ELAIOPLASTS IN IRIS: A MORPHOLOGICAL STUDY

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INTRODUCTION

ELAIOPLASTS are a heterogeneous group of intracellular bodies presenting the characteristics of fatty substances to a marked degree but not recognizable as ordinary types of plastids, chondriosomes or vacuoles. There is no general agreement in the literature regarding their structure, origin, development, classification or chemical composition. They have been variously described as aggregations of lipoid globules, as modified or unusual types of plastids or vacuoles, as nuclear derivatives, as aggregations of mitochondria-like bodies or as independent structures. They have been linked with various physiological processes such as assimilation, excretion or degeneration.

Much of the confusion regarding elaioplasts is due to the use of inadequate techniques and to a consequent lack of accurate information about the early developmental stages of these bodies. Many of the discrepancies are also due to failure to visualize and interpret correctly the full range of morphological variability of chondriosomes and plastids.

The investigations described in this paper were undertaken to clarify our conception of the elaioplasts in *Iris* and to compare these bodies with those in other monocotyledons and in liverworts. By using an improved technique critical evidence has been obtained to show the early stages in the development of the elaioplasts in *Iris* and the changes which these bodies undergo in different tissues and at different seasons.

In addition to the morphological study an extraction and preliminary analysis of the so-called oil in the elaioplasts of *Iris* and some physiological experiments on the metabolism of two types of Iris rhizome, one of which contains abundant elaioplasts, have been made. The results of these investigations are being published elsewhere.

HISTORICAL RÉSUMÉ

Since the middle of the last century papers have appeared from time to time describing cytoplasmic bodies associated with oil. The writers have used various names for these structures which through usage have become more or less interchangeable. Thus they are termed elaioplasts,

oléoplasts, oléosomes, ölkörper, oil bodies, Zellenbläschen, Zellenkörper, fatty bodies, elaiosferer, oelplastids, oléoleucites, éléments oléifères and système oléifère. Sometimes the terms are restricted in their application. For example ölkörper is used only for oily bodies in the liverworts, and elaioplast is kept for those in the monocotyledons. But recently with an increasing tendency to consider all of these oily bodies essentially similar, one name is often used to designate all of them.

The earliest references to elaioplasts are found in the writings of Mirbel (35) in 1835, of Gottsche (13) in 1843, of Holle (24) in 1857, of Hofmeister (23) in 1867 and of Ward (49) in 1883. But the first adequate descriptions of oil bodies were published in papers by Pfeffer (38) in 1874 and by Wakker (47) in 1888. These, together with a paper by Lidforss (32) in 1893, provide a description of the three main types of mature oil bodies; from this later authors have diverged little. Although often resembling one another, the three main types present certain distinct features which are further emphasized by their restriction to a given group of plants.

Pfeffer (38) described oil bodies characteristic of the liverworts. In common with such bodies in general, they are highly refractive structures which stain brilliantly in "fat" dyes, such as alkannin, and which are more or less soluble in 95% alcohol and in fat solvents such as ether. They are distinguished from other oil bodies by their almost complete solubility in alcohol, by a characteristic residual ring left after treatment with alcohol, by their location commonly in the peripheral cytoplasm but within the chloroplast-bearing layer, by their presence in practically every species of the group, by their appearance commonly in every cell of a plant and by their permanency as cell structures. The Marchantiales present a contrast to other elaioplast-bearing hepatics in the restriction of the oil bodies to special cells scattered throughout the thallus and in the location of a single large oil body in the center of each of these cells. Oil bodies in the liverworts vary in shape from round to spindle-shaped as a rule, though some are irregular in form. They vary in color from colorless to dark brown and in appearance from granular to segmented or homogeneous.

The oil bodies described by Wakker (47) differ from those in the liverworts in their location near the nucleus, in their invariably granular appearance, in their often irregularly lobed shape, and in their character of being more or less temporary cell structures. Elaioplasts of this type are often yellowish in color and are marked by their reaction with some reagents which cause an extrusion of the oil and leave a characteristic net-like structure. Although reported from most tissues, they

are often restricted to certain ones. Raciborski (41) and Beer (4) found them only in flower or fruit tissues, while Politis (39) described them in these tissues and in those of bulbs. Oil bodies of this type are further restricted to a few groups of flowering plants. Lists published by Zimmermann (51) and by Politis (39) record them in groups of species in the Orchidaceae, Liliaceae, Amaryllidaceae, Iridaceae and Malvaceae, while Beer (4) found them in one of the Compositae.

The third type of oil body described by Lidforss (32) is characterized by its homogeneous appearance, by its spherical shape and by its unrestricted location in the cell. It is reported from leaf tissues of flowering plants and is of common occurrence in this group.

Besides these three classes of oil bodies there are isolated descriptions of elaioplasts that are not included in any of the types described. Such are the reticulate, highly refractive structures saturated with an amber-colored oil described by Keene (26, 27) in two molds. Such also are the yellow, green or black oil bodies near the nucleus found by Hieronymus (22) in some algae.

In 1888 Wakker (47) demonstrated by abnormal plasmolysis that the oil bodies in the monocotyledons and in the liverworts are located in the cytoplasm. He showed that, although these structures often protrude into the vacuole, they are never located in it as Pfeffer (38) and Rattray (42) had thought. Later investigations have substantiated Wakker's observations and extended them to include all types of elaioplasts.

There is no general consensus of opinion on the structure of the non-homogeneous oil bodies. Pfeffer (38) described them as aggregations of homogeneous oil globules, a view expressed in modern times by Guilliermond (20), by Meyer (34) and by Kozlowsky (28). Other students have described a stroma with embedded oil globules. This view was first expressed by Wakker (47). It was elaborated upon by Zimmermann (51), who pointed out less refractive inclusions which he termed vacuoles or portions not producing oil. Later Beer (4) and Politis (39) described the elaioplasts in *Gaillardia* and in the monocotyledons as aggregations of smaller bodies, each composed of a stroma with included oil globules. A more elaborate structure was postulated by Woycicki (50) and by Keene (26, 27). Woycicki described elaioplasts in *Vanilla* with central oily drops surrounded by a mucilaginous layer which in turn was covered by a granular layer. Keene described a somewhat similar structure in the oil bodies of *Sporodinia* which showed a denser reticulate center and a coarser reticulate outer portion. The presence of an unfixable stroma in the oil bodies of the liverworts in

contrast to the fixable one in those of the monocotyledons was pointed out by Küster (29). Later Gargeanne (9) and Dombray (7) attempted to show that this unfixable stroma was a fluid or a semi-fluid.

The question of an enveloping membrane has been raised with reference to the oil bodies in the liverworts. Pfeffer (38) inferred the presence of a membrane from the characteristic ring left after treatment with alcohol. Küster (29) demonstrated in 1894 that this ring is an artefact. Gargeanne (9) repeated the demonstration but maintained that, although the ring is an artefact, the bodies possess a true membrane homologous with the tonoplast of the vacuole. Later writers have not agreed with Gargeanne in recognizing a membrane. The presence of a membrane about elaioplasts in the monocotyledons has been described only by Raciborski (41) who considered the stroma at times to be reduced to a surrounding layer.

The development of the oil bodies is also a disputed point in the literature. Pfeffer (38), Rivett (43), Lidforss (32), Chalaud (5), Meyer (34) and Guilliermond et al. (20) have considered the formation of elaioplasts to be a process of aggregation of small drops in the cytoplasm with more or less fusion. Kozlowsky (28) has further stated that the drops are first extruded from the chloroplasts. A second theory has been postulated by Wakker (47), by Küster (29) and by Harper (21). They consider that a stroma appears first as a shadowy, wrinkled structure in which refractive oil drops appear later. Gargeanne (9) stated that the oil drops are secreted by a surrounding membrane, while Dombray (7) described the deposition of substances from the cytoplasm and their transformation by the cell sap as a catalyser. Another theory is expressed by Hieronymus (22) and by Beer (4) who described elaioplasts formed by the aggregation of degenerating plastids with the production of oil. Somewhat similar is Woycicki's (50) theory of the aggregation, partial degeneration and fusion of mitochondria-like bodies forming oil. Keene (26, 27) postulates the formation of a reticulate structure in homogeneous bodies with the later fusion of several of these bodies. Still another theory by Politis (39) and by Raciborski (41) describes the development of elaioplasts by the growth of refractive drops and the subsequent fusion of the bodies so formed.

The division of elaioplasts has been noted in a few instances. Raciborski (41) in 1893 described a fragmenting of the bodies after they had passed maturity and a breaking off of bud-like protrusions. Again in 1914 Politis (39) described division of the elaioplasts. Politis considered division not merely an incidental or degeneration phenomenon, but a method of increasing the number of these bodies. Besides the

budding already described by Raciborski, Politis described passive division of the body by the cell wall during cell division.

The history of oil bodies after they have reached maturity has been studied. In the liverworts they are generally thought to remain unchanged even after the death of the cell, although Dombray (7) noted a decrease in size, fusion of the oily globules and aggregation of the oil bodies before death. Elaioplasts in the monocotyledons are generally thought to degenerate some time after reaching maturity. Wakker (47) described their disappearance in older tissues of *Vanilla*. Beer (4) and Woycicki (50) described a resolution of the oil bodies into scattered oily spheres. Politis (39), on the other hand, described the disappearance of the oil first, leaving a vacuolated protein mass which might later disappear also.

Movement has been noted in connection with elaioplasts. In 1893 Zimmermann (51) first recorded the rotation at times of oil bodies in the monocotyledons, a phenomenon observed also by later investigators. A second type of motion consisting of Brownian movement of the globules within the oil bodies appears in oily structures in the hepatics. Gargeanne (9) described this as an injury phenomenon, but recently it has been noted by Dombray (7) as a normal condition in the elaioplasts of some species.

The chemical composition of the elaioplasts and particularly of the oily portion has received much attention. The theories advanced are based chiefly upon microchemical reactions. Dombray (7) has interpreted microscopical observations in the light of analyses of extraction products. Two opposing theories regarding the composition of the oil have been formulated. In one the oil is said to be chiefly a mixture of essential oils. This is the view recently expressed by Popovici (40) and by Rivett (4) in her description of the oil as a mixture of essential oils with small amounts of protein and fatty oils. Dombray (7) stated that the oily substance was a mixture of essential oil and "tannoides." The opposing theory considers the oil to be composed chiefly of fatty oils. This is the opinion of most investigators. Pfeffer (38) described the oil as a mixture of fatty oil with some water and protein and with traces of wax and resins. Later Küster (29) designated the oil in the elaioplasts of liverworts as a fatty oil resembling castor oil. Lidforss (32) identified the oil in the homogeneous oil bodies of flowering plants as a non-drying oil containing fatty acids of the type $C^n H_{2n-2} O_2$. The stroma, if present, is generally considered to be a protein, a view first expressed by Zimmermann (51).

There is little agreement among investigators concerning the origin

and identity of oil bodies. Raciborski (41), Küster (29) and Gavaudan (10, 11, 12) have considered them to be cell systems independent of the vacuome, chondriome or plastidome and originating more or less *de novo* in the cytoplasm. Wakker (47) considered them to be special plastids, while Beer (43) and Hieronymus (22) described them as degenerating plastids. Kozlowsky (28) has stated that they are products of the chloroplasts. That they are special or transformed vacuoles has been postulated by Keene (26, 27), by Dombray (7), by Gargeanne (9) and by Rivett (43). Woycicki (50) and Lundström (33) have described oil bodies originating from mitochondria-like bodies. Politis (39) has ascribed a nuclear origin to them.

A relationship between elaioplasts in the monocotyledons and crystal formation has been suggested. Wakker (47), Politis (39) and Monteverde (36) stated that there is no connection between the oil bodies and the calcium oxalate crystals found in the same plants. But Warlich (48) considered them to be interdependent structures, while Woycicki (50) in 1929 described crystals forming in some of the elaioplasts in *Ornithogalum*.

Many writers have ventured theories on the physiological and biological significance of the oil bodies. In general they have considered those in the liverworts and also the homogeneous ones in the flowering plants to be excretions. But those in the monocotyledons they term assimilation products, although Raciborski (41) stated them to be excretions. Various other theories have been offered. Beer (4) in 1909 demonstrated that the bodies in *Gaillardia* are degeneration products of the plastids with the secondary function of producing color. Hieronymus (22) and Lundström (33) suggested that the bodies are protective in function, a theory opposed by Dombray (7).

In concluding the summary of the literature on oil bodies it should be noted that these structures do not include the ölplasma described by Leiner (31) and by others of Tschirch's school, nor do they include the oil cells described by Lehmann (30) and others. The former (ölplasma) deals with oil in the cytoplasm — chiefly of fatty seeds. The subject is well summarized in the account by Tunman and Rosenthaler (46). The phenomenon of the appearance of oil in special oil cells involves the transformation of large portions of the cytoplasm or secretion from the modified cell wall rather than the appearance of oily bodies in the cytoplasm.

In addition to the literature on oil bodies, some reference should be made to the literature on the structure of Iris cells. The most recent and complete studies are those by Guilliermond (15–20) and by Dan-

geard (6). They have developed a method of vital observation especially adapted to this type of study. They have pointed out the presence in Iris cells of vacuoles and their inclusions, of cytoplasm, of oil globules, of chondriosomes of various types and of plastids. In particular Guilliermond has described the chondriosomes and plastids and their developmental stages. He has noted the presence of oil globules in most plastids and chondrioconts in *Iris*. These globules which he has found associated more often with young or degenerating types of plastids he considers to be lipoids separating out from the plastid substance. He has described the development of plastids from mitochondrial types differentiated from other mitochondria by their potentiality for plastid formation. He has described the formation of chloroplasts from an intermediate chondriocont stage by budding and fragmenting. Other phases of studies carried out on *Iris* include the action of hypo- and hypertonic solutions on chondriosomes, observations of the amoeboid movements of chondrioconts, and the identification of an oily body in the vacuoles of certain cells as a phenol compound.

MATERIAL

The plants used in my studies of elaioplasts included numerous irises, some liverworts and a few representative flowering plants. They were obtained from several sources. The major part of the study was made on colonies of *Iris versicolor* and of an *Iris pallida* of hybrid origin which grew in abundance near the laboratory. For work on living tissues it was desirable to have the plants as close at hand as possible. It was also desirable to locate single colonies in a natural habitat for the basic study of variations. In this way differences due to season, development, etc., were less likely to be confused with those due to location, to abnormal habitat or to individual variations.

