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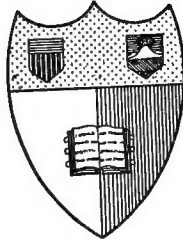
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On the elaioplasts in mono- and dicotyle



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Three papers by IOANNES POLITIS, Honorary Assistant
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University of Pavia.

Translations by U. S. Dept. Workers

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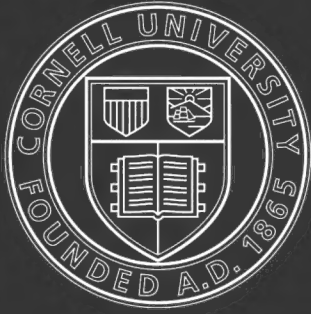
The titles of the papers are as follows:

On the Elaeoplasts in Mono- and Dicotyledonous Plants.
Translation of: sugli elaioplasti nelle mono- e
dicotiledoni. Atti Ist. Bot. Univ. Pavia, 11,
14:335-361. pl. 13-15. 1914;

On Special Cellular Bodies Which Form Anthocyanine.
Translation of: Sopra speciali corpi cellulari
che formano antocianine. Atti Ist. Bot. Univ.
Pavia, 11, 14:363-376. pl. 16-18. 1914;

On a Special Cellular Body Found in Two Orchids.
Translation of: Sopra uno speciale corpo cellulare
trovato in due orchidee. Translation Atti
Ist. Bot. Univ. Pavia, 11, 14:377-385. pl. 19.
1914.





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Politis, Ioannes, Honorary Assistant in the Botanical Institute of the Royal University of Pavia.

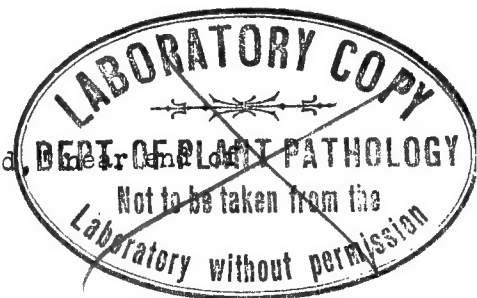
ON THE ELAIOPLASTS IN MONO- AND DICOTYLEDONOUS PLANTS. Translation of: Sugli elaioplasti nelle mono- e dicotiledoni. Atti Ist. Bot. Univ. Pavia, II, 14: 335-361. pl. xiii-xv. 1914.

In 1888 Wakker (6)^a noted for the first time within the cytoplasm of the epidermal cells of young leaves of Vanilla planifolia and of V. aromatica latifolia some special bodies very strongly refractile to light, to which he gave the name elaioplasts (formed from olio), because they consisted of a fundamental plasmic substance in which were found included a fatty or oily substance. About five years later Zimmermann (9), having found in the perianth of Funkia coerulea similar bodies, took up the study of the question and examined many species belonging to a large number of families among the Monocotyledoneae. Studying the elaioplasts from a morphologic point of view and that of their diffusion, the result of this research was that he found the elaioplasts in five other genera, of which three belonged to the Liliaceae, one to the Amaryllidaceae, and one to the Orchidaceae.

The first, however, to study the development of the bodies in question was Raciborski in 1893 in the genera Ornithogalum, Albuca, Funkia, and Gagea.

Raciborski (5) noted that in these the elaioplasts appeared as little spheres strongly refractile to light, always close to the cell nucleus; that they did not participate in nuclear division and that they multiplied by neoplasia from the cytoplasm. In Ornithogalum stachyoides the elaioplasts would multiply, according to the author, by gemmation.

^a Reference is made by number to "Literature cited, Herb. Acad. Sci. Friburg translation.



A little later Zimmermann (10) established the presence of elaioplasts in the stele and in the subepidermal assimilatory tissue of *Psilotum*, in the internal epidermis of the lobes of the perianth of *Maxillaria picta*, in the epidermis and in the parenchyma of the perianth and in the floral axis of *Beschorneria bracteata*.

Finally, Kuester, comparing the elaioplasts to the oily bodies of mosses, found that the former differed from the latter by their behavior in their stroma and in the oily substance, but were similar to them in structure.

With the work of Kuester (3) concludes the series of publications which, so far as I know, have been written on elaioplasts, which, as one sees, were encountered in only a few genera of monocotyledons, namely, five of the Liliaceae, two of the Amaryllidaceae, and three of the Orchidaceae.

In the dicotyledonous plants nobody has found elaioplasts. ^a

Last year I encountered in the epidermal cells of scales of functionally active bulbs of *Hippeastrum aulicum* some spherical bodies strongly refractile to light, joined together in the form of bunches of grapes, which in their physical properties and in their microchemical reactions were identical to those which Wakker, Zimmermann, and Raciborski described under the name of elaioplasts.

This fact led me to the following researches the results of which are summed up in this contribution.

a The cellular organs observed by R. Beer (On elaioplast - Ann. Bot. XXIII, 63-1909) in various parts of the flower of a *Gaillardia* can not, in my opinion, be ascribed to elaioplasts, but to chromatophores, since in the elaioplasts neither chlorophyll nor other pigments are formed.



I have studied many species belonging to several different families of the vegetable kingdom and have established the presence of elaioplasts in 24 new genera belonging to the Monocotyledoneae, such as the Liliaceae, Amaryllidaceae, and Orchidaceae, and also to the Iridaceae, and later also in certain dicotyledonous plants, namely, in all species which I have studied belonging to the family Malvaceae.

I have found elaioplasts in the following species:

Liliaceae: Aloë rhodacantha, Dc., Drimia undulata Jacq., Yucca filamentosa Linn., Ruscus racemosus Linn.

Amaryllidaceae: Clivia nobilis Lindl., Eucharis subdentata Benth., Himantophyllum cyrtanthiflorum Groenl., Himantophyllum miniatum Groenl., Hippeastrum aulicum Herb., Hippeastrum vittatum Herb., Hippeastrum reticulatum Herb., Haemanthus albiflos Jacq., Haemanthus coccineus Linn., Sternbergia Fischeriana Rupr., Sternbergia lutea Orph., Sternbergia macrantha J., Polianthes tuberosa Linn.

Iridaceae: Watsonia humilis Mill.

Orchidaceae: Brassia brachiata Lindl., Cattleya Harrisoniae Paxt., Laelia anceps Lindl., Lycaste aromatica Lindl., Lycaste Skinneri Lindl., Miltonia Clowesii Lindl., Tetramicra bicolor Rolfe, Cymbidium aloifolium Sw., Cymbidium Lowianum Reichb., Oncidium sphacelatum Lindl.

Malvaceae: Hibiscus Rosa-sinensis Linn., Hibiscus herbaceus Vell., Hibiscus liliiflorus Cav., Hibiscus tricolor Dehnh., Hibiscus syriacus Linn., Althaea rosea Cav., Malva rotundifolia Cav., Malva sylvestris Linn., Gossypium arboreum Parl., Boethea cauliflora Nees.

The study of the elaioplasts in these species was undertaken by me from the microchemical standpoint and from the morphological and biological points of view, and I hope my observations may complete the contributions reported in preceding papers.

Method: In the study of the plasmatic stroma of the organ in question I have used as fixative solutions an alcoholic solution of corrosive sublimate, alcoholic solution of picric acid, and a solution of absolute alcohol containing 5 per cent of acetic acid.

In the monochromatic staining I have made use of fuchsine, eosine, aurantine and erythrosine, and later have preferably used Zimmermann's mixture (iodine green and fuchsine).

With these reagents I was able to demonstrate that the stroma is eminently erythroise and nearly always presents an affinity for stains similar to that of the nucleolus.

In the study of the distribution of the elaioplasts in diverse parts of the single species, I availed myself almost exclusively of fresh material, since these bodies because of their strong refractility and the characteristic forms which they present are easily recognizable, and it would, I believe, be unnecessary to have recourse to special stains. However, in those cases in which the elaioplasts might be confused with the nucleus or with other bodies included in the cytoplasm I used osmic acid, a solution of potassium iodide, Sudan III, and Scarlet R.

With the first of these reagents, employed also by Zimmermann, the elaioplasts in a few minutes turned brown or blackish.

With the second of these reagents, the starch granules took on, according to the length of time the reagent was allowed to act, a violet color or azure blue more or less intense. The protoplasm stained a golden brown,

the nucleus and the plastids, brown; the elaioplasts, on the contrary, a more or less intense brown, remained distinct from the nucleus, however, because of the intensity of the stain and because of its [the elaioplast's] form and diverse structure, particularly because of its mass subsequent to the continued action of the reagent, and the drops of some unstainable oil.

With Sudan III the elaioplasts stain intensely red.

I used for this latter reagent an 80 per cent alcoholic solution and to get better results first gently heated the sections before subjecting them to the action of the reagent, since the oily substance of the elaioplasts, which is easily soluble in alcohol, becomes somewhat insoluble when, as noted by Raciborski, it is later heated. Ordinarily a few moments of immersion in the staining solution suffices because of the intense staining of the elaioplasts. The sections taken from the stain are then carefully washed with water and examined in water or in glycerine.

Finally I have obtained singularly instructive preparations using Scarlet R (2) in the same manner as I did Sudan III.

Special Part.

Polianthes tuberosa Linn.

The elaioplasts are found in this species in the cell cytoplasm in nearly all of the young epidermis and are of special importance because of their notable size, which they attain in the adult stage, a fact which facilitates greatly their study.

I observed in living cells [a structure] strongly refractile to light and possessing a color which varied from pale yellow to brown.

