, rings, networks, etc. (fig. 13, a; 14, a) and are sometimes observed about the nucleus and larger plastids. If the cell remains alive, the mitochondria are decolorized after about thirty minutes of observation, the clumps and aggregates become dissociated (fig. 13, b; 14, b), and the mitochondria resume the appearance of unstained mounts, continuing their typical motion in the cytoplasm as separate units. Thus, in this material, staining with Janus green B has a definite paralyzing effect upon the mitochondria, which disappears simultaneously with decolorization and is apparently an effect of the change in surface tension. After three hours of staining, autolytic processes begin to be evident in most of the cells.

Oil globules (fig. 12, o) are fairly abundant, their motion being conspicuously faster than that of mito-

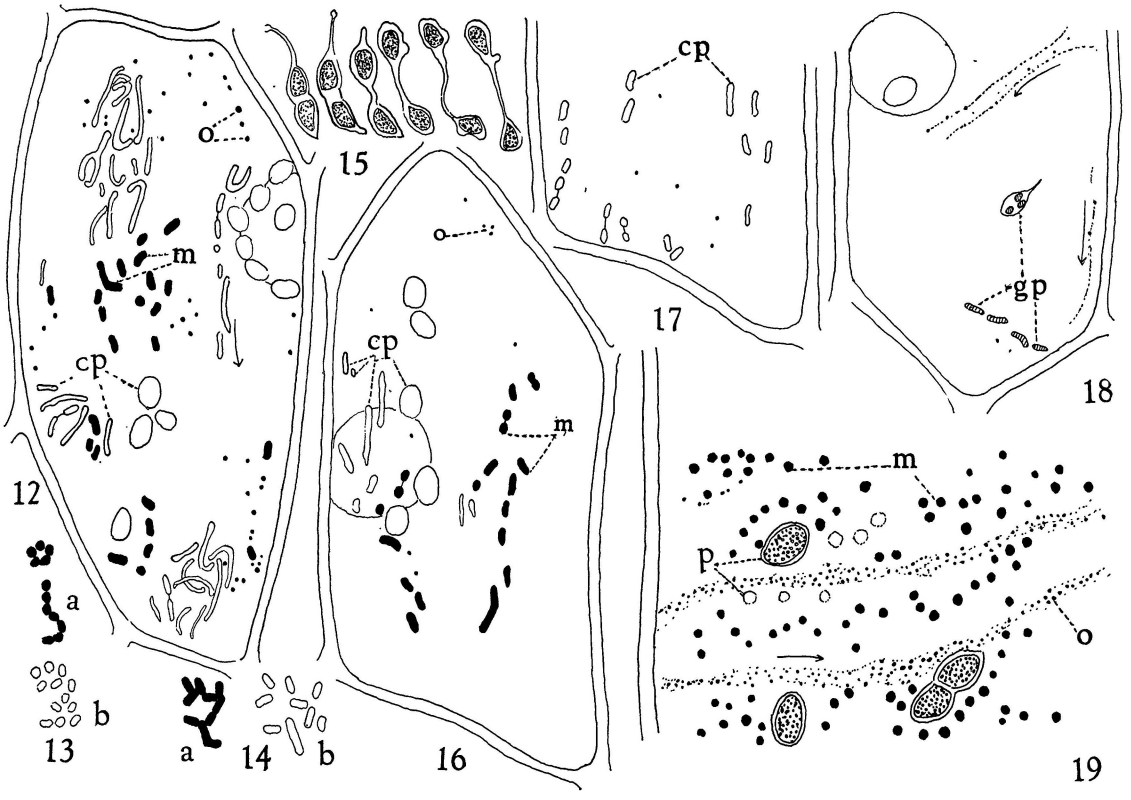

Fig. 12–19.—Fig. 12–14. *Lactuca sativa* L.—Fig. 12. Uppermost epidermal cell from the midrib of a leaf at the innermost focus after Janus green B reaction. m, mitochondria; cp, colorless plastids; o, oil globules.—Fig. 13, a, aggregation of mitochondria into ring and beaded chain; b, restoration of normal distribution after decolorization.—Fig. 14. Same as figure 13, except that the mitochondria are granular.—Fig. 15–18. *Apium graveolens* L. var. *dulce* D.C.—Fig. 15. Changes recorded during two hours of observation in a chloroplast.—Fig. 16. Uppermost epidermal cell at the innermost focus after Janus green B reaction. m, granular and rod-shaped mitochondria; cp, colorless plastids; o, oil globules.—Fig. 17. Portion of the cell, as in figure 16, at uppermost focus; layer of cytoplasm adjacent to the cutinized cell wall. cp, colorless plastids and absence of mitochondria.—Fig. 18. Cell similar to that of figure 17 after culture upon an isotonic sucrose solution for six days. gp, plastids which have acquired chlorophyll.—Fig. 19. *Spinacea oleracea* L. m, mitochondria after Janus green B reaction; p, plastids; o, oil globules. Camera-lucida drawings × *ca.* 1,333.

chondria; and Janus green B has no paralyzing effect on their motion.