As a rule the material was used as soon as it was collected. But in some instances it was kept in water or in wet sand in the greenhouse for later observations, or it was transplanted to garden beds. In the early part of the study a few plants of *I. pallida* and of *I. versicolor* were transplanted to pots in the greenhouse to supplement the outdoor material. Although some interesting observations were made on these plants, they grew so poorly that this method of providing material was abandoned. Fortunately, it was not necessary to rely on greenhouse or garden material at any period.

The *Iris versicolor* was taken from a swampy field at the corner of Weston St. on the Cambridge-Concord turnpike about an hour's drive from the laboratory. The *Iris pallida* hybrid, a garden plant, grew in

beds within a few rods of the laboratory. Both of these species were sufficiently near at hand to be obtained as they were needed for examination.

The other irises used as supplementary material and for a general survey of the genus were obtained from several places. A group of native West Coast species was studied in California. Three of these, *I. macrosiphon, I. Douglasiana* and *I. longipetala,* grew naturally within a few hours' drive of the laboratory. But *I. Hartwegii* and *I. missouriensis* had to be brought to Palo Alto from the eastern part of the state. A large number of other species were obtained from the Missouri Botanical Garden, from the Brooklyn Botanical Garden and from the New York Botanical Garden. Those at the Missouri Garden were examined *in situ,* but the ones from New York were brought to Boston for examination.

For a list of the species of *Iris* studied see the table on page 246.

In addition to the Iris plants, a group of rhizomatous plants and a number of liverworts were obtained. The former were studied at the Missouri Botanical Garden for the most part, although a few were collected around Boston. Two species of *Vanilla,* the plant used by Wakker (47) in his classical studies on elaioplasts, were obtained from Panama. The liverworts were collected in the New England woods for study in Boston or they were sent from Oregon to the California laboratory for use there. The hepatics were kept in the laboratory in moist glass containers over a period of weeks.

A list of the flowering plants studied is given on page 248 and one of the hepatics on page 254.

TECHNIQUE

In choosing a method for the morphological examination of the elaioplasts one fundamental requirement was kept in mind. It was desirable to observe the bodies in as unaltered a condition as possible in order to discover their normal development and variations due to seasonal, environmental or specific differences in the plants examined.

At the present time there are two methods used in the study of cytoplasmic bodies. The first of these is the fixation technique introduced in the later decades of the nineteenth century and developed to the highest degree in the complicated "mitochondrial techniques" and "silver or osmic impregnation methods." Essentially it consists of killing and fixing blocks of tissue in reagents that solidify proteins and fats, rendering them insoluble in specific fluids, and then staining sections differentially. Incidentally the technique involves a rather complicated process of embedding and one or more dehydrations.

The other method is that of examining untreated tissue either with or without the aid of vital dyes. Although untreated tissue was used before the introduction of fixatives, it was superseded by them. Recently the so-called vital technique has been revived and developed, notably by the Dangeards and by Guilliermond in France and by Bailey in America. Guilliermond has described a technique for vital staining in his studies of the vacuome and has contributed data on various aspects of injury and death in his studies of the chondriome. Bailey (1) in his investigations of the cambium has tabulated criteria that can be used in distinguishing living from dying or dead cells. Bailey and Zirkle (2, 3) have clarified the vital staining technique by their investigation of the toxicity of a large number of dyes, of the most suitable media in which to use the stains, of the staining properties of different dyes and of the varying reaction of vacuoles to given stains.

Both of these methods were tried in the study of *Iris,* but that of fixation was eventually discarded because of the difficulties involved. The vacuoles in the rhizome were found to contain large quantities of a substance that precipitated with fixatives and stained deeply, obscuring the sections, while the elaioplasts in the rhizomes of *Iris versicolor* contained quantities of "oil" that either was dissolved or was extruded in large masses obscuring the cell structure. In the one or two instances where this did not occur, a good fixation was obtained in mature but not in meristematic cells. The fixation images in sections of rhizome meristem were not comparable with those obtained in root-tip meristems, nor could they, as in the case of the root-tips, be identified with structures clearly seen in similar "living" cells. A third difficulty, that might in time have been overcome, lay in the persistent plasmolysis of cells in the rhizome meristem and in leaf tissue. For these reasons it was felt that the fixed material did not give an image of unaltered cells, nor could it be relied upon for comparative work. Better results were obtained with the "vital" technique where dead and dying cells could be observed and where those that survived for some hours without undergoing lethal changes seemed to present a more reliable picture of an unaltered condition. Consequently after some months of unsuccessful experimenting with fixatives and dehydrating reagents and with different hydrogen ion concentrations of single fixatives, the method was entirely abandoned and the "vital" technique alone retained.

Although fixation methods were finally discarded, it should be noted that in certain instances satisfactory results were obtained in this way. Thus the mitochondrial fixatives and stains proved successful for root-tips where they apparently produced little or no alteration in the cell

structure. Likewise, since chromic and osmic acids fixed the elaioplast "oil," occasional slides were obtained of mature rhizome tissue quite comparable with that examined "vitally." Other fixations, although they did not give exact images of the cytoplasmic contents of the cell, proved useful in determining the structure of the oil bodies. The fixatives that proved most successful for the occasional rhizome slides were 0.5% osmic acid solution and Flemming's weak solution followed by Flemming's triple stain. The most satisfactory of the mitochondrial fixatives was ammonium Erliki solution (25 cc. each of 1% solutions of ammonium and potassium bichromates plus 25 cc. of an 8% solution of formaldehyde) followed by Milovidov's modification[1] of Volkonsky's stain. With these fixatives the usual dehydrating and paraffin embedding schedules were satisfactory. A third instance of useful fixation was found for the mitochondrial fixatives. These, although not entirely successful except for root-tips, did fix mitochondria throughout the plant sufficiently well for a rough survey of the distribution of these elements.

The "vital" method was preferred and finally used exclusively because it presented a more reliable picture of unaltered cell structures. Although this was the main consideration, there were other factors that made the "vital" technique especially favorable for the study of developmental and other changes within the cell. Of primary importance was the possibility of observing fluctuating changes of a moment's duration, as well as those more permanent ones associated with age or season. This was possible only with a technique which left the more or less fluid contents of the cell unchanged. The "vital" method provided such a technique. Another factor favoring the "vital" method was its practical simplicity. Although some skill was required in sectioning, after this was obtained the actual preparation required but a few seconds. Not only was this a saving of time but it was possible to examine material as it was brought in, a method that enabled one to proceed quickly with the study. A third factor of importance was the applicability of the method without modification to all kinds of material. In a comparative study of tissues and plants this was an essential requirement for the technique.

As used in this study the "vital" technique was essentially that developed by Bailey (1) for the study of cambium. The material was

[1]Stain in acid fuchsin over flame for 5 min.; stain in 0.5% aurantia in alcoholic solution for 20 min.; stain in gentian violet; differentiate in alcohol. [Milovidov, P.F. Sur les méthodes de double coloration du chondriome et des grains d'amidon. — Archiv. Anat. Micro. (24), 1:9. 1928.]

sectioned, placed in appropriate solutions and examined immediately and at intervals. For distinguishing the living from injured or dead cells criteria were established based upon comparisons between obviously injured cells and those that survived for some hours before showing signs of injury. The only differences in the technique for *Iris* lay in the details of sectioning and of preparing solutions and in the possibility of more firmly establishing criteria for living cells by comparisons with mounts of thin, unsectioned tissue.

The sectioning was done with a "Gem" razor blade freehand, or, for some rhizomes, with a Thomson-Spence sliding microtome. Although the microtome sections were more uniform in thickness and more convenient for mature rhizomes, they were less satisfactory with the other tissues. Apparently a thinner blade produced less injury in rhizome meristems, while it was the simplest means for sectioning leaf, flower or root tissue. The razor blade was used for mature rhizomes also when a microtome was not available. In either case, sections were obtained varying from one to several cells in thickness. Measured by the microtome, sections of mature rhizome varied from 30 μ to 50 μ or more, while those of the smaller-celled meristem were 15 μ to 20 μ or less.

The solutions in which these sections were immersed consisted of a basic solution plus one or more of the "vital" dyes, or merely of the basic solution alone. Of the three fluids tried, water, nujol and sucrose solution, the sucrose solution in a five to ten per cent concentration, proved most satisfactory.

The dyes most commonly used were Neutral Red, Janus Green BB, Chrysoidin Y and Benzene-azo-alpha-naphthylamine. Although Chrysoidin Y is the only one of these dyes which stains the elaioplasts, the light staining of the vacuole with Neutral Red throws the cytoplasm into relief and makes its structures more clearly visible. The other dyes in combination with Neutral Red and Chrysoidin Y have a clarifying effect. None of these dyes stain the immature oil bodies, while the staining of the mature oil bodies by Chrysoidin Y is but temporary. Almost all dyes will stain dead, mature oil bodies. In practice, only traces of the dyes were used (one drop of a concentrated aqueous solution to 25 cc. of sugar solution). Staining is better and more rapid when the sucrose solution is made alkaline with Clark's buffers (pH 8.2 to 8.6) which shorten the staining period from an hour or more to fifteen minutes or less. Since most stains, even in small amounts, are toxic after a time, sections that it was desired to keep were removed to pure sucrose solutions. In this way cells were kept "living" for twelve hours or more.

An essential part of the technique was the establishment of criteria

for distinguishing living from dead or dying cells. By comparing obviously injured cells with those which survive sectioning for some hours without sign of injury, such criteria have been established for cambial tissue. By the same method criteria were found for Iris cells. In addition unsectioned roots, bracts and flower parts of *Iris* and the thin leaves of a *Potamogeton* were examined. Living cells in *Iris,* like those in cambium, are marked by the following characters: regular cyclosis, absence of Brownian movement in the cytoplasm and a staining of the vacuole in the presence of Neutral Red. Two additional criteria were found for living cells of *Iris,* namely, a pulsation of the cytoplasm in isodiametric cells and the amoeboid movement of the chondriosomes. Both of these phenomena are essentially a swelling or contraction of parts of the structure involved. The pulsation, for example, is the swelling of one part of a protoplasmic thread at the expense of another, a phenomenon involved in changes in the concentration of the substance at a given point. The pulsation of the cytoplasm occurs principally in isodiametric cells where there is no streaming. The amoeboid movement of the chondriosomes may occur in any cell. Both criteria proved valuable as indications of the condition of the cells. Dead cells of *Iris,* as of the cambium, show one or more of the following characteristics: coagulation of the protoplasm, a general formation of granules in the cytoplasm, staining of the nucleus and cytoplasm in the presence of dyes, increasing opacity of the whole cell and Brownian movement in the cytoplasm. Dying cells in *Iris* were found to show the following characters: jerky or irregular streaming and Brownian movement within the plastids. Parallel phenomena were found in the irregular streaming and degenerating plastids of some epidermal, bract and flower tissues.

The validity of these criteria for distinguishing living from dying or dead cells should be considered. The possibility of injury lies in the sectioning, in the action of the solutions in which the sections are placed, in the pressure of the cover glass used in mounting sections and in the strong light used for microscopic observations. In establishing criteria, the use of unsectioned material eliminated the possibility of injury due to sectioning, while the examination of water plants in the water of their natural habitat provided a check upon the effects of the solutions used in the study of *Iris.* A similar check upon the effects of pressure from the cover glass was provided by removing it. The possibility of injury due to strong light alone remains. That strong light will produce injury and death is clear, but the effects are slow in appearing and, if the light is removed in time, they are temporary. They can be taken

into account in establishing criteria for distinguishing living from dying or dead cells. That there are undetected, instantaneous changes is improbable, for the reactions in plants are in general slow. The effect of the light appears chiefly in the slowing down of streaming, and, if exposure is continued, unmistakable signs of death such as coagulation of the cytoplasm finally are observed.

It should be noted that the observation of minute details of cytoplasmic structures can be carried on only with the aid of the best high-powered microscopic equipment. For the observation of sections mounted in aqueous media a water immersion objective is essential. Without such equipment, many of the details of structure described in the following section cannot be seen.

OBSERVATIONS

Description of Elaioplasts in Rhizomes of Iris versicolor

Elaioplasts occur typically in the parenchyma of the rhizomes of *Iris versicolor*. They appear in every cell as granular, highly refractive masses with a decidedly yellowish cast (Fig. 1). The individual elaioplasts are almost spherical in shape and seem to be composed of closely compacted globules approximately one micron in diameter (Fig. 2). They are relatively constant in size within a given rhizome, generally averaging 10 to 13 microns in diameter. Although in some material they may be twice this size, they are never as large as the nucleus which has a diameter of the order of 40 to 50 microns. Often a hundred or more of these elaioplasts will be found in a single cell, aggregated for the most part into one large mass. Sometimes there are as few as twenty to a cell, but often they more than half fill the cell lumen, obscuring the nucleus and protruding into the huge vacuole.

All evidence shows that the elaioplasts are located in the cytoplasm. Although they protrude into the vacuole, protoplasmic threads are often observed to spread at their surface as if to include them (Fig. 1). Occasionally one is seen moving in the streaming protoplasm. The study of similar bodies in the root, where they obviously are included in the cytoplasm, substantiates these observations.

Microchemical tests indicate that the bodies are mainly lipoid in character. They stain brilliantly in "fat" dyes such as Sudan III, alkannin and nascent indophenol blue.[1] They are almost completely

[1]For this technique see Zweibaum, J. Sur la coloration des graisses dans la cellule vivante. Comp. Rend. Soc. Biol. 1923. — Zweibaum, J. and G. Mangenot. Application à l'étude histochimique des végétaux d'une méthode permettant la coloration vitale et post vitale des graisses de la cellule végétale. Comp. Rend. Soc. Biol. 1923.

soluble in lipoid solvents such as ether, chloroform and carbon tetrachloride. They also dissolve largely in 95% alcohol, a solvent for some oils. They are insoluble in hydrochloric acid, sulphuric acid and potassium hydroxide, although they are more or less structurally disorganized by these reagents. They are not volatile at 100°C., which indicates the presence of lipoids rather than essential oils.