When viewed in their sections made in diverse directions one arrives at the conclusion that the elaioplasts are of spheroidal form. This form changes frequently with the growth of the elaioplasts becoming irregular. They possess a granulose or spongy structure and consist of a fundamental proteinaceous substance in which one finds included an oily substance. These bodies ultimately presented the following reactions:

They are easily soluble in alcohol, ether, carbon bisulphide, and benzine; insoluble in acetic acid and chloral hydrate.

With tincture of Alkanna they stain red, with Sudan III intensely red, and with copper acetate after prolonged action they take on no particular color.

On the other hand, the fundamental proteinaceous substance is recognized by the brown color which it assumes with the iodine preparation, by the rose red reaction which is produced by Millon's reagent (especially if it is applied by warming), by the yellow color which it acquires when treated with nitric acid (or with nitric acid and ammonia), and finally by the red color which it takes on when stained with Raspail's reagent.

So far as concerns the development [of the bodies], it is to be noted that in the epidermal cells of the apex of a very young floral axis of this species (Polianthes tuberosa) the elaioplasts appear as very minute spherical bodies strongly refractile to light, close to the cell nucleus. They multiply by neoplasia from the protoplasm. Not infrequently, however, it follows that they [the elaioplasts] participate in the process of cell division by the bipartition of their plasma, a process which takes place passively.

In the culminating point of the process of nuclear division, in the division, that is, of the chromosomes, and, in general, in the phenomena which occur in the process of kariokinesis, the elaioplasts do not take an active

part and, therefore, the above-indicated division, I believe, has no fundamental importance. On the contrary, it remains as a passive division with the bipartition of the plasma after the manner followed in this case. In fact, during nuclear division the elaioplast remains inert and almost to the moment in which the new membrane which begins to form comes against it. Then it bends and takes on a semilunar form. The extremity of the thin membrane forms and then splits into incurved points generally in two unequal parts.

With increase in size of the cell the elaioplast grows in volume and at complete development exceeds the dimensions of the nucleus, becoming often four or five times larger than the latter. Later the elaioplast of the epidermal cell, where it is found at a certain distance from the extremity of the apex of the floral axis, begins to undergo phenomena of degeneration. In fact, it diminishes in volume, becomes impoverished in oily substance, and presents some scattered vacuoles in its mass among which often there is to be distinguished a large central vacuole. Finally in the cell, as development advances, nothing more than a residuum of the elaioplasts can be found, and frequently also this comes to be completely reabsorbed in the cytoplasm in which it is immersed.

It is to be noted that in this species the elaioplast remains almost always throughout its life close to the cell nucleus.

In the species in question the elaioplasts are encountered in the epidermis and in the parenchyma of the perianth, of the floral axis and of the peduncle, of the stamens, of the pistils, and of the young leaves; they are absent in the ovule, in the seed, in the adult fruit, and in the roots.

Haemanthus albiflos Facq.; H. coccineus Linn.

In all the species of this genus which I have studied the elaioplasts appear, on microscopic examination, in the adult stage, under various forms, which may be considered as variations of a fundamental type of globose form. They frequently appear continuous, although in reality their form results from the union of many minute spheres which may be separated from one another by pressure on the cover glass of the preparation. Each of these spheres consists of a plasmatic stroma in which is found included an oily substance. With osmic acid (solution 1 per cent) the elaioplasts become almost instantaneously black (Pl. XIV, fig. 4). After treatment for a few seconds with Sudan III the single spherical elements which constitute the elaioplasts coalesce and from their mass oily drops appear, while the mass and the drops assume an intense red color. The oily substance resists the action of acetic acid and of chloral hydrate, but dissolves easily in absolute alcohol, ether, chloroform, and benzine. It, however, resists the action of these latter solvents following heating or following the action of osmic acid. The elaioplasts attain greater dimensions than those of the cell nucleus. They have a light yellow color and are found in the adult stage close to the cell nucleus or a little distance therefrom. Their manner of development is comparable to that described for Polianthes tuberosa.

The elaioplasts of this species are found in the epidermis of all the scales of the bulb, in both epidermises of the leaf, and finally in the epidermis of the floral axis and of the perianth.

Himantophyllum miniatum Groenl., H. Cyrtanthiflorum Groenl.

In these species I had occasion to study, in addition to the vegetative organs, also floral organs. With the aid of methods indicated by Wakker, that is, plasmolyzation of the cell, I was easily able to demonstrate that the elaioplast could be found in the cytoplasm. In this case a better reagent is a 15 per cent aqueous solution of potassium nitrate, with the addition of a small quantity of eosine. With such a solution, which has a marked power of disintegration, I brought about the plasmolysis and the contraction of the interior protoplasm from which the elaioplasts separated, which remained for a long time visible and maintained their primitive form. In the cells of the ovary, in process of development, the elaioplasts in the younger stage appeared as little aggregates of rotund or oval bodies endowed with strong refractility and frequently were close to the cell nucleus. Each group did not exceed the size of the cell nucleus.

Later, however, when the flowers have reached almost their ultimate size and begin to open in the epidermal cells of the ovary, which are preferably chosen for the study of the development of the elaioplasts, these will wax in volume and in number and each group consequently becomes greater but never attains to the dimensions of the cell nucleus. In this stage, which may be the culminating point of their development, the elaioplasts begin to lose their original form and primitive structure and show phenomena which, I believe, are to be regarded as degenerative.

On the contrary, all the smallest oval or rotund bodies appear at first to flow together, forming a single mass. The fusion takes place gradually in a manner which may follow all the minute phases of the total disappearance of the characteristic structure of the elaioplast. This mass,

at first homogeneous, then aggregated or grumose, is finally granular, having a very irregular form frequently with tree-like ramifications (Pl. XV, fig. 1). It is refractile and presents yellow reflections. In its interior it presents in turn one or more central globules, which are at the starting point of the tree-like ramification. Such forms show all the reactions of the proteinaceous substance and are encountered in epidermal cells of all parts of the open flower but absent in the epidermis of the leaves and of the floral axis, in the tissue of the lobes of the anther as well as in the stem and in the pollen grains. It is also useless to seek for them in the seed, in the ovule, in the root tips, and in the deeper tissues of all the other organs.

Hippeastrum vittatum Herb., H. reticulatum Herb.

The bulbs of this species were examined both in the dormant and functionally active stages. In the external epidermis of the scales of the bulb during this period of dormancy the elaioplasts assume varied forms (Pl. XIII, figs. 4, 5), which may be considered as representing the ultimate or final phases of their development; in fact, they appear as single masses without any structure, often very large, strongly refractile to light, colorless or light yellow.

Such a mass is of most irregular form and may appear now grumose and lobate, now granulose with tree-like ramifications, and finally as a collection of very minute rounded bodies. Such masses are immersed in the cytoplasm. They are generally found close to the cell nucleus and show the following reactions:

With a solution of iodide of potassium they stain intensely brown, treated with nitric acid and then with ammonia they take on a yellow color.

The color reaction to Millon's reagent when warmed is brick red.

Treated with sulphate of copper and then with potassium hydrate, they take on a violet tint.

The substance, therefore, of the mass under examination is of a proteinaceous nature. Moreover, as is shown, it yet remains to be established, with the aid of the microchemical reactions indicated by Zacharias (6) (in his last work on the chemical constitution of protoplasm and of the nucleus), to which of the proteinaceous substances which enter into the constitution of the cell protoplasm can be ascribed that of the following bodies:

(1) In a 10 per cent solution of sodium chloride they are insoluble and do not expand.

(2) They swell and are insoluble in hydrochloric acid diluted according to the following proportions: Four parts of hydrochloric acid to 3 parts of water.

(3) They remain unchanged in a 20 per cent solution of magnesium sulphate.

(4) In methyl green (used on sections made from fresh material as well as on those fixed by immersion in absolute alcohol) they show no color.

(5) With the mixture methylene blue plus fuchsine S [1 volume (250 ccm. H₂O plus 0.25 g. fuchsine S) plus 1 volume (250 ccm. H₂O plus 0.25 g. methylene blue)] used on sections treated for 12 hours in 5 per cent hydrochloric acid and then in absolute alcohol, the bodies and the nucleoli stain intensely red; the chromatin of the nucleus blue, the plasma of the nucleus brilliant red.

(6) In sections treated with carmine acid the body and the nucleoli remain colorless and the chromosomes stain red.

(7) Submitted to the action of glycerinated pepsin for a period of 20 hours, the bodies and the nucleoli swell up and are not dissolved.

Such reactions lead one to the conclusion that the fundamental substance of the elaioplasts behaves towards the above-named reagents like the substance of nucleoli.

As already said, the elaioplasts encountered are found under the forms above described in the cells of the epidermis of the scales of dormant bulbs; in actively functioning bulbs; ~~in the contrary,~~ they separate or appear in various stages of a dissolutional process (Pl. XIII, fig. 3, é), while in long cells they are accompanied by new elaioplasts (Pl. XIII, fig. 3, c). These last are of notable size, colorless, and refractile, and result from the union of small spheres, disposed like grapes. Each of these little spheres consists of a plasmatic stroma and of an oily substance. The stroma shows all the reactions indicated as characteristic of proteinaceous substance. And, where the sections are fixed in an alcoholic solution of corrosive sublimate, they may be stained with fuchsine, erythrosine, and eosine.