The colorless plastids occur as oval bodies present in greater or smaller numbers, and as very slender, threadlike forms, 0.3 μ to 0.5 μ in width and 1 μ to 12 μ length (fig. 12, cp), which exhibit continuous motion. Janus green B has no effect upon plastids: they do not become colored, and their motion is not retarded by the toxicity of the dye.

Apium graveolens L. var. dulce D.C.—"Explants" from the outer surface of the stalk leaves of celery survive in a sucrose solution for six to seven days. When floated upon a dye solution the mitochondria of the subepidermal cells become colored after ten to twenty minutes. For the study of subepidermal cells the material was mounted with the cutinized cell wall downward; for that of epidermal cells the mounting had to be reversed. The size and shape of mitochondria vary according to the material. In some cases they are spherical, with a diameter of 0.8 μ to 1 μ; in others rod-shaped mitochondria, 0.8 μ in width and 1 μ to 6 μ in length, may be observed in addition to the spherical forms. The coloring is uniformly blue, and decolorization takes place after five to fifteen minutes. In preparations stained more than one hour a dark-blue precipitate appears in the vacuole, and the granules become involved in rapid molecular movement and aggregate into irregularly branching bodies of considerable size. Such precipitates are typical for the A-type vacuoles subjected to vital staining with basic dyes (Bailey, 1930). The formation of precipitates can be postponed by staining the cells for a short period of time (ten to fifteen minutes), examining under the microscope until the color disappears from the mitochondria (about five to ten minutes), and then floating the material upon an isotonic sucrose solution without the dye for about thirty minutes. The coloring of the mitochondria in such preparations is frequently even better, and the cells do not show injury. Decolorization occurs after twelve to fifteen minutes. The experiment may be repeated for four hours without any signs of precipitation of the dye in the vacuoles. After this time, however, the mito-