The reaction of the elaioplasts to heat and to many reagents in which they are insoluble is marked by the extrusion of the lipoid in drops (Fig. 5). The reaction occurs relatively slowly so that it can be watched. A net-like residue remains which is not distinctly lipoid in character. The drops characteristically remain in contact with the net and are flattened on their attached side. The reaction occurs with heat, picric acid, dilute sulphuric acid, Gram's solution, etc.

Injury to the cell typically produces active Brownian movement of the globules within the limits of the elaioplast which eventually bursts, freeing the globules within the cell lumen. A similar phenomenon occurs in elaioplasts which escape from cut cells. It can be induced by mechanical pressure.

Structure of Elaioplasts in Rhizomes of Iris versicolor

The structure of the elaioplasts in the mature rhizome is that of a matrix with embedded globules. This is best shown in sections of fixed material, for the globules in fresh material are so refractive and so closely packed that it is difficult to distinguish any structure clearly. With osmic acid and some of the chrom-osmic fixatives the globules are preserved *in situ* (Fig. 3b). They clearly show a network of a different substance between them. With other fixatives the globules are never preserved, but a net-like structure with lacunae of the approximate size of the globules remains (Fig. 3a). This can be seen best by the use of mitochondrial or plastid fixatives such as ammonium Erliki and an appropriate stain. It is well shown, too, by Wakker's method for double staining elaioplasts with anilin blue and alkannin after fixation of sections in picric acid. In this case, the extruded globules are stained red and the matrix appears as a purple network with blue interstices.

The behavior of the bodies in fresh material supports the observations on their structure as seen after fixation. The globules show no tendency to fuse, a fact which indicates a separation by the presence of at least a surface film. In injured material, they move apparently unchanged in a liquid portion of the intact body. Further proof of a matrix is found in developmental forms and in homologous oil-bearing

bodies in other species. Here the matrix is often so abundant as to be clearly visible in untreated material. Such is the case in very young cells of the rhizome, in some cells of the root-tip, in rhizomes of *Iris pallida* and of *Iris Hartwegii,* etc. The matrix is also clearly shown in the root during the degeneration of elaioplasts. Here before death the refractive globules disappear leaving only a net with lacunae. This net is very similar in structural appearance, although not in shape, to the net-like image of rhizome elaioplasts in fixed material.

The globules were identified as the material which gives the elaioplast as a whole its lipoid characters. They show the reactions previously described for the elaioplasts and additional ones equally characteristic of lipoids. They stain in the "fat" dyes. This is apparent in intact bodies, but it is more clearly seen with the moving globules in disintegrating ones. They are highly refractive, a property seen in both intact and disintegrated elaioplasts. They disappear from sections treated with "fat" solvents such as carbon tetrachloride, ether, etc., but they may be preserved in sections treated with "fat" fixatives such as osmic acid and chrom-osmic mixtures. They are completely soluble in alcohol. This was demonstrated with globules in suspension in alkaline water. Upon the addition of 95% alcohol a homogeneous fluid resulted indicating the complete solution of the globules.

The matrix was shown to be of a different substance from the globules. It appears to be more like the cytoplasm in composition. Unlike the globules it requires no special fixative for its preservation. At least a portion of it is insoluble in alcohol and lipoid solvents such as carbon tetrachloride, for it sometimes remains intact after the use of these reagents. It is not refractive, for this character can be seen in young tissue and in injured cells to be a property of the globules only. Nor is it stained to any extent by the "fat" dyes such as Sudan III, etc. This is evident in elaioplasts with globules in Brownian movement where the stain is largely confined to the globules. That the matrix is of a plasma substance was suggested by the difficulty of staining it differentially from the protoplasm. This view was substantiated later by the identification of the elaioplasts with the plastidome and chondriome.

No evidence of a differentiated membrane about the elaioplasts could be found. None could be seen in fresh material, nor has any been brought out by reagents or fixation techniques. The only observation that might be interpreted as indicative of a membrane was the "bursting" of injured elaioplasts already described. But no fragments of membrane remained. It is more probable that the sudden freeing of the globules depended upon changes in the matrix which made it miscible with the surrounding medium.

Seasonal Variations in Elaioplasts in Iris versicolor

Certain variations in the form and structure of the elaioplasts are due to the seasonal appearance of starch. In New England the elaioplasts are without starch from early November through June. By July or August the starch begins to appear, while by September or early October a maximum development has been reached. The disappearance of the starch then begins and proceeds rapidly. By early November no traces of it can be found.

The starch can be identified with Gram's solution and polarized light. In the former the grains stain a blue to a bluish-black, a reaction typical of starch in the presence of iodine. In polarized light they appear as bright grains with a black maltese cross on each.

The type of starch formation in rhizomes of *Iris versicolor* is characteristic and constant. Each elaioplast develops several included grains (Figs. 4b and c). Counts made in early October showed commonly from 8 to 12 grains, with a recorded range of 1 to 16 per elaioplast. Although the grains are always grouped more or less centrally within the globule-filled portion, they form bulges in the otherwise rounded contour of each elaioplast (Fig. 4). The individual starch grains are approximately isodiametric. They show the central hilum characteristic of this shape of grain when it is included within the plastid. In size they are small, generally 6 to 7 microns in diameter as measured in material collected in early October.

Climatic differences in the disappearance of the starch from the growing point of the rhizome are indicated. In material from the vicinity of Boston and of New York the starch disappears completely in the winter. But in plants grown in the Missouri Botanical Garden it may be found about the growing point in March, although completely absent from the rest of the rhizome.

The disappearance of the refractive globules of the elaioplasts has not been observed. Numerous observations have been made from September to May, during which time they remain in abundance. They are likewise present in the rhizome during June, July and August, although a less thorough study has been made of their behavior during those months.

Development of Elaioplasts in Rhizomes of Iris versicolor

By tracing back to the meristem, the elaioplasts in the rhizome were found to develop from mitochondria-like primordia by increase in size, in visibility and in the number of contained globules (Fig. 19). In the youngest cells there are small, irregular, shadowy proplastids with two or three included non-refractive globules. In increasingly older cells

these bodies become more distinct and larger with a greater number of included globules. At the same time the globules become refractive and the whole body even more irregular in contour. Later with further increase in size and in the number of included globules, the irregular contour is lost. The cells then contain the granular, smoothly rounded, mature elaioplasts characteristic of the rhizome.

The young elaioplasts are distinguished by the following characters. They have more matrix in proportion to the number of globules than the mature forms. They do not stain after death to any degree in Sudan III nor in any other anilin dyes in contrast to the brilliant staining of the mature elaioplasts. They are restricted to a small region about the growing point, while the youngest stages are found only in the cells of the growing point. They are all irregular in contour, but this irregularity is emphasized in the intermediate forms which are almost nodulose.

The youngest stages show characters ordinarily associated with mitochondria. They are about the size of Iris mitochondria, ranging from this up to several times their size. They are indistinctly visible like much of the chondriome with a peculiar fading and reappearing quality. Thus a period of clear definition of these shadowy forms will be followed by a fading and disappearing. This, in turn, after a few minutes or after several hours may be succeeded by another period of clear definition, and so on. In general, although not always, these forms show included non-refractive globules. This is a character shared by the rod-shaped mitochondria of the species. In the young elaioplasts there is no definite arrangement of the globules which in the rod-like mitochondria always form a single row.

The formation of starch occurs in any of the young or mature forms of plastids. It was found during the season of its formation in all of them. In the young forms the starch grains ordinarily protrude from the globule-filled mass of the elaioplast, in contrast to the completely included grains of the mature elaioplast.

No evidence of increase by division was found in mature or developmental stages. No division was seen at any time, although material was collected from September to June and kept under observation for hours at a time. In the rhizome tissue even the "dumb-bell" figures so often cited as evidence of division were absent.

Degeneration of Elaioplasts in Rhizomes of Iris

No evidence of degeneration was found in the rhizomes of *Iris versicolor*. Elaioplasts are found unchanged and in abundance even in the oldest living cells.

Two isolated cases of degeneration of elaioplasts similar to those in *Iris versicolor* have been noted in the rhizomes of other species. One of these is an abnormal condition produced in a slowly dying plant. The second is a normal phenomenon in otherwise morphologically unchanged cells. It is apparently unassociated with the death of the cells, for no other signs of degeneration appear. This phenomenon occurs consistently in the cortex of the rhizomes and in the epidermis and sub-epidermis of the roots of *Iris macrosiphon* var. *californica*.

In *Iris macrosiphon*, elaioplasts in the mature cells of the cortex of the rhizome appear as large lipoid spheres (Fig. 14c). These spheres are marked by their large size, by their distinct yellow color and by their brilliant staining in "fat" dyes, Sudan III, etc. They may be demonstrated to be in the cytoplasm by coagulating the surrounding protoplasm with fixatives (Fig. 15). The study of developmental forms which can be seen to be carried in the streaming protoplasm offers further proof of their inclusion in the cytoplasm.

Stages in the formation of the lipoid spheres from mature elaioplasts can be seen in cells not far from the growing point. The process consists of the formation of homogeneous spheres by the fusion of the globules and the disintegration of the matrix (Fig. 14). A single elaioplast resolves itself into one or more of these spheres. In older cells still further fusion occurs for the spheres in them are larger and fewer. In these cells each sphere probably includes the substance of more than one elaioplast.

A similar formation of lipoid spheres can be observed in epidermis and sub-epidermis of the root-tip (Fig. 18). The phenomenon is identical with that in the rhizome, although starch is present in the root elaioplasts. It shows more clearly than in the rhizome the steps in the resolution of the elaioplasts. The fusion of the globules proceeds for some time before the apparent structure of the elaioplast is lost. The final degeneration products include starch grains as well as lipoid spheres. The grains and spheres remain distinct in the cytoplasm, although the starch is indiscriminately scattered among the lipoid spheres.

Proof that the formation of lipoid spheres in *Iris macrosiphon* is a degeneration phenomenon is based on two points. First, the structure of the elaioplast characteristic of the functioning body is lost. There is no evidence that the lipoid spheres can produce starch as the elaioplasts do, or function actively in any way. Secondly, the phenomenon occurs in tissue which tends to die and slough away. In the root, the epidermal cells in which the spheres form are short-lived. This is less evident in the cortex of the rhizome where the cells may live for a season or more

after the formation of the spheres. But even in this tissue the outer cells die and the formation of lipoid spheres is more marked in the outer cells. It is not found in the inner cells of the cortex or elsewhere in the rhizome.

A second case of degeneration was found in rhizome cells of *Iris tectorum* (Fig. 7). In a slowly dying plant the elaioplasts appeared closely compacted in each cell into one or two masses. The rounded contour of each elaioplast was lost, while the matrix seemed to have become more plastic. The identity of each elaioplast was lost in the mass which appeared as a single granular body with indistinct partitions within it (Fig. 7b). Where starch was present the grains were included in the composite mass. This condition has never been found in healthy plants.

Oil-bearing Plastids in Rhizomes of other Species of Iris

Oil-bearing plastids are found in the rhizomes of practically all species of *Iris*. They show the same fundamental structure and development as those in *Iris versicolor* just described. But they differ from one another in their formation of starch. Two clearly marked types based on the mode and time of starch production occur.

The first type is that found typically in *Iris versicolor* (Figs. 1, 2 and 4). It has already been described. In contrast to the second type, it is marked by the disappearance of starch during the winter dormant season, by the formation of several starch grains in each plastid and by the inclusion of the starch within the plastid. Plastids of this type vary considerably, but they usually show at least two of the general characters. In some species of *Iris* the starch persists more or less throughout the winter; in others it may persist one season and not the next, and in still others, such as *I. versicolor,* it always disappears. The inclusion of the starch in mature plastids, although not in the younger forms, is complete in most instances. But in some cases the starch grains tend to protrude slightly. This is more often the case in the cortex, although it may characterize the whole rhizome. An extreme case accompanied by an unusually reduced number of lipoid globules in the plastids (Fig. 12d) was found in one of two collections of *Iris Hartwegii.*

The second type is characterized by the persistence of the starch through the dormant season, by the formation of one, large, asymmetric starch grain or sometimes two in each plastid and by a conspicuous protrusion of the grain from the globule-filled portion of the plastid (Fig. 9). Caplike elaioplasts attached to one end or side of the large starch grains are typical of these plastids. Often the lipoid globules are larger than

in the plastids of *I. versicolor*, while the matrix is abundant enough to be clearly seen between them. A typical example of this type of plastid is found in rhizomes of *Iris pallida* (Fig. 8).

Although the disappearance of starch is not general in this second type, it has been noted in one or two instances. The starch disappears from the main part of the rhizome of *Iris pumila* during flowering (Fig. 11), although it persists around the growing point. The solution of the starch leaves peculiarly cup-shaped elaioplasts (Fig. 11c). A second case of the disappearance of starch may occur under abnormal conditions. It was induced in the rhizomes of *Iris pallida* placed in the greenhouse during the winter. It is accompanied by a lack of vigor and the disappearance from the rhizome cell vacuoles of substances ordinarily present at that season. The change in the vacuoles is apparent in fixed material in a lack of the precipitate characteristically produced in them by reagents during the winter. The plastids in the rhizomes of the greenhouse plants resembled the spherical ones of winter material of *Iris versicolor* except for their smaller size and fewer numbers.

The distribution within the genus of the two types of rhizome plastids has been found to follow closely the recognized taxonomic grouping. The homogeneous and closely related groups show the same type of plastid, while a heterogeneous group such as the Apogon shows both types. In the latter case aberrancies from the prevailing type in the group are often correlated with anomalous taxonomic characters. Sufficient material has been examined to show definitely the condition in the two largest groups, Pogoniris and Apogon, and in several of the smaller groups, Evansia, Regelia and the Pardanthopsis and Gynandiris species. An indication of the prevailing type in each of the other groups may be found in the notes made on a few representative species.

Rhizomes have been examined largely during the late winter. In late winter the pallida type of plastid shows its characters clearly, while the versicolor type is generally without starch at that season. An indication of the mode of starch formation in the latter type of species can often be obtained from the persistent starch in the plastids about the growing point. In this study, such observations have been supplemented by notes made during the starch-forming season.