The oily substance is resistant to the action of concentrated hydrochloric acid, of acetic acid, of a 50 per cent caustic potash solution, and of chloral hydrate. It is easily soluble in alcohol, ether, chloroform, xylol, and benzine. It becomes black with osmic acid and ultimately stains with Sudan III, Scarlet R, alkanine, and cyanine.

Similar elaioplasts in the adult stage are found in non-determined positions, which have no relation to the cell nucleus. They are endowed, in addition to a passive movement impressed upon them by the cytoplasmic streaming, with a movement of their own. They appear, in fact, to cross the cell cavity, to arrive at the wall, hesitate for a few moments, then recross the cell by returning to the point from which they departed. Such a movement is sometimes slow, sometimes rapid, and often interrupted by a rest of greater or less length. Contemporaneously with the movement of translation, they show a rotatory movement.

Besides those which appear in the bulbs, elaioplasts are found in the epidermal tissues of the ovary.

They, in open flowers, are of spheroidal and irregular form, resulting from the union of colorless or pale yellow spherical bodies, ^{and dimensions} the number/ of which are variable. Their size is often very considerable, since in complete development they often approach and even surpass, the dimensions of the nucleus. They have a development similar to that of the elaioplasts of the Liliaceae, described by Raciborski. In their senile stage their greatest degree of refractility diminishes and they appear sluggish.

With their interpidity appear numerous vacuoles which give them a granular or spongy aspect and which, on becoming greater, often form a single vacuole which, increasing in size, terminates by bringing about the disappearance of the elaioplasts.

Hippeastrum aulicum Herb.

In the cells of the external epidermis of the scales of some dormant bulbs of Hippeastrum aulicum was found immersed in the cytoplasm a body which, so far as I could determine, had not hitherto been observed; and I present here a summary of the results of a study undertaken by me as to the chemical and structural constitution of the same.

The body is endowed with a refractile elevation; it is found situated in the cell in a non-determinate position and has dimensions which vary from 10 to 20 microns, and in certain cases even more. It possesses a spherical form and comprises a central part, c, and a peripheral part, b (Pl. XIII, fig. 9).

The central part is of variable form and dimensions, homogenous^e structure, and vacuolated. This latter character is that which predominates and presents bimodality in the diverse phases of the development of the body, following which the vacuoles are unequal but of uniform type, sparse throughout the mass of the central part, or superior in size to a single central vacuole surrounded by others of smaller dimensions. In not infrequent cases in which they show marked vacuolization (Pl. XIII, fig. 6) it may be noted that the central vacuole, increasing and dissolving under other circumstances, terminates in a manner comparable with the central part. In such a case the body presents nothing other than a very large vacuole limited by the peripheral portion.

This is less refractile in the central part, presents a finely granulose structure, and envelops the central part like an involucre.

Finally, so far as concerns the relation between the central part and the peripheral part, I observed that in the fixed and stained preparations the first is almost always separated from the second by means of a colorless halo (Pl. XIII, fig. 9, a).

Staining Methods. In the examination of the body under consideration I find fresh material desirable or fixed material treated with different reagents. As fixatives I have employed absolute alcohol containing 5 per cent of acetic acid, platinum chloride plus acetic acid (Merkel), saturated solution of corrosive sublimate, acetic acid plus osmic acid plus platinum chloride (Hermann), Flemming's solution, Rabl's solution, Keiser's solution, chromic acid, alcoholic or aqueous solutions of picric acid, osmic acid.

Among these I have obtained the best results with the first four.

With regard to the stains, without enumerating all the reagents which I used, I note here only those which have given good results.

With Flemming's triple stain (safranin plus gentian violet plus Orange g.) the nucleoli and the bodies stained red and the chromatin of the nucleus violet.

With the triple stain Ehrlich-Biondi-Heidenhain, used on material fixed with absolute alcohol plus 5 per cent acetic acid, the nucleus and the body stained intensely red.

With acid fuchsine, eosine, erythrosine, and aurantia, I obtained a beautiful stain of the body and of the nucleus. With such stains it is to be noted that the central part of the body stains more intensely than the peripheral part.

Microchemical Studies. The bodies present the following reactions:

In water, aside from even at high temperature, the body does not disappear.

In absolute alcohol, even after prolonged immersion, it is resistant.

In sulphuric ether, it is insoluble.

In a solution of iodide of potassium, the central part stains an intense brown and the peripheral part golden brown.

With Millon's reagent, if agitated and heated, the central part takes on a marked brick red color and the peripheral part stains a light rose yellow.

With Raspail's solution, the central part takes on an intense rose color, while the periphery is stained less intensely.

Treated with a solution of Trommer's reagent (copper-alkaline solution), the body assumes a violet color, and this color becomes more distinct in the central part of the body.

With nitric acid and ammonia, the body assumes a yellow color; with Sudan III, it stains weakly; with osmic acid it becomes light brown; with acetate and chloride of iron and with bichromate of potassium, the body presents no particular color change; with copper acetate in aqueous solution (after some days) it shows no coloration.

Among the reagents thus listed from the reactions obtained through the use of Millon, Raspail, and Trommer's solutions, there remains no doubt as to the proteinaceous nature of the substance which forms the central part of the body, and these reactions indicate the participation of a substance similar in nature to the formation of the peripheral part of the body.

The reactions with ferric salts, with potassium bichromate, and with copper acetate, exclude the presence of tannic or resinous substances. The stain reactions obtained with Sudan III and osmic acid do not make known the presence of the smallest quantity of oleose substance or the proteinaceous nature of the body, considering the fact that even crystals of protein react to these stains (1).

Conclusions. The body in question, occupying the same position as the elaioplasts, of a composition analogous to them, and being found, moreover, in the genus *Hippeastrum*, where these latter [the elaioplasts] were found, I believe to be nothing more than a form of their [the elaioplasts'] development.

Eucharis subdentata Benth.

So far as regards the study of the history of the development of the elaioplasts in this genus, I have been unable to examine the floral axis in process of development, or the bulbs in germinative activity, and am unable to make any pronouncement.

I examined solely the bulbs following the stage of blooming and encountered the elaioplasts in the external epidermis of their scales.

They occur immersed in the cytoplasm and are generally of notable dimensions. The color varies from yellow to brown and in form the elaioplasts are roundish or irregular.

Often drops of oil may develop from their mass. Examining one by one of the scales, I noted that in all cases they [the elaioplasts] are not of irregular form and variable size and that in no case are they wholly lacking.

Such elaioplasts, when treated with Millon, Raspail, Trommer's reagents, give a proteinaceous reaction, while when treated with osmic acid they turn black; they stain intensely red with Sudan III and Scarlet R.

Sternbergia Fischeriana Rupr ., S. lutea Orph., S. macrantha J.

If one examines the bulbs of these species in the stage of their functional activity one will find in the cells of the external epidermis of their scales colorless elaioplasts which are strongly refractile to light and which in the adult stage attain much larger dimensions (Pl. XIII, fig. 11). Such elaioplasts are of a more irregular form and result from the union of numerous little spheres which, together, comprise fundamental proteinaceous and oily substances.

Ornithogalum caudatum Facq.

All that I have noted here for the elaioplasts of this species may be found in the work cited from Raciborski and is as follows:

"The elaioplasts are found isolated or bound together in the large epidermal cells of the walls of the ovary and lying near the nuclei."

Having had ~~left~~ my disposition abundant material, I undertook also in this species the study of the elaioplasts and will speak briefly of the method of their development in the storage organs.

In the epidermal cells of the scales of the bulb, whether they be adult or young, provided it is in the period of vegetative activity, one finds the elaioplasts, which at complete development present themselves as little spheres strongly refractile to light, united in one or more groups, immersed in the cytoplasm, and situated in a position having no relation to the nucleus. They are colorless or light yellow and move actively in a manner already indicated.

If one examines the same bulb during its dormant period, it is easy to discover in the cytoplasm of all or of some of the epidermal cells a body of homogeneous and vacuolated structure, sometimes grumose, very voluminous, and which fuchsine, eosine, and aurantia stain actively. It shows all the characteristic reactions of proteinaceous substance. Such bodies present a form which is extremely variable (Pl. XIV, figs. 3, 6, 8); they are of a color which varies from yellowish to brown and often present a central granulose part which stains more intensely at the periphery when treated with a solution of iodide of potassium. They do not behave otherwise than do the elaioplasts which, after a short period of functional activity, degenerate and take on later the aspect and form alluded to. In fact, by the end of the flowering period the elaioplasts which one finds in the external epidermis of the scales of the bulbs begin to show phenomena of degeneration. Before the little spheres which constitute the body dispose themselves somewhat disorderly they lose their form and coalesce, thus contributing to the formation of a single unified mass.

The fusion occurs at the points of contact and occurs contemporaneously throughout the elaioplast, or gradually, beginning from fixed points. In this period of its evolution it is possible to find sometimes in the plasma, and more especially in the neighborhood of the mass of the elaioplast, one or more spherical refractile bodies, which become black when treated with osmic acid, react to Sudan III, to cyanine, and to Alkanna, and at the same time show all the reactions of proteinaceous substance. Such bodies probably represent the elements of the elaioplast separated from its mass, first having been derived through fusion, or probably formed by neoplasia.

The forms under which the mass appears following the fusion of the spherical bodies are, as has been said, extremely variable. In fact, such a mass may appear as a large sphere (from which are sent out one or more tree-like ramifications) (Pl. XIV, fig. 9, f, g), spheroid, ellipsoid, and even grumose, thus presenting prominent lobes.