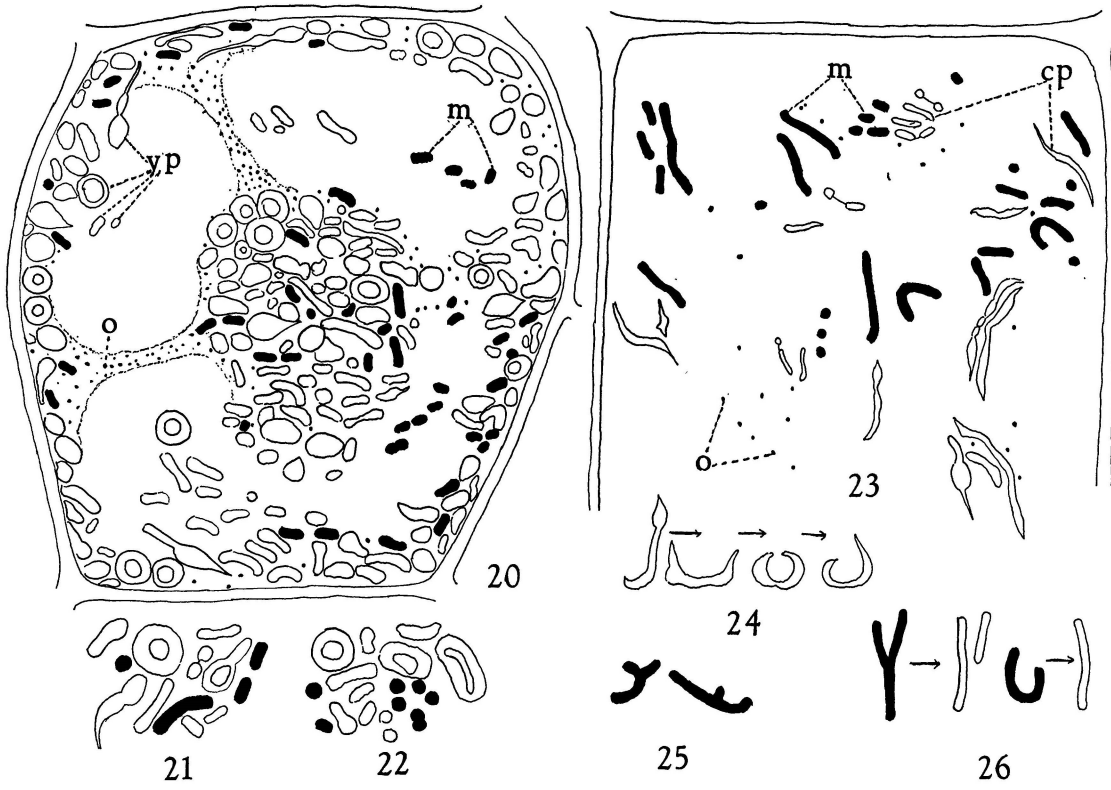

Fig. 20–26.—Fig. 20–22. *Narcissus Jonquilla* L.—Fig. 20. Epidermal cell from the perianth after Janus green B reaction. m, mitochondria; yp, yellow plastids; o, oil globules.—Fig. 21. Variety Victoria.—Fig. 22. Variety King Alfred.—Fig. 23–26. *Narcissus Tazetta* L. var. *papyraceus* hort. after Janus green B reaction.—Fig. 23. A cell from the inner surface of a sheath leaf enclosing an old flower stalk. m. mitochondria; cp, colorless plastids; o, oil globules.—Fig. 24. Successive changes of a colorless plastid.—Fig. 25. Branching of mitochondria in very old material.—Fig. 26. Paralyzing effect of Janus green B upon mitochondria (black), and restoration of normal shape after decolorization. Camera-lucida drawings.—Fig. 20, 23–26 × *ca.* 1,333.—Fig. 21–22 × *ca.* 2,000.

chondria fail to color, although the cells remain alive for more than forty-eight hours. Similarly, mitochondria cannot be stained in living material which has been floating upon a sucrose solution for twenty-four hours. Thus, Janus green B, in concentrations used, is not lethal for mitochondria, and the death of the cells after two to four hours of staining is a result of accumulation of the dye. In the uppermost layer of epidermis the mitochondria (fig. 16, m) are similar to those from the subepidermal cells, except that their number may be smaller; and they are usually absent from the layer of cytoplasm adjacent to the cutinized cell wall (fig. 17).

The plastids exhibit marked polymorphism, and their number and the types present are subject to variation in different samples of material. In the portions of the celery stalk which show a slight green coloration the chloroplasts may occur as oval bodies, in size and shape typical for other tissues of this plant and closely adherent to the nucleus. In some samples the oval chloroplasts may display long, attenuated, colorless protrusions; in such cases changes in a single form, as illustrated by figure 15, were observed during two hours. In still other material, oval chloroplasts may be absent, and large, green, pliable plastids may be seen scattered in the cytoplasm. If the material containing the latter type of plastids is floated upon a sucrose solution without the dye, in a container exposed to sunlight for about twenty-four hours, the irregular plastids may assume the perfectly regular shape and size of the typical chloroplasts and group themselves about the nucleus. Thus the position of the oval-shaped plastids about the nucleus is apparently an effect of the physical forces of attraction exerted upon oval bodies and does not indicate a functional relationship. In the completely bleached portions of the stalk the green plastids are absent. The number and the type of the colorless plastids (fig. 16, cp) are again subject to variation in different samples. They may be represented either by the oval leucoplasts or by slender filamentous forms, similar to those described for other materials. The latter type may be distributed throughout the cytoplasm, including the layer adjacent to the cutinized cell wall, where forms 0.7 μ in width and 1 to 4 μ in length, which do not color with Janus green B, are regularly observed (fig. 17, cp). Evidence that the small bodies described (fig. 17, cp) represent small forms of plastids can be obtained by culturing the

"explants" upon a sucrose solution, according to a technique similar to that described for iris. The chlorophyll has been observed to develop after six days in the small plastids, as well as in the oval leucoplasts (fig. 18, gp). The specificity of Janus green B toward mitochondria is once again illustrated in this material.