The pallida type of rhizome plastid occurs characteristically in Pogoniris, Regelia and Oncocyclus. These are homogeneous groups which together with Pseudoregelia form a unit of closely related species. The same type has been found in a Juno Iris and in a Xyphium Iris. In the latter case it occurs only in tissue about the vascular bundles but not in the large parenchyma cells which are filled with starch. It is also found

in Pardanthopsis, in the closely related hexagona sub-group of the Apogons, in the anomalous Apogon, *I. verna,* and in one of the variable Apogon spuria group, *I. spuria ochroleuca.*

The versicolor type of plastid appears in the Evansia group and in the majority of the Apogons. In the latter it characterizes the following sub-groups: Sibirica, Laevigata, Longipetala, Californian, Tripetalous, Spuria and Ensata. Its distribution is more limited than that of the pallida type, for the Evansia and Apogon sub-groups include but one-third of the species. The pallida type appears to characterize the other two-thirds of the genus. Since the Evansia and Apogon sections include all of the American irises, the versicolor type is predominantly the type found in North American species. The only exceptions are the anomalous Apogons cited in the preceding paragraph and one to be described later. A similar predominance of the versicolor type is to be found in the Asiatic species. The American and Asiatic species contrast in this respect with the more strictly European and Mediterranean species, which belong chiefly to groups showing a pallida type of plastid, notably Pogoniris and related groups and the bulbous forms.

The absence of oil-bearing plastids has been noted in five irises. In these cases the rhizome cells are filled with starch. The starch is of two types paralleling in distinguishing characters and in distribution the two kinds formed by the oil-bearing plastids. One is present as large, single, asymmetrical grains similar to those in the pallida type of oil-bearing plastid. They characterize the anomalous Apogon, *Iris unguicularis,* the Reticulata Iris and Gynandiris. The last of these is not always included in the genus; in the formation of starch and no lipoid in its corms it resembles the closely related genus, *Moraea.* The Reticulata is a group closely related to the Xyphium, which shows similar starch grains and a few oil-bearing plastids of the pallida type. The second starch grain type resembles the starch grains of the versicolor plastids in their small size, in their isodiametric shape and in their formation in groups within a single leucoplast. Like the versicolor type of plastid they are found in Apogon Irises, the Japanese Iris and *Iris Sintenisii.* The former is a hybrid of *I. laevigata,* one of a group characterized by the versicolor type of plastid, and another member of the same group. The other *Iris* belongs to the Spuria sub-section, a group of intergrading and variable forms, for which no single characteristic type of plastid was found.

For the type of plastid found in individual species, the reader is referred to the table on page 246 and also Figs. 12 and 13. The table also includes data on the material, its source, the season of examination, etc.

TABLE OF IRISES EXAMINED SHOWING THE TYPE OF RHIZOME PLASTID AND THE SOURCE OF THE MATERIAL FOR EACH SPECIES[1]

Species	Section	Group	Type	Source of Material	Parts	Season Collected	See Pl. 133, Fig. 13
I. pallida Lam. ×?	Pogoniris	Pallida	2	Bussey Garden	All	All	Qa
I. pallida variety	Pogoniris	Pallida	2	Mo. Bot. Gard.	r, l	March	—
I. Cengialti Amb.	Pogoniris	Pallida	2	Mo. Bot. Gard.	r, l	March	Qf
I. pumila L.	Pogoniris	Pumila	2	Mo. Bot. Gard.	r, l	March	Qe
I. pumila variety	Pogoniris	Pumila	2	Mo. Bot. Gard.	r, l	March	—
I. Korolkowi Regel	Regelia		2	Brooklyn Gard.	r	April	Pb
I. Hoogiana Dykes	Regelia		2	Brooklyn Gard.	r	April	Pa
I. susiana L.	Oncocyclus		2	Brooklyn Gard.	r	April	O
×*I.* "*Zwannenburg*" Hort.	Oncocyclus		2	Mo. Bot. Gard.	r, l	March	—
I. alata Poir.	Juno		2	Brooklyn Gard.	bulb	April	N
I. Xyphium L.	Xyphium		2	Florist Florist	f, l bulb	Feb. March	— M
I. dichotoma Pall.	Pardanthopsis		2	Mo. Bot. Gard.	r, l	March	R
I. foliosa Mack. & Bush	Apogon	Hexagona	2	Mo. Bot. Gard.	r, l	March	Ic
I. fulva Ker	Apogon	Hexagona	2	Mo. Bot. Gard. Brooklyn Gard.	r, l r	March April	Ia —
×*I. hexagona* Walt. ×?	Apogon	Hexagona	2	Mo. Bot. Gard.	r, l	March	—
I. vinicolor Small	Apogon	Hexagona	2	Brooklyn Gard.	r	April	Ib
I. verna L.	Apogon	Verna	2	Virginia	r	May	H
I. spuria Pall. var. *ochroleuca*	Apogon	Spuria	2	Mo. Bot. Gard.	r, l	March	Ee
I. halophila Pall.	Apogon	Spuria	1	Brooklyn Gard.	r	April	Ea
I. ensata Thunb.	Apogon	Ensata	1	Brooklyn Gard.	r	April	C
I. setosa Pall.	Apogon	Tripetalous	1	Brooklyn Gard.	r	April	Fb
I. setosa var. *canadensis* Foster	Apogon	Tripetalous	1	Brooklyn Gard.	r	April	Fa
I. Douglasiana Herb.	Apogon	Californian	1	Brooklyn Gard. California	r r, l, o	April Aug.	Da —
I. Hartwegii Baker	Apogon	Californian	1	California Brooklyn Gard.	r, l, o r	Aug. April	— Dc
I. macrosiphon Torr. var.	Apogon	Californian	1	California	r, l, o	Aug.	—
I. tenax Dougl.	Apogon	Californian	1	Brooklyn Gard.	r	April	Db
I. longipetala Herb.	Apogon	Longipetala	1	California	r	Sept.	—
I. missouriensis Nutt.	Apogon	Longipetala	1	California	r	July	—

TABLE (*Continued*)

Species	Section	Group	Type	Source of Material	Parts	Season Collected	See Pl. 133, Fig. 13
I. virginica L.	Apogon	Laevigata	1	Mo. Bot. Gard.	r, l	March	Bb
I. versicolor L.	Apogon	Laevigata	1	Mo. Bot. Gard.	r, l	March	Ba
				Boston	All	All	—
×*I. robusta* E. Anders.	Apogon	Laevigata	1	Mo. Bot. Gard.	r, l	March	—
I. pseudacorus L.	Apogon	Laevigata	1	Bussey garden	r		Bc
				Arnold Arbor.	r		—
				Mo. Bot. Gard.	r, l	March	—
I. Kaempferi Sieb.	Apogon	Laevigata	1	Brooklyn Gard.	r	April	Bd
×*I. Wilsoni* Wright ×?	Apogon	Sibirica	1	Brooklyn Gard.	r	April	Ad
I. sibirica L.	Apogon	Sibirica	1	Mo. Bot. Gard.	r, l	March	Aa
I. prismatica Pursh	Apogon	Sibirica	1	Duxbury, Mass.	r, l		Ae
				N. Y. Bot. Gard.	r	April	—
I. orientalis Mill.	Apogon	Sibirica	1	Brooklyn Gard.	r	April	Ac
×*I.* "*Quest*" Hort.	Apogon	Sibirica	1	Mo. Bot. Gard.	r, l	March	—
I. Clarkei Baker	Apogon	Sibirica	1	Mo. Bot. Gard.	r, l	March	—
I. chrysographes Dykes	Apogon	Sibirica	1	Brooklyn Gard.	r	April	Ab
I. cristata Ait.	Evansia		1	N. Carolina	r	May	—
				Mo. Bot. Gard.	r, l	March	Ja
I. gracilipes A. Gray	Evansia		1	N. Y. Bot. Gard.	r	April	Jb
I. tectorum Maxim.	Evansia		1	N. Y. Bot. Gard.	r	April	Jc
				Mo. Bot. Gard.	r, l	March	Jc
I. lacustris Nutt.	Evansia		1	Brooklyn Gard.	r	April	Je
I. japonica Thunb.	Evansia		1	Brooklyn Gard.	r	April	Jd
I. unguicularis Poir.	Apogon		3	Brooklyn Gard.	r	April	G
I. reticulata Bieb.	Reticulata		3	N. Y. Bot. Gard.	bulb	April	L
I. sisyrinchium L.	Gynandiris		3	Brooklyn Gard.	corm	April	K
I. Sintenisii Janka	Apogon	Spuria	4	Brooklyn Gard.	r	April	Eb
I. laevigata Fisch. ×?	Apogon	Laevigata	4	Mo. Bot. Gard.	r	March	Be

1—versicolor type of rhizome plastid
2—pallida type of rhizome plastid
3—rhizome plastid with single large starch grain and no oil
4—rhizome plastid with several small starch grains and no oil
r—rhizome, l—leaf, f—flower, o—root

[1] Nomenclature according to Dykes, The Genus Iris.

OIL-BEARING PLASTIDS IN RHIZOMES, BULBS ETC. OF OTHER PLANTS

No oil-bearing plastids have been found in any other rhizomes or bulbs examined. None are present in either of the species of *Moraea* examined, a closely related genus replacing *Iris* in the southern hemisphere. Nor are there any in the many Araceae, Bromeliaceae, Commelinaceae, Liliaceae and Scitamineae examined. Rather, all of these plants contain large asymmetric starch grains in their storage organs.

The chloroplasts in all of these plants characteristically contain more or less refractive granules. In general, such appears to be the condition in all of the monocotyledons and in many of the dicotyledons. Indeed it seems to be true even of some of the lower forms such as the liverworts and mosses, although in these the granules are often not refractive.

The following is a list of the species of monocotyledons examined. The species are grouped according to families.

ARACEAE: *Acorus Calamus* L., *Aglaonema* sp., *Arisaema triphyllum* (L.) Schott, *Dieffenbachia* sp., *Nephthytis* sp., *Philodendron Selloum* C. Koch, *Philodendron cordatum* Kunth, *Schismatoglottis crispata* Hook. f., *Schismatoglottis rupestris* Zoll. and Mor., *Spathiphyllum* sp.

BROMELIACEAE: *Ananas macrodontes* E. Morr., *Billbergia* sp., *Cryptanthus* sp.

COMMELINACEAE: *Palisota* sp.

IRIDACEAE: *Moraea iridioides* L., *Moraea* sp.

LILIACEAE: *Allium* sp., *Hemerocallis* sp., *Ornithogalum umbellatum* L., *Yucca filamentosa* L.

ORCHIDACEAE: *Vanilla planifolia* Andr., *Vanilla pompona* Schiede.

MUSACEAE: *Strelitzia* sp.

ZINGIBERACEAE: *Alpinia nutans* Rosc., *Amonum* sp., *Hedychium* sp.

MARANTACEAE: *Calathea* sp.

OIL-BEARING PLASTIDS IN OTHER PARTS OF THE PLANT OF IRIS SPECIES

The observations in this section apply to any species of *Iris* unless otherwise stated. A careful study has been made of the conditions in *Iris pallida* and in *Iris versicolor*. Additional notes have been made on other species.

The formation of oil globules is characteristic of plastids throughout the tissues of plants of the genus *Iris*. The globules are not always so numerous as those in the rhizome plastids of *Iris versicolor* where they are developed to an unusual degree. An extreme example of a limited formation of globules is found in the chloroplasts of the guard cells where

the matrix of the plastids is relatively abundant and clearly visible. Nor are the lipoid globules usually the only observable product of the plastids. Ordinarily starch is also present, while in some plastids chlorophyll or a yellow pigment is formed.

The elaioplast condition described for rhizome plastids of the versicolor type may occur in any of the uncolored tissues. It is dependent upon the absence of starch and pigment and upon a large production of oil. Such conditions are found at times in the rhizome, in the root and in uncolored leaf and flower tissues.

In the rhizome and root elaioplasts occur generally throughout the tissues of these organs. They are restricted to certain species and, at least for the rhizome, to certain seasons. There is no connection between their presence in the rhizome of a species and their appearance in the root of the same species. For example elaioplasts were found in the rhizome of *Iris versicolor* but not in the root (Figs. 2 and 35). On the other hand, they were found in the root of *Iris pallida* but not normally in the rhizome (Figs. 9 and 30). An example of their formation as a seasonal phase of the leucoplasts in the rhizome has already been described for *Iris versicolor*. Whether or not they also form a seasonal phase for leucoplasts in the root has not been investigated.

In the leaf and flower the elaioplasts are restricted to a few cells. Often they are but transitional forms appearing for a very brief time. Such is the case in the flower where they may occur in the course of the development of the chromatophores. Because of their limited occurrence in a few cells it is usually easy to identify them with the leucoplasts or chromatophores in neighboring cells. In these tissues they do not develop the brownish color so characteristic of the rhizome elaioplasts in *Iris versicolor*. Instead they remain entirely colorless.

The development of elaioplasts can be induced under unfavorable conditions. An example of this has already been cited in their formation in rhizomes of *Iris pallida* grown in the greenhouse (p. 244). In this case they were formed by the dissolution of the starch leaving only the oil-bearing plastid. By growing plants in semi-darkness chloroplasts can be prevented from forming pigment or starch. They then appear as elaioplasts. In neither of these cases is an increase in the number of oil globules involved. Nor has the formation of unusual numbers of elaioplasts been observed as a result of abnormal conditions.

The oil-bearing plastids in other parts of the plant show essentially the same features as those described for the rhizome. They differ from those in the rhizome in minor characters, also in the absence of a general elaioplast phase except in the root and in the formation of pigments. In

addition they show in some cases chondriome types as an intermediate stage in their development from the proplastids. In some tissues, notably in the flower and in epidermal tissues, the mature plastids often show further changes involving chondriome types not found in the rhizome. These points will be taken up separately in the succeeding paragraphs.

Minor differences between the plastids in other parts of the plant and the type found in the rhizome and root appear in the lesser production of lipoid globules and in their complete lack of color in colorless tissue. Correlated with the smaller number of globules is a greater stability. This is shown in their greater resistance to injury by mechanical pressure and to distortion or destruction by reagents. Their lack of color when not pigmented can be seen in colorless leaf tissue in marked contrast to the strongly yellowish cast in the equally unpigmented rhizome plastids. This is particularly well shown in *Iris versicolor*.