Thus does one see how in this degenerating elaioplast there develops a unified mass from the normal form of an aggregate of spherical bodies which represent the perfect development by diverse successive stages, in which the single spherical elements which constitute it come little by little to finally join together. Probably the fundamental substance of the elaioplast during this process undergoes some chemical modification, considering that it, being at first colorless, begins to become yellowish, and ends frequently by becoming intensely brown. Moreover, the oleose substance in the advanced stages of degeneration of the elaioplast appears in the form of minute drops, then diminishes in size, and finally disappears.

Following the disappearance of the oleose substance there remains the proteinaceous substance, which continues for some time to represent the elaioplast, reduced to lumps or granules which later dissolve and disappear.

When the bulb resumes its functional activity there form anew in the epidermal cells of the scale elaioplasts, which multiply through a process of true gemmation as well as by neoplasia (Pl. XIV, fig. 9). In fact, I have observed that they appear as a sphere at the surface of which in complete development there forms a tiny spherical embossment. This growth ends on reaching a certain size (which is generally much inferior to that of the individual which produces it) and afterwards there develops another similar one which behaves nearly in the same manner.

This repeating process occurring again produces a considerable increase in the number of individuals, which occasionally become isolated one from another by continuing to live isolated and independent. In general, they remain united until the end of their life, forming a chain in which they attain the size of the degenerative elaioplast.

Such a chain may also break in two or more parts. There may be observed, moreover, some cases in which the gemmae form contemporaneously at two or more points of the primitive elaioplast (Pl. XIV, fig. 9, d), and then, attaining the size of the mother sphere, one may find attached to it at different points two or more chains of daughter individuals.

Drimia undulata Jacq.

If one examines the bulbs of this species during the period of their active growth one will find the elaioplast in the cytoplasm of all the epidermal cells of the scales. The elaioplasts appear as a group of little spherical bodies endowed with strong refractility of ~~pale~~ pale ochraceous yellow, and variable in number. In examining one by one the scales of various bulbs in diverse condition, depending on the stage of development and age, one would be able to note in the epidermal cells the diverse forms which the elaioplasts assume and which correspond to the diverse stages of their development. Among these will be noted the more important.

✕ The completely developed elaioplast appears in the form of a cluster [grape-like]. Later, in successive stages, the spherical bodies become confluent but form a homogeneous, roundish mass, with ramifications of a granulose substance.

The fusion occurs at points of contact of the spherical bodies and may take place contemporaneously throughout the entire mass of the elaioplast or, gradually, commencing at definite points. Treating this formation with absolute alcohol, the oleose substance dissolves out rapidly and, if we later submit it to a solution of potassium iodide, one will observe a residuum of fundamental proteinaceous substance.

In this species, besides the bulbs, there were examined the young and adult leaves in which, in the epidermal tissues as well as in the assimilatory parenchyma, the elaioplasts were lacking.

Aloë rhodacantha DC.

The elaioplasts occur in this species in the epidermis of the axis and of the floral organs; they are lacking in the deeper tissues of the organs above indicated, in the epidermis and in the parenchyma of the leaves, in the young and adult roots.

Yucca filamentosa Linn.

"There is present, in addition to the cell nucleus, also a peculiar body which stains brown on treatment with osmium." (4).

In the lobes of the perianth of this species one finds an elaioplast in the cytoplasm of each cell of the epidermal tissue. It is of spherical form or nearly so, colorless, or slightly yellowish, of smaller dimensions than the ^{cell} nucleus, and is markedly refractile.

In the youngest stage ~~it~~ is found always near or in the neighborhood of the nucleus; in the adult stage some of them are slightly remote from the nucleus, while still others maintain a median position. With osmic acid they become black; with Sudan III they stain intensely red (Pl. XIII, fig. 10) and show all the reactions of proteinaceous substance, in which respect their structure is more similar to that of the elaioplasts of Polygonum tuberosum.

Their distribution is characteristically irregular; in fact, one does not find them in the young epidermis. In the leaves, both young and adult, with the aid of osmic acid one can find no trace of the elaioplasts. They are found, however, always in the epidermis of the pedicels^{and} of the floral bracts.

Ruscus racemosus Linn.

If one examines the epidermal tissue of the still young scaly leaves of this species, one will find in the cytoplasm of some or all of the cells elaioplasts having, in the adult stage, dimensions which frequently exceed those of the nucleus and a variable form which assumes a form characteristic of the elaioplasts of Ornithogalum umbellatum. Such elaioplasts are colorless and refractile and do not assume a fixed position in the cell.

Watsonia humilis Mill.

From studies made by me it appears that in the Iridaceae the elaioplasts are not diffused things, as in the case of the Orchidaceae, the Amaryllidaceae, and the Liliaceae.

In fact, wherever I have examined many representatives of such families I have found them only in Watsonia humilis.

They appear in this species in the epidermis of tubers. When completely developed they are large (voluminous), of dimensions more or less similar to those of the nucleus, of rotund form, oval or irregular, and pale ochre in color.

In many respects their structure is notably that of a constitution of small and very numerous spherical elements and in the further stages of their development they show vacuoles which render them granulose or spongy.

Sudan III, Scarlet R, Alkanna, and cyanine stain them and osmic acid blackens them.

Miltonia Clowesii Lindl.

In the floral axis and in the perianth of this species, in each of the epidermal cells, and in those of the parenchyma, there is to be found an elaioplast immersed in the cytoplasm situated generally near the cell nucleus. These elaioplasts resemble in physical properties and in microchemical reactions those described above.

Laelia anceps Lindl.

If one examines the epidermis of the floral axis of this species one will find in each cell an elaioplast in the form of a single sphere extremely refractile and presenting in its complete development a reaction identical to the reactions of the preceding species.

Cattleya Harrisoniae Paxt.

The elaioplasts are found in this species in the epidermis of the leaves during process of growth. In the adult stage they appear in groups of tiny spheres extremely refractile to light. Such groups have the form of bunches of grapes and are relatively smaller.

Lycaste aromatica Lindl., L. Skinneri Lindl.

If one examines the leaves of the perigonium of this species one will find in each cell of the epidermis an elaioplast immersed in the cytoplasm or close to the cell nucleus or a little distance from the latter. Such elaioplasts in the adult stage are of irregular form, granular structure, and of a size slightly inferior to that of the nucleus. They show all the reactions peculiar to proteinaceous and oleose substances.

Cymbidium aloifolium Sw., C. Lowianum Reichb.

In this species one finds elaioplasts in the floral axis, in the ovary, in the leaves of the perigonium, in the lip, and the gynostemium.

They are found immersed in the cytoplasm of the epidermal cells and result from the aggregation of very numerous little spheres which are strongly refractile to light (Pl. XV, fig. 6). Their size upon completion of development is most remarkable, since they come to occupy half, or even more, of the cell cavity. Such elaioplasts are of irregular or spherical form and roll around or cover nearly perfectly the cell nucleus (Pl. XV, fig. 5), or they can rarely be found any distance away from the cell nucleus. In the most advanced stage they lose their characteristic structure and become granulose and vacuolated.

Brassia brachiata Lindl.

The elaioplasts are found in this species in the epidermis of the ovary of the floral organs and are wanting in the leaves and in the adult roots. They are spherical or irregular in form and where they have separated in the first stage of their evolution they show in their interior some vacuoles which increase in number, giving to the elaioplasts a granulose appearance (Pl. XV, fig. 4).

Oncidium sphacelatum Lindl.

The elaioplasts of Oncidium sphacelatum resemble more nearly those of the preceding species. They are found in this species in the epidermis of the floral organs. In their final stage they break up and thus multiply themselves (Pl. XV, fig. 3). Thus it follows that in one and the same cell may be often found more than one elaioplast. Such a method of multiplication is described also by Raciborski in Ornithogalum comosum and in O. Eckloni.

Tetramicra bicolor Rolfe.

The elaioplasts of this species occur in the epidermis of the floral axis and of the leaves of the perigonium. They are found in the adult stage generally some distance from the cell nucleus. They are of spheroidal form and of a pale ochre color and present reactions which perfectly parallel those characterizing the elaioplasts of the preceding species.

Malvaceae.

The elaioplasts are found in all species of Malvaceae alluded to, which I have examined in the epidermal cells of young sprouts, of the petiole, of the leaf blade, the floral peduncle, the calyx, and the corolla. They are wanting in the ovule, in the seed, in the adult fruit, and in the roots. They are of grape-like or irregular form and result always from the union of very minute spheres the size and number of which are variable (Pl. XIV, fig. 7; Pl. XV, fig. 2). Each sphere consists of a plasmatic stroma, including an oleose substance. The plasmatic stroma treated with an aqueous solution of iodide of potassium assumes an intensely brown color. Treated with nitric acid and then with ammonia it turns yellow. Treated successively with sulphate of copper and potassium hydrate it takes on a violet color.

The reaction to Millon's reagent is red, especially where the material is heated.

The oleose substance is soluble in 5 per cent alcohol, in ether, and in chloroform. It is insoluble in acetic acid, in chloralhydrate; it stains with Sudan III and with Scarlet R and turns black when treated with osmic acid.

The development of these bodies is comparable to that of the elaioplasts of the preceding species. In fact, in epidermal cells in process of division of the growing point they do not participate in the phenomena of kariokinesis. They appear at first to turn around the nucleus like little refractile spheres which, moreover, increase in number as they progress in their development, increasing slightly in volume. In the advanced stages of their development they are of a granulose structure (Pl. XIV, figs. 2, 5) and present in their interior numerous vacuoles. When the epidermal cells have passed beyond the stage of their complete development it is difficult to find in them any elaioplasts because they, degenerating, have disappeared.