Spinacea oleracea L.—Epidermal cells from the petioles of the leaves of spinach survive for several hours upon sucrose solution. The mitochondria of subepidermal cells become brightly colored after floating upon the dye solution for twenty-five to forty minutes. Since the cutinized cell wall and the deposits of various substances on the surface prevent good visibility, the material had to be mounted with the cutinized cell wall downward. In most of the material examined the mitochondria are spherical, 0.6 μ to 1.2 μ in diameter (fig. 19, m), although short, rod-shaped forms may also occur in certain samples. The coloring is very bright and may be observed not only with oil immersion lenses but even with lower magnifications. The toxicity of the dye is different in various samples. In some cases the motion of the mitochondria is not immediately affected by the staining; in other instances they appear motionless, as if paralyzed, and are absent from the thick cytoplasmic strands carrying the oil globules (fig. 19, o). The decolorization usually occurs after fifteen to twenty minutes of observation, and if the cell remains alive the mitochondria may be colored again after floating upon sucrose solution. More often, however, shortly after decolorization, or even before they begin to vesiculate, they increase in size and become involved in molecular movement. In material stained more than one hour the autolytic processes usually appear in the cells, precipitates become apparent in the vacuole, and the mitochondria begin to aggregate and form irregular chains or clumps. However, in the cells containing a larger number of chloroplasts, mitochondria may appear normal even after ninety minutes of staining. After two hours the accumulation of the dye in the vacuoles becomes very conspicuous, and the reaction is highly confusing.

Narcissus Jonquilla L.—In the epidermis from the outer surface of the perianth tube the mitochondria become colored with Janus green B after about seventy-five minutes. For microscopical examination the material had to be mounted with the cutinized cell wall downward. In certain varieties the mitochondria are either spherical or else granular, 0.8 μ to 1.1 μ in diameter (fig. 22, m); in other varieties short rod-shaped forms 0.8 μ to 1 μ in width and from 1 μ to 4 μ in length were observed in addition to the spherical forms (fig. 20, 21, m). The coloring is a brilliant blue, and the contrast with yellow plastids is striking. Colored mitochondria either are stationary or else move very slowly, and are not carried in the thick cytoplasmic stands containing numerous oil globules (fig. 20, o). The decolorization appears after ten to forty minutes, and the color can be restored after oxidation. The marked polymorphism of the plastids (fig. 20, yp) is well demonstrated in this material, and the differentiation of small, morphologically similar forms of plastids, impregnated with xanthophyll, from mitochondria is very simple. Here again the Janus green B test is specific for mitochondria only.

Narcissus Tazetta L. var. papyraceus hort.—In the subepidermal cells from the inner side of a sheath leaf of a shoot, mitochondria become colored with Janus green B after thirty to forty minutes. They are spherical, granular, and rod-shaped, 0.8 μ to 1 μ in diameter and 1 μ to 12 μ in length. In younger material the spherical and granular forms predominate, whereas in that from the sheath of the sturdy flower stalk the long, rod-shaped forms are abundant (fig. 23, m). The width of the long forms is constant, and the ends are blunt. In noncolored preparations the rod-shaped forms may twist and bend, whereas after the Janus green B reaction they appear paralyzed in the position they had assumed at the onset of the reaction. At the moment of decolorization the twisted forms may straighten out (fig. 26) and continue their undulating, gliding motion, typical for unstained preparations, or remain stationary. The plastids of corresponding length, if they are present in the cell, are less viscous (fig. 23, cp), taper at their extremities and undergo conspicuous changes in width (fig. 24). Their motion is not retarded by the toxicity of the dye. The small forms of plastids are more slender than mitochondria of corresponding length, and in some samples of material were observed to acquire chlorophyll. In very old material mitochondria may reach 16 to 20 microns in length, and occasional branching can be observed (fig. 25).