The formation of pigment, chlorophyll or yellow pigment, occurs ordinarily in the young plastids. But there is no specific stage at which it is developed. In the leaf chloroplasts it often forms shortly after the appearance of starch in the young plastids, although it may not develop for some time. In the chromatophores of the root it sometimes appears before the formation of starch, for example in the rootcap of *Iris versicolor*. At other times yellow pigment appears in plastids which do not form starch, for example in the chromatophores of roots of *Iris pallida*. In many cases yellow pigment is found in chondriocont types of plastids, but its formation is quite unconnected with the phenomena producing these forms. Proof of this is found in its formation in the approximately spherical plastids of the root-tip before they pass into a chondriocont state and in those of the rootcap of *Iris versicolor* where the mature plastids retain a more or less spherical state.

The location of pigment in the refractive globules and also in the matrix and its greater solubility in the former was demonstrated. In the guard cells of *Iris pallida*, where there is little chlorophyll, the green color can be seen to be confined to the globules, while the matrix remains colorless. That it may also be dissolved in the matrix is shown in degenerating chromatophores of the root where color remains in the matrix after the disappearance of the refractive globules.

Intermediate developmental forms of the plastids are found in the root-tip (Figs. 20–24). They differ from the small plastids in other differentiating tissue by the retention for a longer period of the shadowy visibility of the proplastids and by a plasticity amounting in the younger stages to an almost fluid character. They resemble other young plastids

in their origin in the proplastids, in their development by increase in size, in visibility, in the number of included globules and in their final development in many cells into the same type of plastid. In their often elongated shape they resemble the chondrioconts of many authors.

The shadowy character of the younger intermediate forms is evident in the peculiar fading and reappearing already described for the proplastids (p. 241). With the differentiation of the tissue this shadowy quality is lost (Figs. 20–24), but the bodies do not become refractive until a late stage (Figs. 24 and 25). Often the more or less indistinct forms persist for long periods.

The plastic quality of the intermediate forms is shown in their more or less elongated shape and in their movement in the streaming protoplasm. The movement consists of a continuous changing of form (Figs. 20–23). Both movement and elongation are more marked in the younger stages, some of which are almost fluid. In older cells the plastids become less and less elongated with increasing viscosity until they are more or less spindle- or tadpole-shaped. At the same time the motion of the plastid becomes reduced to a moving about of the ends. In the fully differentiated plastid the shape is roughly spherical and there is no movement. Often the plastids remain in the spindle- or tadpole-stage for some time.

The continuous motion of the intermediate types is essentially an amoeboid movement of the plastid (Figs. 20–23). This appears to some degree in all of the intermediate types. In its most exaggerated expression in the youngest stages, it consists of a change in form from a filament, through intermediate stages, to a sphere. Another example characteristic of the plastids before the globules have become refractive is the formation of two swollen ends connected by a thread. In some cases the thread becomes invisible, but it always reappears and shortens to reunite the two ends. In its least pronounced form in the older spindle- and tadpole-shaped forms, the movement is confined to a turning from one side to another of the tapered ends.

That the movement is not wholly connected with cyclosis, although probably aggravated by it, appears likely. In cells where there is no cyclosis, the intermediate forms customarily show a pulsating movement associated with changes in thickness. An example of this is seen in young plastids in the isodiametric cells of the rootcap.

It is worthy of note that in none of these forms has division of the plastids been seen. Many observations have been made at different times and over periods of an hour or more. But even plastids which appear to be divided are seen shortly to be connected by a thread which after a time thickens to reunite the two parts.

The liquid character of the youngest forms is shown in the movement of the globules within the plastid. This consists of a sloshing about of the globules. In older forms this movement does not appear. Any rearrangement of the globules in them is due to the amoeboid movements of the plastid.

The intermediate plastid types develop into leucoplasts in the cortex of the root or into chromatophores in the rootcap. But in other regions of the root and in the elongated cells of the fibrovascular bundles throughout the plant they persist as chondriocont types. The shadowy, very plastic forms are found in the central cells of the root. In other parts of the central cylinder more differentiated forms are found. Similar ones appear in the fibrovascular bundles throughout the plant. In the inner cortex the tadpole- and spindle-shaped forms with refractive globules often remain. The chromatophores of the rootcap often retain their chondriocont-like shape and plasticity after the formation of pigment and oil (Fig. 29b).

Associated with the persistent developmental forms are shadowy leucoplasts and mitochondrial types not ordinarily linked with plastids. In *Iris*, the latter are marked by their gradation into the plastid forms. In the same cells with the persistent chondriome-like plastids, they appear as long filaments with a single row of globules and a twisting or wavy motion in the streaming protoplasm (Figs. 26–27). Some of the filaments are shadowy, while the granules in others are refractive. The filaments with refractive granules sometimes show thickened ends which contain more than one row of granules.

It may be noted here that in addition to the proplastids and filamentous chondriosomes, the more usual types of mitochondria, that is globular or rod-shaped forms, are found in *Iris*. They appear in all cells but are concentrated about the apical meristems and in tissues of the leaf and root. They are less evident in the cells of the rhizome. Two observations are worth recording. The so-called spherical mitochondria were observed to be more or less fluctuating in form and only approximately spherical. The rod-shaped ones were seen to contain globules.

Changes in plastids after "maturity" are found in epidermal cells and in the flower. The changes in both cases are marked by an increasing fluidity of the matrix and by the disappearance of the refractive globules. In the epidermis of the root and in the flower the changes culminate in the death of the cell. But in the leaf epidermis the plastids remain as more or less shadowy chondrioconts (Fig. 37). These are quite similar in appearance and in motion to the developmental types in the root.

The changes in the plastids in the epidermal cells of the root have

been carefully studied. The more or less spherical leucoplasts or chromatophores first become somewhat elongated (Fig. 31). This is followed in older cells by increasing fluidity of the matrix and the gradual disappearance of refractive globules (Figs. 31–33). Where the plastids are pigmented some of the pigment remains after the globules are gone, but this also tends to disappear. Where these changes progress far, the plastids become shadowy nets much elongated in shape (Fig. 33). In the streaming protoplasm, they are often partially drawn out into long filaments (Fig. 32). In some cases the attenuated portions are bent back upon the rest of the plastid so as to include a small amount of protoplasm (Figs. 30, 33, 34). When an oil globule is included with protoplasm it is often in Brownian movement. The attenuated forms persist until the death of the cell.

During flowering the leucoplasts of the floral tissue were seen to undergo a series of changes similar to those described as occurring in the root. These have not been studied in detail, but the following general changes have been noted. The leucoplasts become pigmented and chondriocont-like in shape. As the flower opens and fades the chromatophores become more and more fluid. At the same time the refractive globules disappear. An advanced stage shows them partially drawn out into long filaments (Fig. 34). Unlike the chromatophores of the root the pigment is retained. These forms remain until the death of the cell. Similar changes occur in the leucoplasts of the bracts.

In *Iris Xyphium* the formation of refractive bosses on the pollen grains from oil-bearing chromatophores has been demonstrated (Fig. 17). In unripened anthers the pollen shows no markings except the small refractive dots forming a part of the wall structure. The grains are surrounded by the tapetal fluid in which are numerous oil-bearing chromatophores. In the shed pollen the grains show not only the refractive dots but the closely appressed chromatophores which appear as granular, yellowish, refractive bosses. No similar observations have been made on pollen grains of other species. Although the latter show refractive spines or a network of refractive structures, these are in every case associated with wall formation. They are in no way connected with the plastidome or chondriome.

All of the plastids described are found to show the following general characters. They tend to aggregate about the nucleus, a character also shown by mitochondria. Unless degeneration is involved they retain the ability of the proplastids to form the pigments and other products differentiating the different types of plastids. They also retain the ability to change from the plastid shape into a chondriocont form and *vice versa*.

ELAIOPLASTS IN PLANTS DESCRIBED IN THE LITERATURE

Of the plants recorded in the literature as forming elaioplasts the following have been examined: *Vanilla planifolia* Andr., *V. Pompona* Schiede., *Marchantia polymorpha* L., *Lunularia cruciata* (L.) Dum., *Pellia epiphylla* (L.) Corda, *Porella* sp., *Bazzania trilobata* (L.) S. F. Gray, *Scapania nemorosa* (L.) Dum., *Cephalozia* sp., *Trichocolea tomentella* (Ehrh.) Dum., *Plagiochila asplenioides* (L.) Dum., *Lophocolea heterophylla* (Schrad.) Dum., two thallose species of the Jungermanniales from Oregon and two leafy species of the Jungermanniales from Oregon.

The two classes of elaioplasts described by Pfeffer, Wakker and later writers were examined. These are the oil bodies characterizing the liverworts and those in *Vanilla,* a classical example of elaioplast-bearing monocotyledons. In both cases certain of the observations of previous writers have been verified and some additional notes made.

The following observations made by earlier writers for *Vanilla* have been verified. The elaioplasts are present as highly refractive granular bodies near the nucleus in cells which contain also leucoplasts and chloroplasts. Structurally they consist of globules of refractive oil in a protein or plasma matrix. They are marked by their brilliant staining in "fat" dyes and by the extrusion of large globules of oil after treatment with various reagents.

In addition it has been noted that the elaioplasts are generally distributed in all the cells of leaf, stem and root tissues rather than restricted to particular tissues in certain parts of the plants.

It has also been observed that the single large elaioplasts are aggregates of smaller granular bodies (Fig. 45). The aggregation is more or less compact. In some cells it is difficult to distinguish the individual bodies, while in other cells they are but loosely grouped or freely circulating in the streaming cytoplasm (Fig. 46). In some cells the smaller bodies could be observed to aggregate into one or more groups from which individuals were carried away from time to time by the streaming protoplasm.

The development of the smaller bodies from non-refractive granular ones can be observed in younger cells of leaf and root. In successively older cells the included globules gradually become more and more refractive until the bodies assume the highly refractive condition typical of mature cells. In the less refractive stages the bodies seldom form compact aggregations. No specific stage has been noted in which aggregation becomes the rule. The formation of compact groups appears possible at any time, although more characteristic of mature tissue.

The rotary movement of elaioplasts described by Zimmermann and others as characteristic of these bodies has been shown to be an injury phenomenon. It is observed in cells which soon show unmistakable signs of injury followed by death. It is not seen in any cells which remain normal in appearance and actively streaming for a period of hours. The movement consists of rotation within a liquid vacuole. It is followed by Brownian movement of cytoplasmic inclusions and a general coagulation or disintegration of the cellular structure, that is by unmistakable signs of death.

In the liverworts the following observations of earlier writers have been verified. Bodies included within the cytoplasm and marked by their refractivity, by their staining in "fat" dyes and by their solubility in alcohol appear generally throughout the group. They are located within the ring of chloroplasts, but, unlike those in *Vanilla,* show no particular affinity for the nucleus. They all characteristically leave a residual ring in solution with alcohol, etc. They vary in color from colorless to dark brown. Two or three classes are distinguishable. The first appears as a single large granular mass almost filling the cell lumen (Fig. 38). It is located in scattered cells throughout the plant body and is characteristic of the Marchantiales. The second and third types are found in the Jungermanniales which they characterize. They are smaller than those in the Marchantiales and are round, spindle- or disc-shaped in form (Figs. 40-44). They grade from a homogeneous type to a very granular one. Commonly there are from one to twenty in a single cell, located more or less characteristically in the peripheral cytoplasm. In this group they are not restricted to particular cells but are found in every cell. Unlike the bodies in *Vanilla* there is little or no tendency for them to aggregate.

The development of the bodies has been observed in the Jungermanniales (Fig. 42). In the younger cells the oil-bodies appear as shadowy, wrinkled, granular bodies. They develop into the mature bodies of older cells by an increase in substance and in the refractivity of the granules. By the time the cells are fully mature, the bodies have become plump and refractive. There is no indication of a vacuolar origin postulated by some writers.

In addition the following new observations were made. The homogeneous type found in the Jungermanniales are sometimes seen with attached granular bodies (Fig. 44). These appear in the younger cells.

The single bodies in the Marchantiales can be shown to be aggregations of smaller ones. This is apparent in younger cells where they are less refractive and less highly colored (Fig. 39). In older cells the

structure is obscured by the dark color. Likewise in older cells the bodies appear to be more closely compacted.

The Brownian movement described by some as characteristic of the bodies in certain species has been shown to be associated with older bodies or with injury. It is never seen in younger tissue, even in cells with mature oil bodies. It appears in some of the older cells of the Marchantiaceae and can be induced in any cell by injury.

DISCUSSION

It has been shown in the preceding observations that the oil bodies in *Iris* are a phase of ordinary plastids. In studying the development and variations of these plastids, many interesting observations have been made which have a bearing upon the status of elaioplasts and upon various problems concerning plastids. In particular the observations provide further evidence of the plastid character of elaioplasts and of a relationship between the various types of oil bodies described in the literature. They also clarify our conception of the interrelationships of plastids and chondriosomes.

1. Significance of Present Study in the Interpretation of Elaioplasts

To identify the anomalous bodies in *Iris* as a seasonal elaioplast phase of plastids adds another instance to the accumulating evidence of the plastid character of oil bodies. This substantiates the theories of Wakker (47), of Beer (4), of Hieronymus (22) and of Kozlowsky (28) who postulate a relationship with plastids rather than with vacuoles or with the nucleus. There is no evidence in any of the observations described in this paper of a vacuolar origin or identity. On the contrary, the structural, developmental and chemical similarities between vacuoles and oil bodies recorded by some authors were not observed in any of the material examined. Nor was there any evidence of a nuclear derivation of the elaioplasts, a theory based upon the similarity in the staining properties of the nucleolus and elaioplasts and in the aggregation of the elaioplasts about the nucleus. Both of these phenomena have been found to be characteristic of plastids in general. The possibility remains that some elaioplasts may be more or less fused aggregations of oil globules which bear no relationship to plastids. The phenomenon was not observed, but the possibility of its occurrence was not disproved.

It is probable that the granular elaioplasts of the monocotyledons and liverworts are types of plastids. They show the same structure as

that of the plastids, that is a matrix with embedded globules. That the stroma in the liverworts is non-fixable is not significant morphologically, although it indicates a chemical difference between the oil bodies in the liverworts and plastids in general. Further evidence of the plastid character of the granular oil bodies in the monocotyledons is found in their similarity in appearance and in general characters to those found in *Iris*. A comparison between the elaioplasts in *Vanilla*, as a classic example of the type found in monocotyledons, and those in *Iris* shows the following characters common to both: presence of refractive granules, brilliant staining in "fat" dyes, extrusion of oil with picric acid, etc., aggregation about the nucleus, yellowish color, plastid structure and the absence of the more usual plastid products such as starch and pigment.