From the results here given regarding the elaioplasts found in the Malvaceae and those hitherto found in the Monocotyledoneae, they resemble these latter perfectly in position, in form, in development, in structure, and in chemical composition.

The examination of the following species failed to show any elaioplasts:

<u>Acalypha hispida</u> Burm.	<u>Caladium violaceum</u> Hort.
" <u>obovata</u> Benth.	<u>Cassia Fistula</u> Linn.
<u>Alpinia nutans</u> Roxb.	<u>Centradenia grandiflora</u> Endl.
<u>Amyris balsamifera</u> Linn.	<u>Ceratozamia robusta</u> Miq.
<u>Andropogon Schoenanthus</u> Linn.	<u>Cineraria amelloides</u> Linn.
<u>Anthurium magnificum</u> Lindl.	<u>Cinnamomum aromaticum</u> I. Grah.
" <u>lucidum</u> Kunth.	<u>Cistus ladaniferus</u> Linn.
<u>Ardisa japonica</u> Blum.	<u>Clerodendron Balfouri</u> Hort.
<u>Aristolochia Gigas</u> Lindl.	" <u>fallax</u> Lindl.
" <u>Pistolochia</u> Linn.	<u>Clusia flava</u> Jacq.
<u>Artemisia Dracunculus</u> Linn.	<u>Cneorum tricocum</u> Linn.
<u>Artocarpus rigida</u> Blum.	<u>Coccoloba excuriata</u> Linn.
<u>Asparagus plumosus</u> Baker.	<u>Coffea arabica</u> Linn.
<u>Bambusa mitis</u> Poir.	<u>Coriaria myrtifolia</u> Linn.
" <u>nigra</u> Lodd.	<u>Crinum ensifolium</u> Roxb.
<u>Begonia foliosa</u> H. B.	" <u>giganteum</u> Andr.
" <u>imperialis</u> Lem.	<u>Croton cascarilla</u> Benn.
" <u>maculata</u> Raddi.	" <u>cornutus</u> Andr.
<u>Bixa Orellana</u> Linn.	<u>Cycas circinalis</u> Linn.
<u>Boehmeria biloba</u> Miq.	" <u>revoluta</u> Thumb.
<u>Bulbine frutescens</u> Willd.	<u>Cyperus alternifolius</u> Linn.
<u>Caesalpinia tinctoria</u> Domb.	" <u>minimum</u> ^s Forsk.
<u>Caladium amabile</u> Versch.	" <u>Papyrus</u> Linn.
" <u>esculentum</u> Vent.	<u>Cyrthanthera magnifica</u> Nees.

- Datura arborea Linn.
Dianthus Caryophyllus Linn.
Dieffembachia picta Schott.
Echeveria coccinea DC.
Eranthemum aspersum Hook.
Eupatorium Morisii Vis.
Fatsia japonica Decne.
Ficus elastica Roxb.
Fittonia gigantea Linden.
Galipea ovata St. Hill.
Gardenia florida Linn.
" tubiflora Andr.
Goldfussia glomerata Nees.
Grevillea robusta A. Cum.
Gynura aurantiaca DC.
Halesia hispida Mast.
Ilex paraguayensis Hook.
Illicium floridanum Ellis.
Ipomea Leari Paxt.
Ixora javanica DC.
Jasminum grandiflorum Linn.
Justicia carnea Hook.
Laurus glandulifera Wall.
Leontopodium alpinum Cass.
Linnaea borealis Linn.
Magnolia grandiflora Linn.
Maranta argyrophylla Linden.
" arundinacea Linn.
" splendida Hort.
Mesembryanthemum barbatum Linn.
" curviflorum Haw.
Myrcia acris DC.
Musa textilis Née.
- Opuntia glaucophylla Wendl.
Origanum Majorana Linn.
Pandanus javanicus Hort.
Pellionia pulchra P. E. Br.
Philodendron giganteum Schott.
Phyllis Nobla Linn.
Picraena excelsa Lindl.
Piper Cubeba Linn.
" longum Linn.
Plumbago grandiflora Tenor.
Plumeria alba Linn.
Polygala myrtiflora Linn.
" umbellata Thumb.
Quercus Suber Linn.
Rivina laevis Linn.
Ruellia Devosiana Hort.
Salvia involucrata Cav.
" officinalis Linn.
Sansevieria guienensis Willd.
Sassafras officinale Nees.
Sempervivum californicum Hort.
Strobilanthes Deyeriana Hort.
Stromanthe sanguinea Lond.
Tamarindus indica Linn.
Thea viridis Linn.
Tournefortia laurifolia Vent.
Tradescantia zebrina Hort.
Zingiber officinale Rosc.
Xanthochimus pictorius Roxbg.
Xylophylla Arbuscula Sw.
" falcata.
" falcata.
" macrophylla Hort.

Relation between Crystals of Calcium

Oxalate and Elaioplasts.

The authors who have occupied themselves with a study of the relation between crystals of calcium oxalate and elaioplasts are the following:

Wakker observed that in the epidermal cells of Vanilla planifolia, contemporaneously with the disappearance of the elaioplasts, there appeared crystals of calcium oxalate. He, however, noted that in V. aromatica latifolia the epidermal cells, although in the young stage being supplied with elaioplasts, lacked the production of crystals in the successive stages of their development and forced ^{him} them to the conclusion that the crystals of the epidermis of V. planifolia could have no direct relation to the elaioplasts.

Warlich (?) subsequently observed that the elaioplasts in Vanilla planifolia in the advanced stages of their development became birefractile and that in their interior there is formed a crystal of calcium oxalate which was later disclosed. Besides this, there is always in the adult epidermal cell accompanying the crystal a birefractile rotund body which shows a black cross. It might probably represent the ultimate residuum of the elaioplast. The conclusion at which this author arrives is opposed to that of Wakker, since he believes that there exists in this case a special relationship between the elaioplasts and the crystals of calcium oxalate. Relative to such an opinion as the above, Zimmermann expresses himself as follows:

These facts, however, appear to me to require confirmation. In any event, it can not be said to be true of the occurrence of such phenomena. Thus Wakker asserts that a cultivated species under the name of Vanilla

planifolia latifolia clearly shows elaioplasts in the epidermis of the leaves, although they exhibit no relationship to calcium oxalate within the cells concerned. Even though I have made observations on the aforementioned *Vanilla* sp. neither in this one nor in *Ornithogalum umbellatum* and *O. nutans* could I demonstrate a single trace of diffraction in the elaioplasts, although I examined them in all possible stages of their development.

With the opinion of this last author I am in agreement also because I have never encountered crystals of calcium oxalate in relation to the elaioplasts in the numerous species in which I followed, to the last stages of their [the elaioplasts'] development.

The Origin and Morphological and Biological Significance of the Elaioplasts.

Raciborski admits that the elaioplasts owe their origin to a secretion of cytoplasm. On the contrary, I contend that they are of nuclear origin. Such an hypothesis is supported both by the fact that the elaioplasts appear during their first stage close to the nucleus and by the fact that the chemical nature of their substance reacts toward solvents and staining reagents like the substance of the nucleus, as I have established.

So far as concerns the morphological significance of the elaioplasts we have the following opinions: According to Wakker, it [the elaioplast] is probably derived from metamorphosis of the chloroplasts. This opinion, as Raciborski observes, is opposed by the fact that they have diverse methods of multiplication.

Zimmermann found in many cases that the elaioplasts showed a manifest similarity to the fungous organisms and as a consequence they do not exclude the question of possible parasitism or symbiosis. Such an opinion was later combated by Raciborski, who, from studies which he made on the development of the elaioplasts in the vegetative organs of the Liliaceae, reached the conclusion that they [the elaioplasts] may be considered as normal organs of the respective cells. I, never having been able to observe the formation of spores in numerous cases in which I observed the elaioplasts, do not believe it is possible to support the opinion of Zimmermann.

On the other hand, if it is a question of parasitism, the spherical bodies which constitute the elaioplasts ought to present the characteristic structure of the cell. Such a structure I have not been able to distinguish in the many preparations which I have made, not even in cases in which they exhibit notable size, even when I used the most powerful immersion objective and the strongest compensating oculars.

If, again, it may be added that it [the elaioplast] may be treated as a parasitic organism, it ought not to have the characteristic chemical constitution which is presented in the elaioplast and should appear anywhere in the cytoplasm and not constantly next to the nucleus.

I have sought also to cultivate the elaioplasts obtained from very thin sections of the external epidermis of scales of the bulbs of Hippeastrum aulicum and Haemanthus albiflos in suitable nutrient media, sugar solutions of varying concentration, Knop's solution, and gelatine. In the attempts made they have not shown the least tendency to grow, and after a few days begin to degenerate.

The researches of Stahl on mosses were done to show that the oleose bodies of these plants serve as organs of defense against the ravages of snails. Raciborski maintains that one may attribute even to elaioplasts such a function, based on observations that the floral axes of such species of *Gagea* and *Ornithogalum* are not eaten by animals even when these are found in dry soil, although the leaves are not respected. Such hypotheses do not to me seem probable since it is to be noted that the elaioplasts develop and function solely during a given period of the evolution of the organs in which they are found, then disappear; hence they would be incapable of protecting any organ whatever during its life against any herbivorous animal. Therefore, even the direct observations exclude completely the possibility that the elaioplasts have any defensive function to perform since, moreover, I have found in the leaves of Malvaceae (in which elaioplasts were present) little pits caused by snails. These [snails] ate also the floral axes of *Funkia coerulesa* and *Polianthes tuberosa* which I fed them.