Discussion.—The confusion and controversial character of contemporary research in the field of plant mitochondria arise from three main factors: (1) inability to distinguish mitochondria from small forms of plastids, and the attribution of properties of one of the components of the cytoplasm to the other; (2) the acceptance by analogy, without critical confirmation respecting plant material, of rare properties of the mitochondria found in tissue cultures of animal cells, and neglecting the fundamental property, ingrained in the organization of the cell, of constancy in shape where the function is similar (Cowdry, 1924); (3) and discrimination on the part of orthodox plant cytologists against the living material technique, and a definite preference for mitochondrial methods developed in accordance with the requirements of proper fixation of the animal cell but often highly disastrous and erratic in their results when applied to plant material. Even the comparatively recent attempts (Zirkle, 1929a) to introduce a more appropriate technique for studying the components of plant cytoplasm, by fixing the material with bichromates on the basic side of pH 4.2 to 5.2, are poorly adapted to the requirements of mitochondrial studies, as such methods do not distinguish mitochondria from small forms of plastids (Zirkle, 1929b; Anderson, 1939) and intro-

duce confusion into the concept of mitochondria. Hence the prevailing perplexity and the current belief in the instability of mitochondrial morphology and behavior.

The evidence presented in this study makes it possible to distinguish the plastids from the mitochondria on the following grounds: (1) the extreme polymorphism of the former bodies and the stability in size and morphology of the latter; (2) the marked variations in the number and type of plastids present in the cells of similar tissues at different times, and the comparative constancy of the mitochondria; (3) the preferential distribution of mitochondria in the layer of the cytoplasm furthermost from the cutinized cell wall; and (4) the ability, specific to the mitochondria of both plant and animal cells, to become colored in dilute solution of Janus green B, to decolorize upon the reduction of the dye to its leucobase and become colored again after oxidation.

The polymorphism of the plastids is typical for both colored and colorless forms. The mitochondria, on the other hand, are constant in size and shape for a given species and tissue. Variations in diameter may be confined to a fraction of a micron in the spherical forms; in species possessing rod-shaped forms, variations in length may range from 1 to 12 microns. This property of plant mitochondria corresponds to similar properties of the mitochondria of the normal animal cell, where functional variations in size usually occur within the limits of certain dimensions and forms, and where branching, the formation of anastomosing plexuses, beaded chains, and other irregular forms are known in material subjected to alterations in the cell culture but only rarely in normal cells (Cowdry, 1924). In slightly injured cells of plant material, mitochondria may assume very bizarre appearances, and in very aged cells occasional branching may occur.

There are marked variations in the number of plastids in the cells. Epidermal cells from the perianth may be literally packed with colorless or xanthophyll-colored plastids, whereas other cells may exhibit only a few plastids. In comparison with that of plastids, the variations in the number of mitochondria in cells of the same tissue are only slight. Similarly, the types of plastids may vary greatly. In accordance with functional requirements of the plant, certain cells may contain only chloroplasts, whereas others may lack colored plastids and possess a variable number of colorless forms.

The distribution of plastids in the cell is fairly uniform, and they occur in both parietal layers of the cytoplasm. The preferential distribution of mitochondria in the layer of the cytoplasm farthest removed from the cutinized cell wall has already been demonstrated by Guilliermond (1919) and by Siwicka-Tarwidowa (1934), and it is confirmed by present studies. The theory of the negative heliotropism of mitochondria is suggestive; but the problem needs additional experimental investigation.

The staining of mitochondria with Janus green B was obtained in all the living material employed (more than fifty different species of plants), provided the conditions outlined in section on methods were strictly observed. The failure of many investigators, even those who have specialized in mitochondrial methods, to obtain the reaction may be explained by faulty technique. In some instances the penetration of the dye was extremely slow (for example in the staminal hairs of *Tradescantia*), and the material had to be slightly crushed to facilitate the penetration. Similar difficulties are often encountered by animal cytologists (Cowdry, 1924). The color of the mitochondria after the reaction is an intense opaque blue, and has nothing in common with the changes in the refractive indices of the cytoplasm, as was recently suggested by Newcomer (1940). In certain materials (spinach, jonquil), where the mitochondria are larger and the color is very intense, the reaction could be seen not only by means of a research microscope but also with any good classroom equipment.