That the homogeneous oil bodies in the liverworts may be classed like the granular types as plastids is suggested. Heretofore no distinction has been made between the two types because of the intergradation occurring between the two extremes. The appearance of attached granular portions in the younger stages of the homogeneous forms substantiates the view that they should be classed with the granular types which, as has been suggested, are plastids.

That elaioplasts are sometimes a phase of functional plastids as well as degenerate forms has been brought out in these studies. Heretofore they have been considered to be degenerate forms or secretions of plastids. In *Iris* they are found as functional plastids, as evidenced in the formation of starch and their apparently continuous presence in individual cells from season to season. That elaioplasts sometimes form by degeneration of plastids involving the production of oil has been shown by Beer (4). There is no evidence that they are ever secretions from plastids.

It is probable that the granular elaioplasts described in the literature are sometimes functional plastids and sometimes degenerate forms. Those found in such organs as leaves, roots and bulbs or those found widely distributed throughout the plant as is the case in *Vanilla* are doubtless active plastids, while those restricted to the more or less evanescent floral tissues are probably degenerate plastids.

The interpretation of the homogeneous oil bodies in the liverworts is not clear. They might be degeneration products, but they might also be an accumulation of normal plastid products within a plastid.

It has also been shown in the studies of *Iris macrosiphon* that elaioplasts of the type described by Lidforss (32) as homogeneous oily spheres may form by the degeneration of oil-bearing plastids. A similar

phenomenon has been described by Beer (4) as a final step in the degeneration of plastids in floral tissue of *Gaillardia*. That the spheres described by Lidforss (32) are likewise degeneration products of plastids can only be surmised. It is possible that they are more or less fused aggregations of oil globules unconnected with plastids.

Evidence of a relationship between the various types of elaioplasts described in the literature has been found in these studies. A structural similarity is seen between the oil bodies in the Marchantiaceae and those in *Vanilla* in that they are both aggregations of plastid-like bodies. I have found no record in the literature of the aggregation of these bodies in the Marchantiaceae, although the phenomenon was noted for elaioplasts in the monocotyledons as early as 1914. In addition to this direct evidence of structural similarity the observations upon the development and variations of elaioplasts in *Iris* have demonstrated that these oily plastids show, at one time or another, the widely varying phenomena which have heretofore been considered distinctive of different types of oil bodies. It has already been pointed out that elaioplasts of the homogeneous type described by Lidforss (32) sometimes result as a degeneration product of a granular type of elaioplast. It has also been found in *Iris* that the oily plastids show at one time or another the following phenomena described in the literature for oil bodies: aggregation and fusion of homogeneous oil globules, aggregation and compacting of plastid-like bodies, aggregation about the nucleus, unrestricted position in the cell, degeneration involving the disappearance of the oil and degeneration involving the formation of oily spheres. In brief the morphological distinctions between the various classes of oil bodies appear to be breaking down, while it is evident that plastids can show widely varying phenomena which, considered separately, might be interpreted as bases for the distinction of fundamentally different types. Further study on this subject is highly desirable. In particular further observations on oil bodies and plastids in *Vanilla, Ornithogalum* and the hepatics are needed, for much of the literature deals with elaioplasts found in them.

An additional point which tends to reduce the number of recorded distinctions between the oil bodies in the liverworts and those in the monocotyledons appears in the permanent character of the elaioplasts in the rhizomes of *Iris*. Heretofore elaioplasts in monocotyledons have been described as temporary structures, while those in the hepatics have been thought to be more permanent. It may be noted here that my own limited studies made on *Vanilla* indicate that elaioplasts are not the temporary structures even in this classical plant that one would infer from the literature.

That conditions producing oil bodies are more or less restricted in their occurrence in the monocotyledons has again been brought out in these studies. Elaioplasts do not appear generally throughout the group, although the appearance of oil-bearing chloroplasts is not uncommon. This study adds another genus and many species to the published lists of monocotyledons in which elaioplasts occur. Although oil-bearing plastids occur in the rhizomes of practically all species, it is noteworthy that the elaioplast condition is restricted for the most part to the Apogon irises of Asia and America. This is the first record that I find of the occurrence of oil bodies in rhizomes, although Politis (39) has described them in bulbs.

Evidence of the function and significance of the oil bodies has been found. In *Iris* the bodies are clearly assimilative organs as shown by their formation of starch. That the oil itself is a reserve food supply is indicated. In certain species it replaces at least morphologically the starch stored in the rhizomes of other species. There is no evidence that the elaioplasts are ever excretions, although they may be at times degeneration products.

There is no evidence of the division of elaioplasts recorded by a few writers. The fragmentation described by Raciborski (41) and Politis (39) is but the separating of the aggregated plastid-like bodies. This can be seen in *Vanilla*. That there is ever a passive division of an aggregated mass of oil bodies by the cell wall is improbable. Neither such aggregations nor a great development of oil was found in the meristems of *Iris, Vanilla* and the hepatics.

2. Significance of Present Study in Interpretation of Plastids and Chondriosomes

With the recognition of elaioplasts as plastids, a study of their variations became a study of the variations in plastids and chondriosomes. No new phenomena have been noted, but significant interpretations of those already recorded in the literature[1] have been made.

Most striking of the phenomena observed was the development of large quantities of oil globules in plastids. The formation of oil globules in plastids has been known for a long time and has recently been emphasized by Guilliermond's (15–20) studies of *Iris*. But even Guilliermond's extensive investigations have not shown an accumulation of oil in plastids comparable to that found in *Iris versicolor* where the quantity is

[1]A summary of the present status of plastids and chondriosomes may be found in books and papers by Schürhoff (44), Sharp (45), Guilliermond et al. (20) and Mottier (37).

so great as to obscure the structure of the plastids and render them unrecognizable for months at a time.

The association of oil globules with young or degenerating forms more frequently than with mature plastids has been suggested by Guilliermond et al. (20). But such is not the case in *Iris* where the largest formation of oil is in the functioning plastids of the rhizomes.

A second phenomenon noted was the plastic quality of chondriosomes and of transitional types of plastids. As evidenced in amoeboid movements this has often been recorded in the literature, while it has been emphasized in the recent studies by Guilliermond and his associates. But I have found in the literature no reference to the extreme plasticity amounting to fluidity such as occurs in some young leucoplasts where the included globules are moved about at random within the plastid.

The significance of the chondrioconts has been brought out clearly in the survey of the variations of plastids and chondriosomes made in this study. The chondrioconts are essentially plastids producing at times all of the visible products of plastids such as starch, chlorophyll or a yellow pigment. They share, too, the plastic qualities of plastids which they display to a much greater degree. They occur in restricted tissues as transitional stages in the formation of plastids from mitochondria-like primordia or as more or less degenerating forms of plastids. Often in the rootcap and in floral tissue they are pigmented, although the formation of pigment is not confined to them. It should be noted that in some tissues chondrioconts persist without assuming the more usual plastid form.

Chondrioconts should not be interpreted as invariably forming a stage in the development of chloroplasts [Guilliermond et al. (20)]. On the contrary my studies show that the majority of chloroplasts and other plastids develop from mitochondria-like primordia without the intervention of a chondriocont stage. Where chondrioconts do form a stage in the development of plastids, the whole chondriocont develops into a plastid. There is no budding or fragmenting of the chondriocont involved. The appearances that have been interpreted as budding in chondrioconts or as evidence of fragmentation are but temporary shapes of the plastic chondrioconts.

It may be noted here that the studies of chondrioconts emphasize Kassmann's (25) observations that plastids do not divide under normal conditions. This is a much debated point in the literature upon plastids.

There was no evidence of vacuole formation in degenerating plastids or chromatophores such as have been described in flowers [Guilliermond et al. (20)]. The appearance which has been interpreted as a vacuole is

rather the inclusion of a small amount of protoplasm as a result of the amoeboid movements of the plastid at this time.

It is worth emphasizing here that the complete degeneration of the plastids may occur without involving the death of the cell. It has already been noted by Beer (4) that such a phenomenon occurs in some floral organs where the life of the mature cells is comparatively brief. I have found no record, however, of the degeneration of the plastids in cells which remain alive for months thereafter, a phenomenon found in the rhizomes of *Iris macrosiphon*.

In general, it may be stated that there is no sharp line of demarcation between elaioplasts, plastids, chondrioconts and mitochondria. In *Iris* they have all been observed to form starch and, with the exception of mitochondria, chlorophyll, oil and a yellow pigment. In some instances several of these products may appear at once, or they may develop in succession, or none of them may form. Nor should any of the chondrioconts and plastids be considered end products of a developmental series originating from mitochondria-like bodies, for until irreversible changes occur such as a resolution into structureless spheres of oil, the shapes assumed are reversible. In other words there is no clear distinction between amyloplasts, leucoplasts, chloroplasts, chromoplasts and elaioplasts; nor is it possible to consider plastids, chondrioconts, proplastids and mitochondria as unrelated cell structures. Rather it appears that these are all forms of the same fundamental cell organ differing only in size and in the chemical products being formed at the time.

3. Significance of the Study of Oil-bearing Plastids in Iris from a Taxonomic Viewpoint

The occurrence of two types of plastids in rhizomes of *Iris* each more or less restricted to certain groups of species appears to be of taxonomic significance. The consistent appearance of the same type in well defined species indicates a character that may be useful in separating species. In addition it should be noted that the substitution of compound starch grains for the elaioplasts in rhizomes of a known hybrid and in one or two questionable species, although not an invariable phenomenon, suggests a possible means of identifying some plants as of hybrid origin.

CONCLUSIONS

1. The anomalous bodies in the rhizomes of *Iris versicolor* are an elaioplast phase of leucoplasts persisting throughout the resting season, but forming starch throughout the actively growing period.

2. Some, if not all, of the so-called "elaioplasts" are plastids in some form or other.

3. Elaioplasts of the plastid type are not necessarily degeneration types: in *Iris* they are functional plastids.

4. The rotary movement of elaioplasts described in the literature is an artefact due to slow death or injury; the Brownian movement described as characteristic of globules in certain liverworts is a degeneration or injury phenomenon.

5. The elaioplasts in *Lunularia* and *Vanilla* are morphologically similar in that they are aggregations of small plastid-like bodies that form oil. This establishes another link between elaioplasts in the liverworts and those in the monocotyledons.

6. There is no sharp line of demarcation between the different kinds of plastids and chondriosomes each of which is a more or less temporary form capable of changing to the other types.

7. At all times the plastids are more or less plastic but particularly so in young tissues, fibrovascular tissue or slowly dying cells.

8. Leucoplasts, chloroplasts and chromatophores do not go through a set series of changes in developing from plastid types characteristic of meristematic tissues. They may pass through various series depending upon the type of mature tissue involved, or they may merely increase in size with probable changes in their physico-chemical structure. They never form by budding of chondrioconts succeeded by separation of the buds so-formed.

9. Chondrioconts may form an intermediate developmental stage in the formation of "mature" plastids, although not necessarily; they may persist in some tissue; or they may be an intermediate stage in the degeneration of plastids.

10. Plastids and chondriosomes in *Iris* all show the structure of a matrix with embedded globules. Pigments are more soluble in the globules than in the matrix, although they are found in both.

11. Two types of degeneration of plastids occur involving (a) an increasing fluidity and a decreasing refractivity or (b) a complete breaking down into large homogeneous spheres of oil. Degeneration of the plastids does not necessarily involve the death of the cell.

12. The formation of a vacuole with at times an included oil drop in degenerating chondrioconts is in reality an inclusion of protoplasm.

13. Different species of *Iris* are characterized by distinct types of elaio-leucoplasts in their rhizomes. The distribution of types follows closely the taxonomic groupings and may be of significance in separating species.

14. The occurrence of such elaioplasts as those in rhizomes of *Iris versicolor* is confined, so far as could be ascertained, to rhizomes of this genus. For the most part they are restricted to rhizomes of certain species, chiefly Apogons of Asia and America.

15. Refractive bosses on pollen grains of *Iris Xyphium* are oil-bearing chromatophores adhering from the tapetal fluid. Other markings found on pollen grains were part of the wall structure.

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DESCRIPTION OF PLATES

Figs. 1–4, 6, 8–10, 38, 39, 45, 46 were made with a camera lucida; the magnifications given for these figures are exact. The other figures were drawn free-hand; the magnifications given for them are approximate.

PLATE 132

Fig. 1. *Iris versicolor* L. Living cells of the rhizome from material collected in December. × 475.
Fig. 2. Individual elaioplasts from cells shown in Fig. 1. × 1600.
Fig. 3. Individual elaioplasts from cells shown in Fig. 1 after treatment with (a) ammonium Erliki fixative, and erythrosin and cyanin; and (b) 0.5% osmic acid. × 1600.

Fig. 4. *Iris versicolor* L. Individual elaioplasts from rhizomes collected in October: (a) surface view; (b) included starch grains; (c) diagram to illustrate position, size, number and shape of starch grains. × 1600.
Fig. 5. *Iris versicolor* L. Individual elaioplasts treated with Gram's solution: (a) material from Duxbury, Mass.; (b) material from Lincoln, Mass. These were drawn at the same magnification.
Fig. 6. Isolated starch grain from elaioplast shown in Fig. 4. × 1600.
Fig. 7. *Iris tectorum* Maxim. Elaioplasts from young cells of rhizomes collected in March: (a) normal plant; (b) dying plant. × 845.