Conclusions.

In the species which I have examined the elaioplasts are found in the adult stage within the cytoplasm, now close to the nucleus, now distant from it. They occur generally in the form of small spheres attached in groups, rarely in the form of single spheres. During their development they are able to assume diverse forms but for the most part in a given species there predominates a single form dependent on the number and size of the little spheres which compose them.

Zimmermann observed that in the perianth of Funkia coerulea the elaioplasts possess an active movement of displacement and of rotation which, also, I, too, was able to demonstrate and which I observed to be characteristic of elaioplasts in the form of grape-like clusters.

In regard to the position of the elaioplasts it is to be noted that with rare exceptions they are found in the epidermis of the floral organs. Their presence in the other organs is inconstant.

Their multiplication may obtain after three months, by neoplasia of the protoplasm; by gemmation; or by division, which takes place passively. The first constitutes the normal method of multiplication, the second is rarer, the third and last represents an exception and accompanies a division of the plant cell. A typical example of the multiplication by gemmation may be observed in the epidermis of the scales of new bulbs of Ornithogalum caudatum; while the passive division occurs during the division of the epidermal cells of the floral axis, of the ovary, and of the perianth lobes of Polygonatum tuberosum.

So far as concerns the biological study of the elaioplasts, from the scope of the surprising and varied stages of their development, and the assignment to them, in so far as possible, of functional significance, my observations were repeated, not only upon vegetative organs of many Liliaceae and Amaryllidaceae, but also on their bulbs examined in diverse conditions with regard to the age of the bulb as well as stage of vegetative activity or dormancy. The authors who preceded me in the study of the elaioplasts were not able to study all the phases of their development since they [the elaioplasts] do not occupy such reserve organs.

From the studies I have made on bulbous species in which I have found elaioplasts the following may be deducted:

(1) That the elaioplasts, besides occurring in vegetative organs, may be found in the external epidermis of all the scales of the bulb.

(2) That the elaioplasts in bulbs, after a short existence, degenerate, then disappear.

(3) That of the two substances of which elaioplasts consist the first to disappear is the oleose substance, while the proteinaceous substance persists for varying lengths of time.

(4) That in dormant bulbs the elaioplasts appear without definite structure and assume varied forms which are to be considered as representing the ultimate phases of their development.

(5) That in each renewal of functional activity of the bulb elaioplasts form anew.

The form of the elaioplasts is singularly and particularly elevated in some bulbs of Hippeastrum aulicum and of Ornithogalum caudatum.

The principal results of my researches, then, may be thus summarized:

(1) I have found elaioplasts in 27 new species referable to 19 new genera of Monocotyledoneae.

(2) I have found that there are elaioplasts even in some Dicotyledoneae (where none were ever found before), namely, in the Malvaceae, the one family from the many examined by me which possess them.

(3) The elaioplasts are not to be considered as parasites (as thought by Zimmermann) nor as organs of defense (as maintained by Raciborski), but as specific organs of the cell in which they form and whose function is that of elaborating oleose nutritious substance.

(4) The fundamental substance of the elaioplast is similar to the substance of the nucleolus.

(5) In bulbs there are formed new elaioplasts with each renewal of their [the bulbs] vegetative activity.

Botanical Institute of Pavia, August, 1911.

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PLATE LEGENDS.

e = elaioplast; n = nucleus; l = leucoplast; c = cytoplasm; g = oil drop.

PLATE XIII.

Fig. 1-2. Epidermal cells of the perianth of Polianthes tuberosa Linn.

" 1. Elaioplast in adult stage stained with Sudan III.

" 2. Elaioplast passively divided.

" 3. An epidermal cell of a scale of the bulb of Hippeastrum vittatum Herb. e = elaioplast in adult stage stained with Sudan III.

e - old elaioplast of which the oily substance has disappeared.

e = the proteinaceous substance begins to dissolve.

" 4,5,8. Hippeastrum vittatum Herb. 5. Elaioplast in adult stage.

4, 8. Elaioplasts in degeneration colored with acid fuchsine (the material was fixed in absolute alcohol containing 5 per cent of acetic acid).

" 7. Hippeastrum reticulatum Herb. Elaioplast in the adult stage treated with absolute alcohol and then stained with acid fuchsine.

" 6,9. Special body found in dormant bulbs of Hippeastrum aulicum Herb. c = central part; b = peripheral part; a = colorless halo; d = central part with large-vacuole.

" 10. Epidermal cells of the perianth of Yucca filamentosa Linn. Elaioplasts colored with Sudan III.

- Fig. 11. Sternbergia lutea Orph. Elaioplast in adult stage.
- " 12. An epidermal cell of the bulb of Drimia undulata Jacq. with an adult elaioplast.

PLATE XIV.

- Fig. 1. Haemanthus albiflos Facq. The little spheres which accompany the elaioplast separate on pressure from the cover glass on the preparation.
- " 2. Hibiscus Rosa-sinensis Linn. An epidermal cell of the ovary with an elaioplast in adult stage.
- " 4. Epidermal cells of a scale of the bulb of Haemanthus albiflos Facq. each one containing an elaioplast in the adult stage, treated with osmic acid.
- " 5. Hibiscus tricolor Dehnh. An epidermal cell of the ovary with an elaioplast in the adult stage.
- " 7. Hibiscus herbaceus Vell. An epidermal cell of the perianth with an elaioplast in the form of a bunch of grapes.
- " 3,6,8. Odd form of elaioplast occurring in the epidermal cells of the scales of the bulbs of Ornithogalum caudatum.
- " 9. Various stages of germination of the elaioplasts of the bulbs of Ornithogalum caudatum.

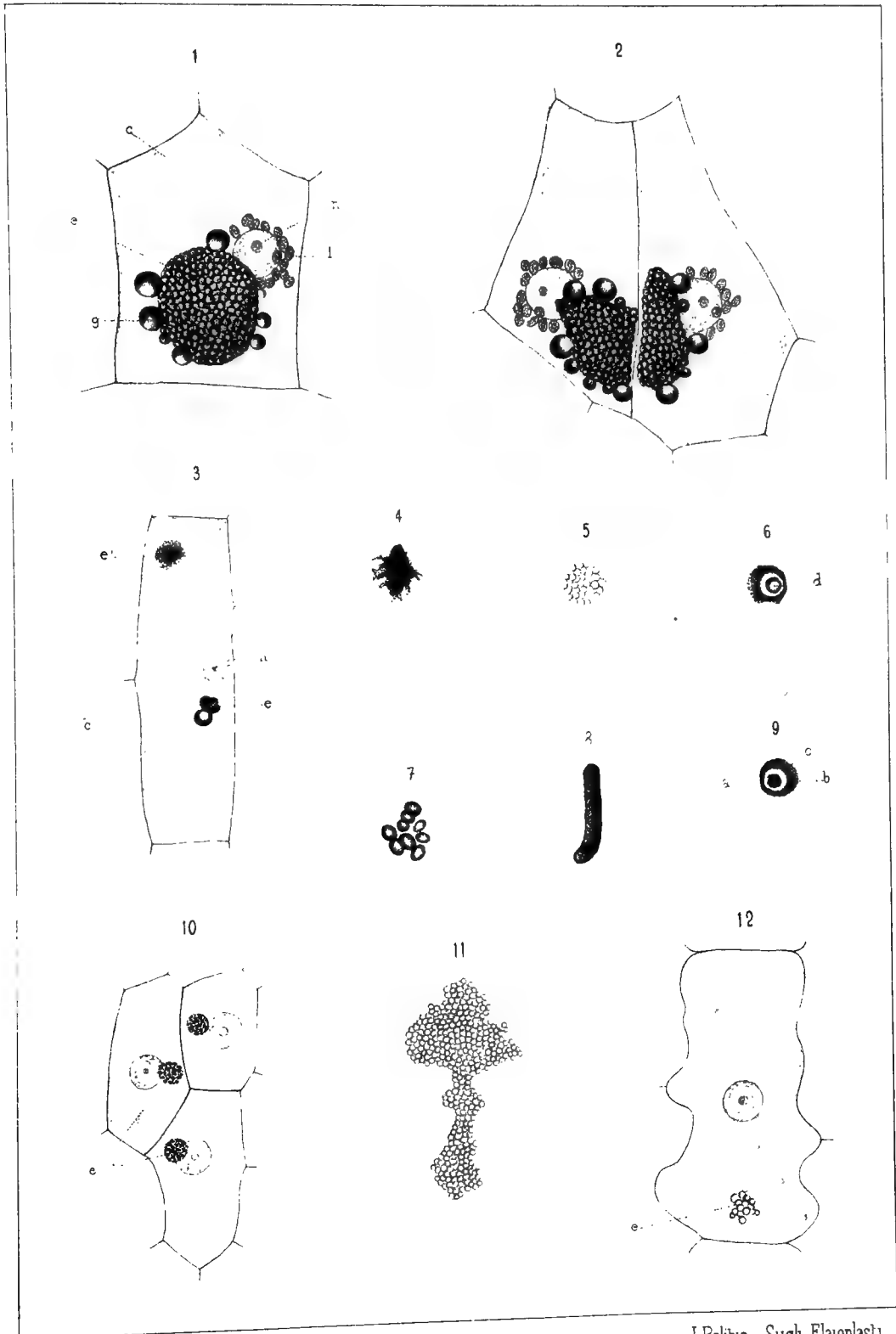
PLATE XV.