The specific coloring of mitochondria with Janus green B represents a special case of vital staining which does not conform to the accepted theory of the action of basic dyes upon the protoplast. It suffices to point out that a specific reaction with a dye of high oxidation-reduction potential implies a different chemical composition in the case of plastids and mitochondria. Of this there is ample evidence in the existing literature. However, the problem needs substantial reinvestigation in view of uncertainties in the identification of the two types of bodies in the literature available. The point raised by my critics (Anderson, 1939, and others) that the two bodies seen in the cells may represent different stages of development of the same body, or even two different reaction stages of the same body, is far more difficult to prove by assuming their identity, as even the evidence of Anderson indicates that in pollen tubes of *Lilium* two types of bodies of different oxidation-reduction potential are present. Unless a tissue can be found exhibiting only bodies which are negative toward Janus green B, the arguments of my critics must be regarded as invalid. Furthermore, if one assumes that the substances of mitochondria and plastids represent two different reaction stages of the same body, the substance of nonreactive plastids must be in a highly oxidized state, a fact which refutes the principal conception of a plastid as a dynamic body (Weier, 1938).

For the indication of the specificity of Janus green B reaction in plant mitochondria, the ability to decolorize after the reduction of the dye and to become colored again with the new oxidation is very important. This property, typical also for mitochondria of animal cell, makes it possible in plant material to distinguish mitochondria from various colored precipitates, which are frequently formed in overstained preparations within or upon the surface of the vacuole and which never decolorize.

The process of decolorization usually proceeds very rapidly in plant material. Only rarely (for example, in *Elodea canadensis,* tulip and narcissus) can mitochondria colored pink with diethylsafranin be observed for any appreciable length of time before the dye is reduced to the leucobase.

The Janus green B reaction is strictly vital. Nevertheless, its effects on the mitochondria of different plants vary. In certain materials (tulip, iris) the mitochondria are not visibly affected for many hours; in others (lettuce, narcissus) the dye has a paralyzing effect upon the motion, which usually is restored after decolorization. In still other plants (celery) accumulation of the dye has a toxic effect upon the cell, and the autolytic processes can be postponed by an early removal of the tissue from the dye and by inducing the coloring of the mitochondria through oxidation of the small amount of dye accumulated in the cell. Finally, in some materials (spinach) the dye is toxic, and the mitochondria soon become vesiculated. The lipophanerotic segregation of otherwise submicroscopically distributed lipoids is usually an indication of slight injury, and in certain instances may be a reversible process; in other cases it may be followed by vesiculation and subsequent coagulation of the protoplast.

SUMMARY

The mitochondria of uninjured living epidermal cells of more than fifty representatives of plants color an intense opaque blue in a dilute solution of Hoechst's Janus green B, and decolorize with the reduction of the dye to its leucobase. Subsequent oxidation of the leucobase of the dye accumulated in decolorized mitochondria can be obtained with a new supply of oxygen by floating the material upon an isotonic sucrose solution without the dye. The ubiquity of mitochondria in the living epidermal cells may be regarded as established.

The decolorization of mitochondria and the restoration of the color through oxidation are properties specific to mitochondria, and permit one to distinguish the latter from various precipitates of the dye which are formed within or upon the surface of the vacuoles in overstained preparations.

Under normal conditions, provided the cells are of the same age and perform similar functions, the mitochondria are highly constant in their morphology. Variations in diameter may be confined to a fraction of a micron in the species possessing spherical forms; variations in length may range from 1 to 12 microns in plants with granular or rod-shaped forms. The variations in the number of mitochondria from cell to cell of the same tissue are not very marked. The preferential distribution of mitochondria in the layer of cytoplasm furthermost from the cutinized wall is possibly correlated with negative heliotropism.

Extreme polymorphism of the plastids, together with marked variations in the number and type present in the cells of the same tissue, is typical for both colored and colorless forms. The reaction of the plastids of living cells toward staining with Janus green B is uniformly negative.

The identity of small forms, which do not color with Janus green B, with plastids can be proved from the study of material in which one of the neighboring cells exhibits colorless forms only, whereas another cell shows morphologically similar forms impregnated with pigment, or from an artificial culture of explants and the development of chlorophyll in the forms which do not react with the dye. Janus green B may be considered as virtually specific for the mitochondria of plants as well as animals.

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