PLATE 133

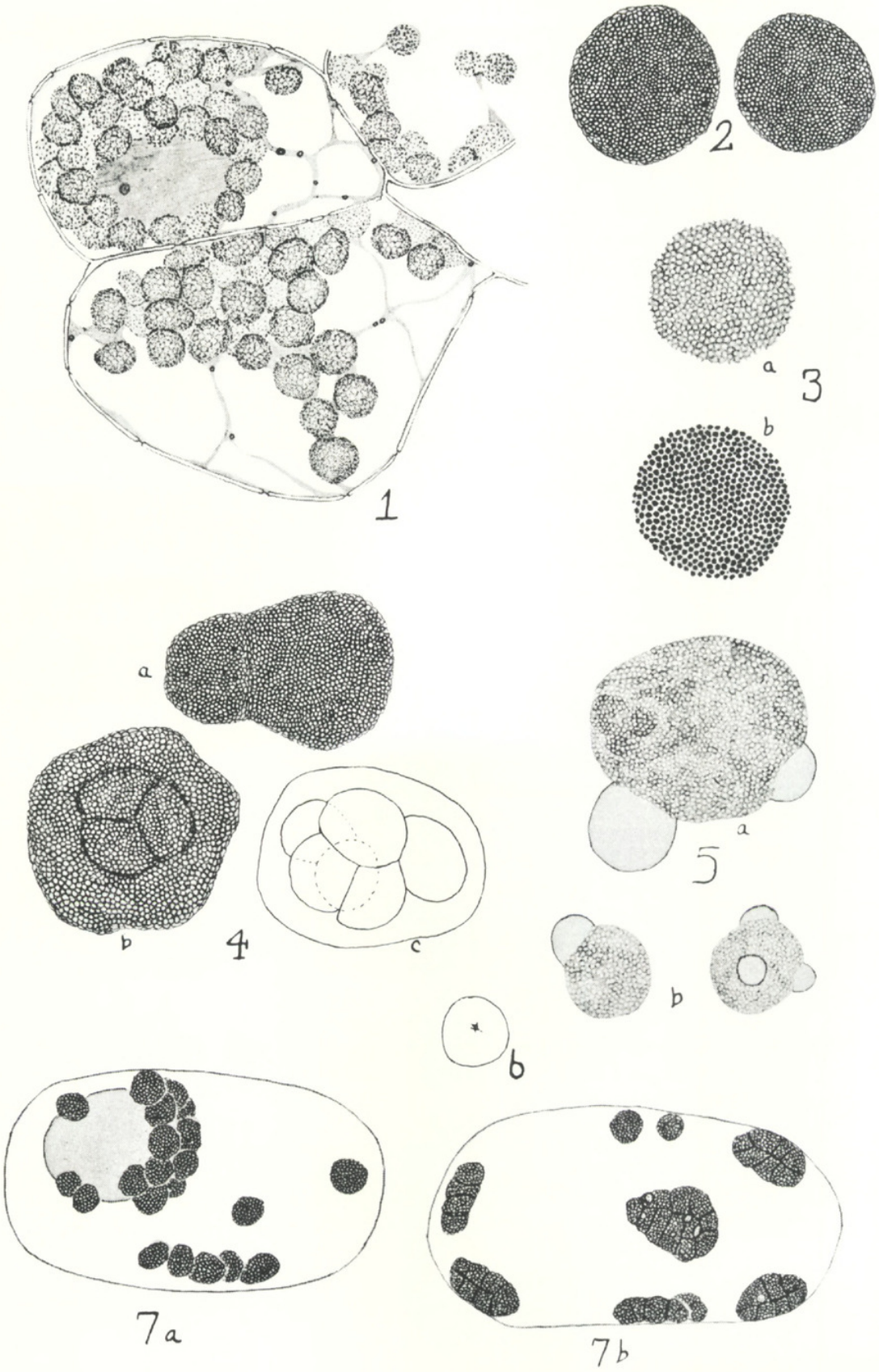
Fig. 8. *Iris pallida* Lam. × ? Living cell of the rhizome from material collected in December. × 475.
Fig. 9. Individual oil-bearing plastids from cell shown in Fig. 8 showing plastids: (a) without starch; (b) with starch; (c) is a diagram showing the relative positions of plastid and starch. × 1600.
Fig. 10. Individual starch grains from plastids similar to those shown in Fig. 9. × 1600.
Fig. 11. *Iris pumila* L. Oil-bearing plastids from living cells of a rhizome collected at St. Louis in March: (a), (b) and (c) are plastids from successively older cells. × 1270.
Fig. 12. Oil-bearing plastids from living cells of rhizomes of the following California species of *Iris*: (a) *I. missouriensis* Nutt.; (b) *I. Douglasiana* Herb.; (c) *I. longipetala* Herb.; (d) *I. Hartwegii* Baker. Material collected in California in August. × 1270.
Fig. 13. Diagram showing the types of oil-bearing plastids found in *Iris* species in March. See table p. 246 for names.

PLATE 134

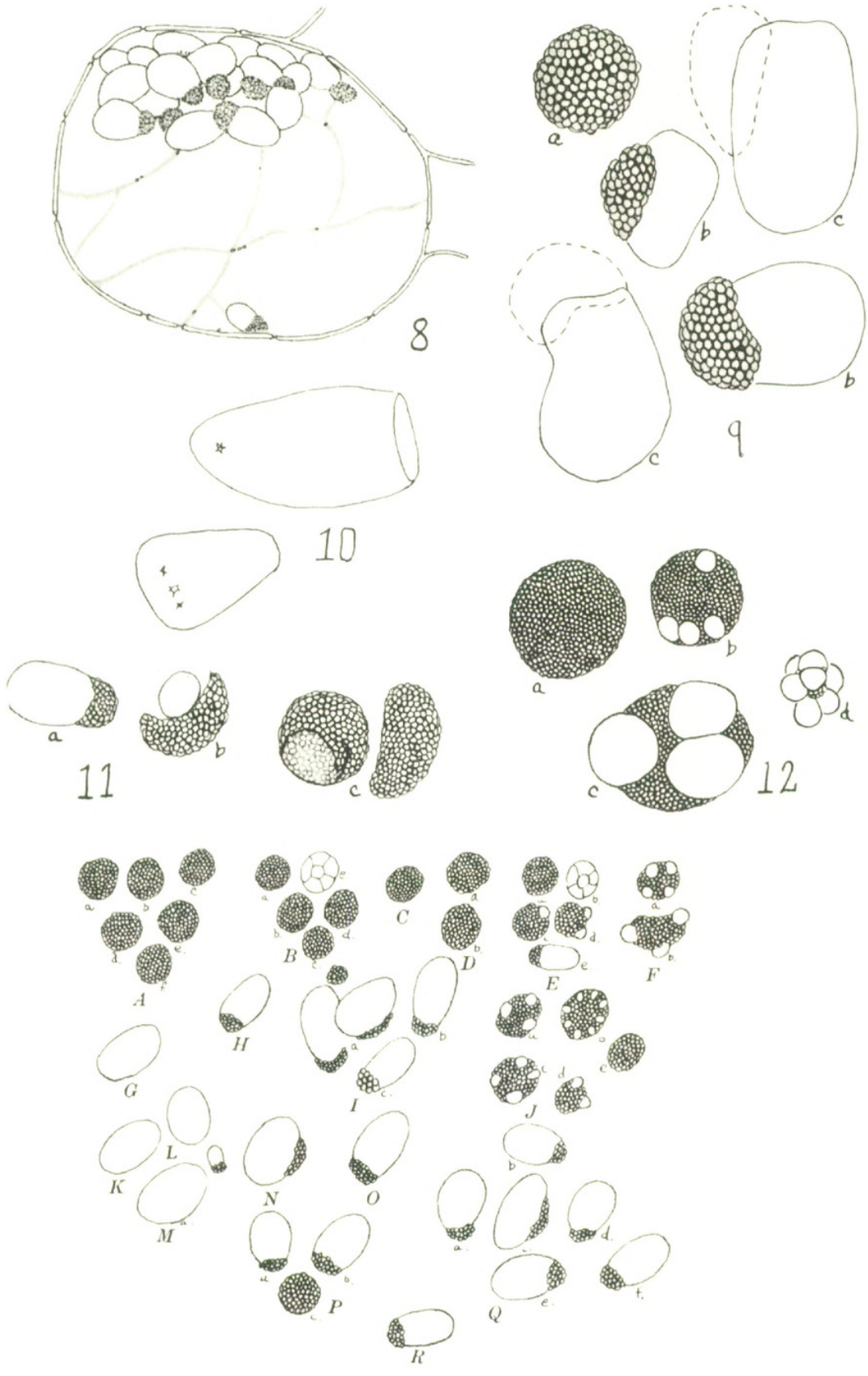
Fig. 14. *Iris macrosiphon* Torr. Elaioplasts in living cells from the cortex of a rhizome collected in July: (a), (b) and (c) are taken from successively older cells. × 1245.
Fig. 15. Elaioplasts shown in (a) Fig. 14b and (b) Fig. 14c treated with Gram's solution to show the surrounding cytoplasm. × 1245.
Fig. 16. *Iris Xyphium* L. Optical section of living pollen grain. × 500.
Fig. 17. *Iris Xyphium* L. Untreated pollen grains in surface view: (a) in tapetal fluid of immature anther; (b) from a ripened anther. × 475.

PLATE 135

Fig. 18. *Iris macrosiphon* Torr. Oil-bearing plastids in living epidermal cells of root-tip: (a), (b), (c) and (d) are from successively older cells. × 1280.
Fig. 19. *Iris versicolor* L. Elaioplasts from living cells of the meristem of a rhizome: (a) from one of the youngest cells; (b) and (c) from successively older cells. × 1620.
Fig. 20. *Iris pallida* Lam. × ? Plastids from living, elongated, differentiating cells of root-tip: (a) successive observations on a single plastid to show fluctuating variations in form; (b), (c), (d) and (e) similar observations on four additional plastids. × 1620.
Fig. 21. Plastid similar to those in Fig. 20 but from an older cell. × 1620.
Fig. 22. Plastid similar to that shown in Fig. 21. × 1620.
Fig. 23. Plastid similar to that shown in Fig. 21. × 1620.
Fig. 23a. Plastid similar to that shown in Fig. 21. × 1620.



ELAIOPLASTS IN IRIS



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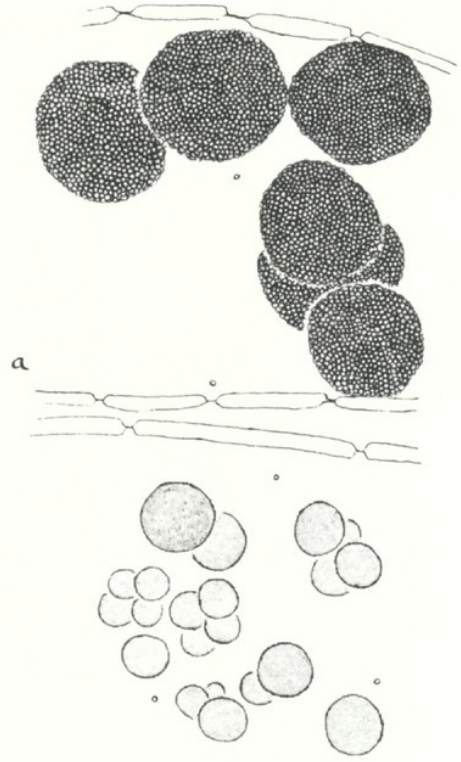

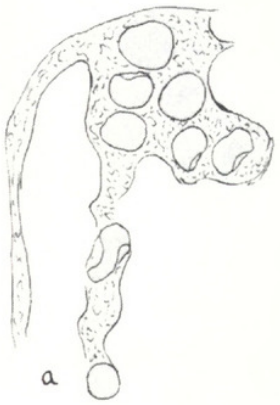

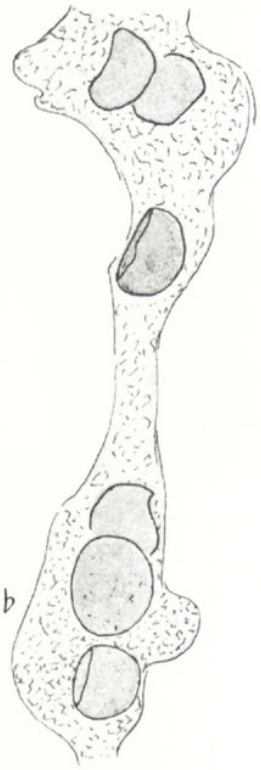

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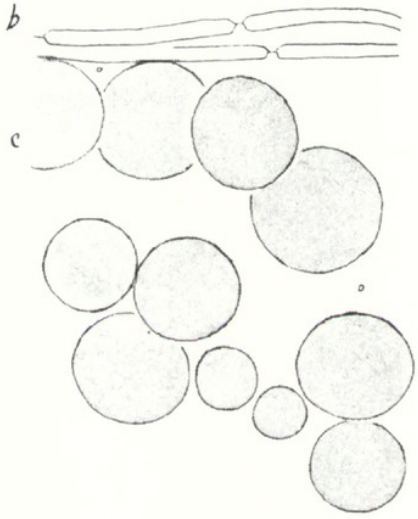

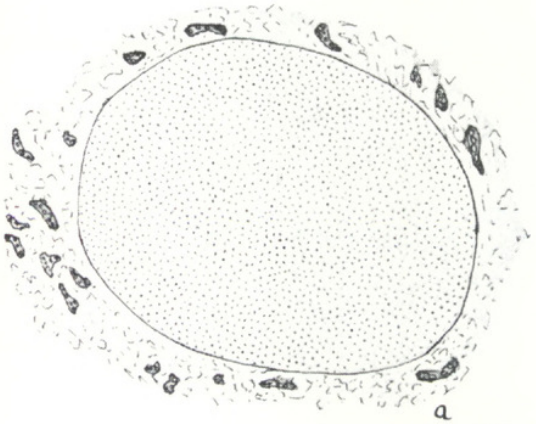

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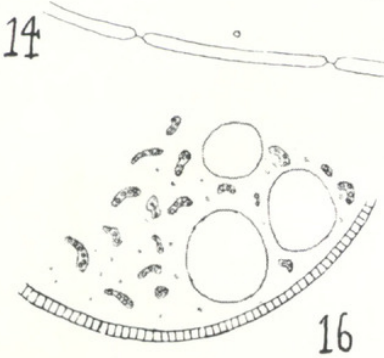