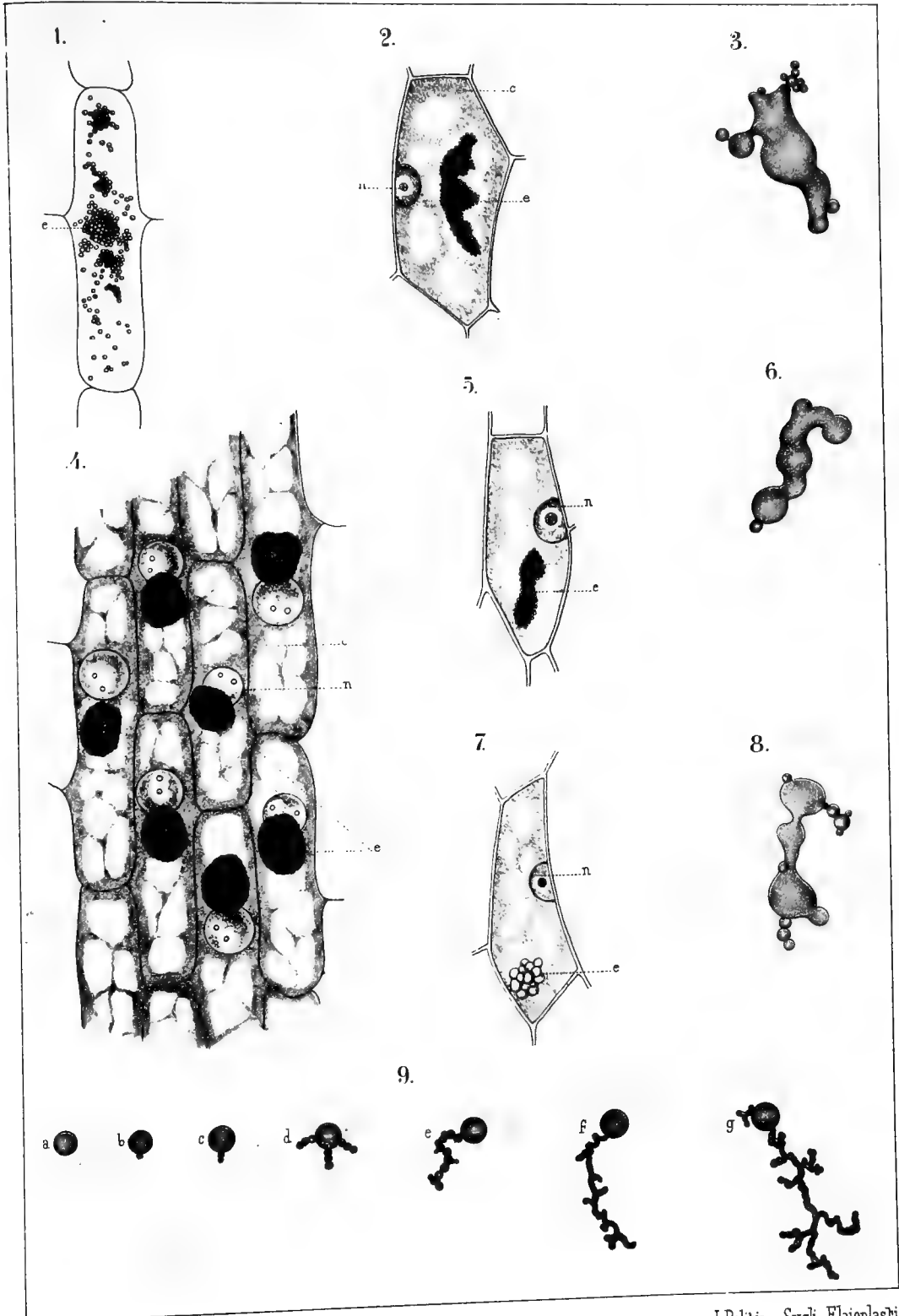
- Fig. 1. Himantophyllum miniatum Groenl. Cells of the epidermis of the ovary with elaioplasts in process of degeneration.
- " 2. Hibiscus liliiflorus Cav. Epidermal cells of the ovary each of which contains an elaioplast close to the cell nucleus.
- " 3. Oncidium sphacelatum Lindl. Epidermal cells of the ovary containing elaioplasts in advanced development. Each of them is fragmented, and thus we have several elaioplasts in each cell.
- " 4. Epidermal cells of the ovary of Brassia brachiata Lindl. with elaioplasts of granulose appearance.
- " 5,6. Cymbidium Lowianum Reichb. Epidermal cells of the ovary (fig. 5) and of the perianth (fig. 6). In the first the nucleus remains completely covered by the elaioplast which is stained black, after brief treatment of the sections with osmic acid 1 per cent.

Note: -- Figures 4, 6, 7, 8, 9 of Plate XIII were drawn with No. 3 ocular and oil immersion objective 1/2 Zeiss; all the others were drawn with No. 9 objective, and No. 4 Koristka ocular.

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Translated by H. B. Humphrey.

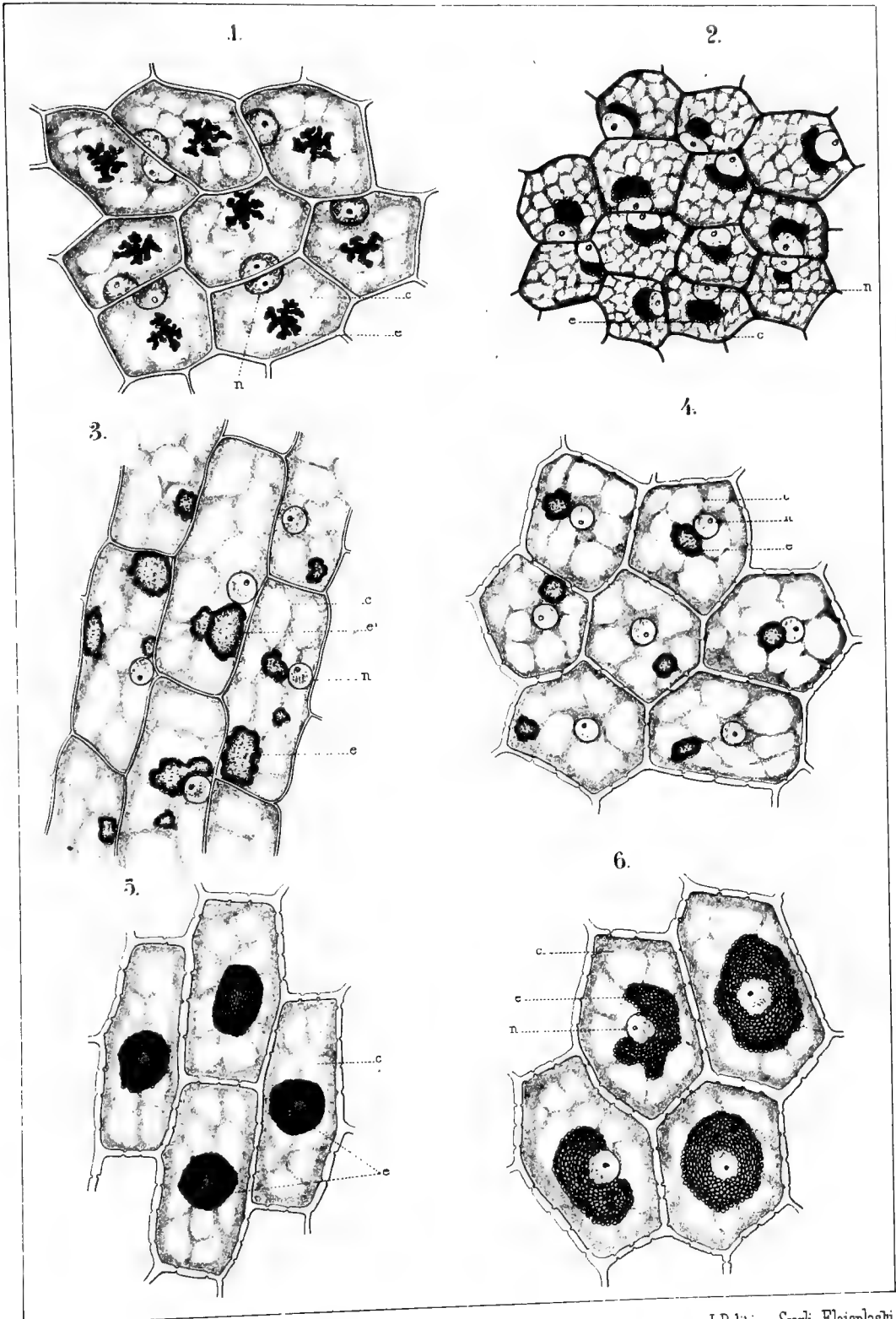




l'Autore del.

Lit Tacchinardi e Ferrari Pavia

I Pohbis - Sugli Elaioplasti



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ON SPECIAL CELLULAR BODIES WHICH FORM ANTHOCYANINE.^{a/} Translation of: Sopra speciali corpi cellulari che formano antocianine. Atti Ist. Bot. Univ. Pavia, II, 14: 363-376. pl. xvi-xviii. 1914.

Following the complete abandonment of the hypothesis as to the chlorophyllian origin of anthocyanine, many authors turned their attention to the substances which they found in plant cells, especially to sugars and tannins. They sought to establish the existence of some genetic relation between them and the anthocyanine pigments.