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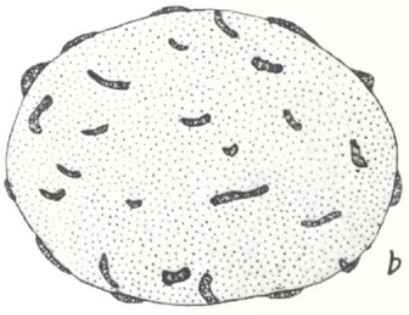
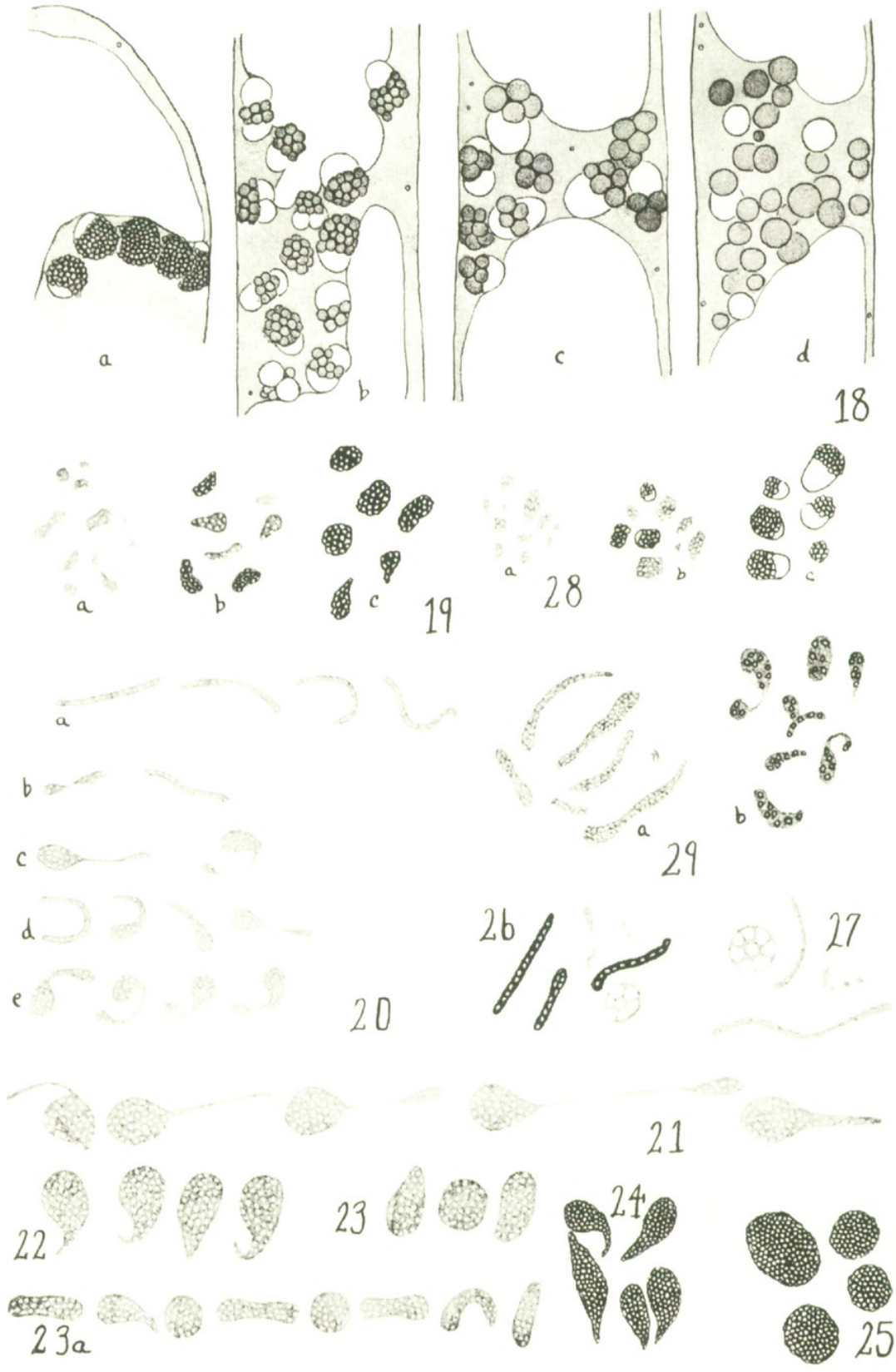
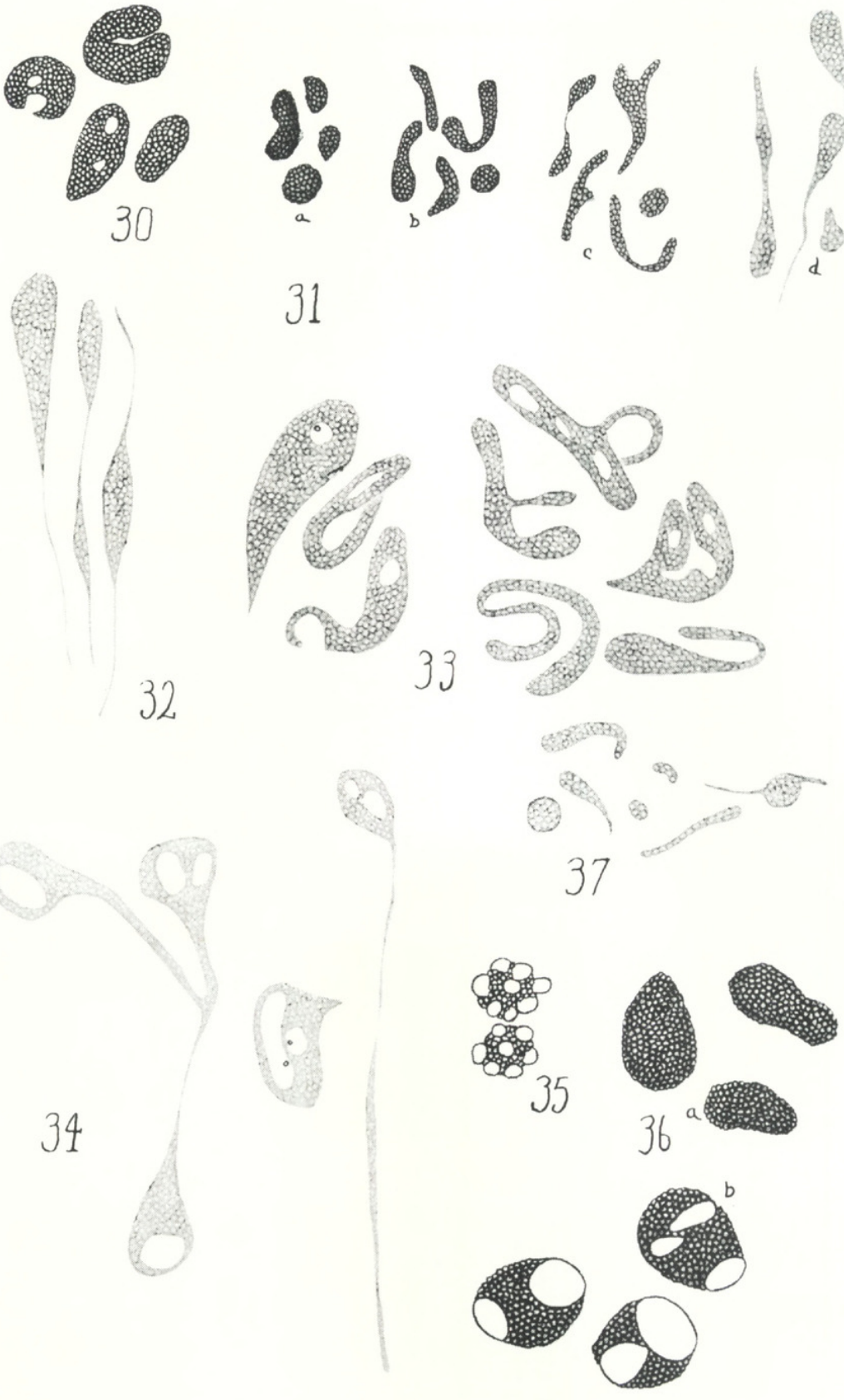


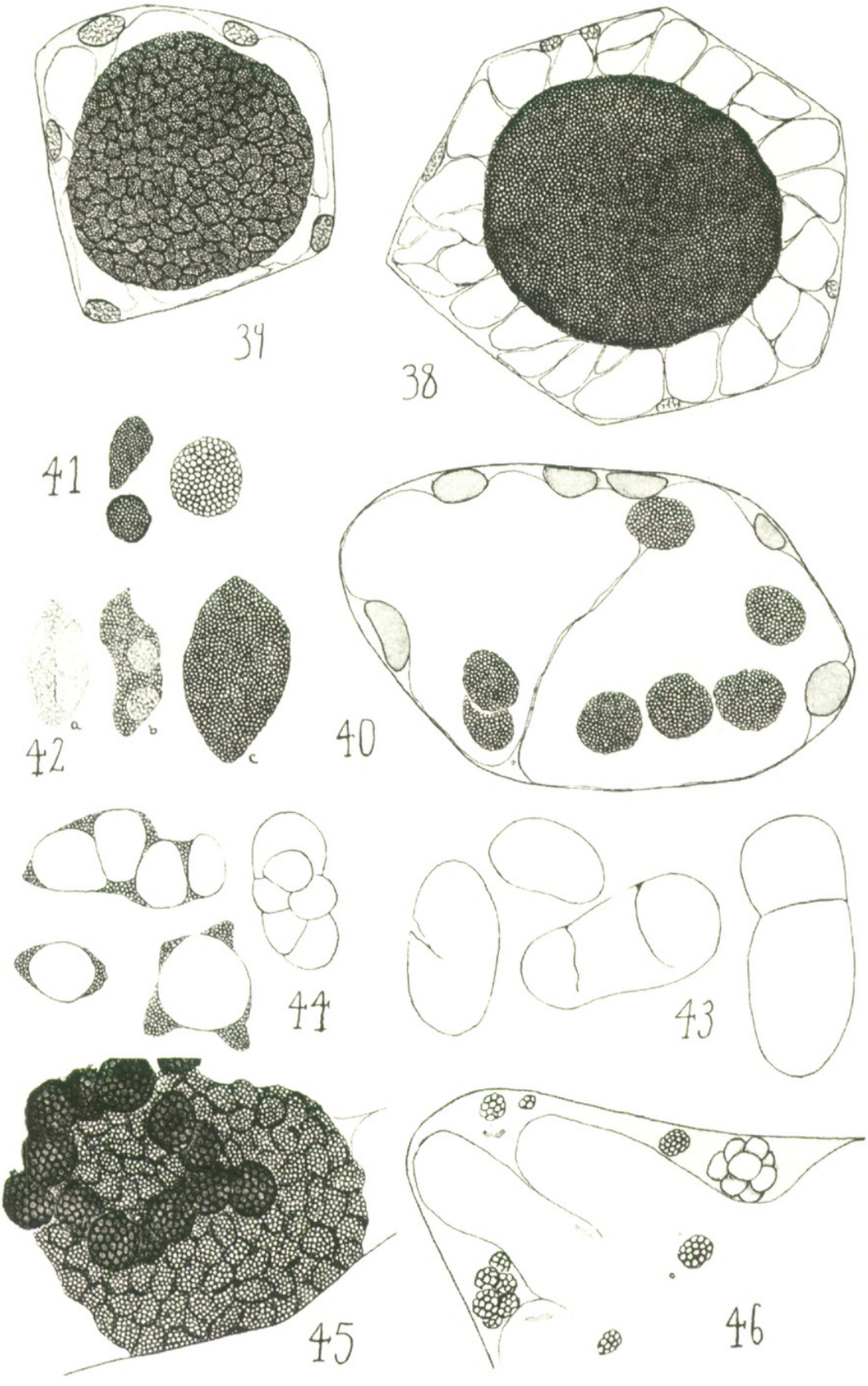
ELAIOPLASTS IN IRIS



Elaioplasts in Iris



ELAIOPLASTS IN IRIS



ELAIOPLASTS IN IRIS

Fig. 24. *Iris pallida* Lam. × ? Elaioplasts from living cells of cortex of root-tip. × 1620.
Fig. 25. *Iris pallida* Lam. × ? Elaioplasts from living cells of cortex of older root. × 1620.
Fig. 26. *Iris pallida* Lam. × ? Plastids and chondriosomes from a single living cell of the central cylinder of a root-tip. × 1620.
Fig. 27. Plastids and chondriosomes from another cell of the central cylinder. × 1620.
Fig. 28. *Iris pallida* Lam. × ? Oil-bearing plastids from living cells of the meristem of a rhizome: (a), (b) and (c) are from successively older cells and show the appearance of starch. × 1620.
Fig. 29. *Iris pallida* Lam. × ? Chromoplasts from living cells of the root-cap: (a) and (b) are from successively older cells. × 1620.

PLATE 136

Fig. 30. *Iris pallida* Lam. × ? Elaioplasts from living cells of cortex of root-tip. × 1650.
Fig. 31. *Iris pallida* Lam. × ? Chromatophores from living cells of the epidermis of the root-tip: (a), (b), (c) and (d) from successively older cells. × 1650.
Fig. 32. *Iris versicolor* L. Plastids from living cells of the epidermis of a root-tip. × 1650.
Fig. 33. *Iris pallida* Lam. × ? Chromatophores from living cells of the epidermis of a root-tip. × 1650.
Fig. 34. Chromatophores from living cells of the epidermis of a flower of a Pogoniris, probably of *I. variegata* L. × 1650.
Fig. 35. *Iris versicolor* L. Oil-bearing plastids from living cell of cortex of root. × 1650.
Fig. 36. *Iris versicolor* L. Chloroplasts from living parenchyma cells of a leaf: (a) without starch; (b) with starch. × 1650.
Fig. 37. *Iris versicolor* L. Plastids from living cells of the epidermis of a leaf. × 1650.

PLATE 137

Fig. 38. *Lunularia cruciata* (L.) Dum. Living elaioplast-bearing cell from a mature thallus. × 1080.
Fig. 39. *Lunularia cruciata* (L.) Dum. Living elaioplast-bearing cell from the younger tissue of a mature thallus. × 1080.
Fig. 40. One of the Jungermanniales. Living cell from a mature leaf. × 1250.
Fig. 41. Oil bodies from living cells of leaves of three different species of the Jungermanniales. × 1250.
Fig. 42. One of the Jungermanniales. Oil bodies from living differentiating cells of stem: (a), (b) and (c) from successively older cells. × 1250.
Fig. 43. One of the Jungermanniales. Oil bodies from living cells of mature plant. × 1250.
Fig. 44. One of the Jungermanniales. Oil bodies from living cells of younger tissue. × 1250.
Fig. 45. *Vanilla Pompona* Schiede. Elaioplast and chloroplasts from living cell of a leaf. × 915.
Fig. 46. *Vanilla Pompona* Schiede. Elaioplasts, chloroplast and chondriosomes in living cell of cortex of root-tip. × 1720.

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