Wigand (19, 20)^{b/} at first admits that the tannic substance should be considered as a chromogen (generator of color), because there seems constantly to appear such a substance in the cell in which later anthocyanine pigments come to be organized and also because he notes that the presence of these latter coincides with that of the tannin in floral leaves and in red autumn foliage.

The conclusions reached by this author we will find more amply confirmed in the works of Wiesner (18), Tschirch, Aufrecht, Kutscher, Detmer, Reinke, Pick, Molisch, Dennert, Bauer (1), etc.; in recent times in those of Overton (12), Buscalioni and Pollacci (1), Mirande (g) and Laborde (7).

Buscalioni and Pollacci (1) supposed, however, that to the oxidases belonged the task of transforming certain substances in the anthocyanine pigment, while to the reductases probably belonged the function of determining their dissolution.

^{a/} See also: Atti Acc. dei Lincei, xx, giugno 1911.

^{b/} Reference is made by number to "Literature cited," near end of translation.

The hypothesis that the formation of the anthocyanine compound is related to the phenomena of oxidation found much support in the works of Mirande, Molliard (11), Miss Wheldale (17), V. Grafe (4, 5), Combes (2), etc., and was applied latterly in a particular manner by Palladin (14). According to this author, there exists in all plants chromogens and oxidases. The oxidases settled upon the chromogens and may give origin to anthocyanine pigments, and these may break up by the diminution of the process of ^ooxidation and by the acceleration of the processes of reduction.

More recent research led Palladin (13) to admit the existence of compounds to which he gave the name prochromogens and from which, through the intervention of an enzyme, there might be derived a chromogen. These latter may be produced in large numbers when the functional activity is more intense, namely, in the spring, while during a great part of the growth period they may form in small quantities by completing the process of oxidation.

With respect to the relation which exists between the anthocyanine and sugars, the research of Overton (12), which was fully confirmed by that of Molliard (10) and Palladin, revealed the existence in some plants of a direct relation between the production of anthocyanine and the accumulation in their tissues of sugars resulting from culture.

Finally, Combes(3), following on his research on gaseous changes during the formation and disappearance of anthocyanic pigments, was led to consider these substances as glucoside compounds, which originated in a more oxidizable ambient or fluid than that normally present and is not differentiated from that which forms under ordinary conditions according to their stage of more advanced oxidation. This point of view conforms with the opinion maintained for a long time by many authors, namely, that the anthocyanic compounds are to be considered as glucosides, an opinion which found new confirmation in the recent researches of Portheim and Scholl (16), and in those of V. Grafe(4, 5).

These are in a few words the principal results of the numerous and important studies that have been undertaken relative to the mechanism of the formation and chemical nature of anthocyanic compounds.

For a long time the fact has been observed that anthocyanine is not found in solution, solely, in the cell sap, but in rare instances also it may be found in the form of little spheres. (6, 9).

As to the origin, chemical constitution, structure, development, morphologic and biologic significance of such colored corpuscles, accurate research, so far as I am able to say, is lacking.

In the flowers of Billbergia nutans Wendl., I have encountered the fact which relates to the formation of anthocyanine and which, I believe, also is important in making one acquainted with the complicated structure of the protoplast.

In this plant in the part of the petals colored azure blue, in each cell of the epidermis and of the parenchyma, is to be noted a granulose protoplasm, and immersed in it are abundant round or oval chloroplasts (sometimes active and full of starch, sometimes a little changed, preferably close to the cell wall), a relatively small nucleus, and, finally, a characteristic body, to which may be attributed a particular biologic significance. I have given it the name of cyanoplast or generator of anthocyanine.

The cyanoplasts, nicely bounded by the cytoplasm which surrounds them, are distinguished principally by their intense azure blue color. They are of variable dimensions and attain, in complete development, considerable dimensions, while at first they are hardly visible. In general, it is to be noted that they are to be found toward the margin of the petals (Pl. XVI, fig. 2), and major dimensions approach those of the cyanoplasts which are found in the epidermal tissue. They are generally spherical, show an involucre the cavity of which is occupied by an azure blue pigment, not resembling in any respect the nucleus or the chromatophores; in each cell may be found constantly from the first a single cyanoplast

Microchemical examination. The azure blue pigment which is found in the involucre of the cyanoplasts presents the following reactions:

With acids it stains red. With alkali it takes on a greenish yellow color. With nicotine (1, p. 356) in dilute solutions it becomes green. It decolorizes easily with oxygenated water and with sulphurous anhydride. It is slightly soluble in cold water and dissolves quickly in warm water, and slightly acidulated water.

Through the microchemical examination of the pigment of the cyanoplast one finds present a substance which behaves toward acid, alkali, alkaloids, solvents, and oxidizing substances like anthocyanine. Therefore, we may conclude that the pigment referred to belongs to anthocyanine and should not be confused with other pigments.

While it is easy to determine the principal chemical properties of the pigment of the cyanoplast, it is difficult to determine the chemical nature of its involucre.

On the whole I have tried to find out if the cyanoplast possesses a plasmatic stroma as do the chromatophores and the elaioplast. Such a stroma I was unable to find, although, using many methods of fixation, such as picric acid, chromic acid, acetic acid, absolute alcohol in an aqueous or alcoholic solution of corrosive sublimate, I have not been able to fix and stain it. In all attempts made with the above-named liquids the cyanoplasm dissolves and disappears.

Verifying the absence of a plasmatic stroma, I tried to determine the nature of the involucre of the cyanoplast.

The mode [means] of resistance of the cyanoplast to attack by the several solvents, such as water, alcohol, neutral salt solutions, acids and dilute alkalis, and potassium iodide solution, shows that in them it is possible to distinguish a peripheral region which differentiates from the color substance by a greater resistance to the action of the determinative solvent.

If one uses a solution of potassium iodide, one will note that it acts so slowly on the cyanoplast that it is possible to follow with the microscope the various phases of its dissolution. The action of the reagent becomes manifest with the yellowing of the mass of the cyanoplast within which there appears one or more cavities. With progress of the action of the reagent, these latter will increase in size and terminate in a manner which results in the development of a great vacuole bounded by a dense wall which later dissolves and disappears.

If one uses the same reagent containing some drops of hydrochloric acid, the cyanoplasts stain red and shine on the yellow background of the protoplasm in a manner which enables one to follow, even better, the above-alluded-to phases of their dissolution.

These reactions and others which I have been able to make with dilute alcohol, with very dilute alkali, and especially with neutral salt solutions serve to show the independence of the two substances which constitute the cyanoplast.

Nevertheless, as will subsequently be shown, there are those instances in which the cyanoplasts appear to be colorless, namely, deprived of said pigment, and, in such cases, we are able to investigate microchemically as to the chemical nature of their involucre and of the substances contained within it.

Such colorless cyanoplasts are of homogeneous structure, oily appearance, and possess high refractility. They show the following reactions:

In water, at ordinary temperature, they vacuolize and end by dissolving completely. They dissolve quickly in absolute alcohol or in alcohol diluted to 50 or 75 per cent, and likewise in ether, in chloroform, in 1 per cent hydrochloric acid, in very dilute alkali, in neutral salt solutions, and, finally, in glycerine. With a solution of potassium iodide they stain

yellow^w and then disappear, dissolving slowly. With Millon's reagent, at first cold, then gently heated, no definite reaction takes place. With sodium coralline, aniline blue tincture of Alkanna, Sudan III, and cyanine, they do not stain. With 1 per cent osmic acid, they turn black almost instantly, and, following this treatment, they show an evident resistance to the solvent action of water and dissolve less readily in alcohol. With ammonium, potassium, and sodium carbonate, and with potash and ammonia, they become yellow. With ferric acetate and with ferric chloride, they become blackish blue. With bichromate of potash in concentrated solution, they stain reddish brown. Methylene blue in aqueous solution they absorb in a short time. (15).

The complexity of the reactions above brought out is not such as to lead one to any safe conclusions as to the nature of the substances which constitute the involucre of the cyanoplast. On the other hand, one may conclude that they do not belong to proteinaceous substances. Moreover, the reactions obtained through the use of ferric salts, potassium bichromate, alkali, alkaline carbonate, and methylene blue show that a chromogen of tannic nature is present in this involucre. But, since, following the transformation of the tannin in the anthocyanine, the involucre does not disappear but rather is rendered the more evident, there follows the deduction that in its formation it may contribute to the substances which contribute to its still unknown constitution.

Development. At the stage in which the corolla begins to emerge from the calyx cup, if one examines the epidermis (ventral and dorsal) and the parenchyma at the margins of the azure blue petals, one will be able to find all the stages of the development of the cyanoplasts. They appear at first as very minute spherical bodies scarcely visible, colorless or pale blue (Pl. XVII, fig. 1), immersed in the cytoplasm, without showing any proper

Later, retaining their spherical form, they increase in size and soon assume a more intense color, attaining in complete development dimensions relatively greater (Pl. XVII, fig. 2).

In this period of their evolution the cyanoplasts indicate marked modifications, in so much that they lose their globose form, assuming odd, extremely variable shapes (Pl. XVII, fig. 3), and reveal at the same time a very delicate membranous involucre, within which there is found included the colored substance. This occurs in the form of a central globule of considerable size or many spherical or oval corpuscles of variable size, which may be found indefinitely distributed within the involucre or else borne toward the extremity of the prolongation which occurs in this stage. The number of such corpuscles varies according to the dimensions of the cyanoplast. This latter, moreover, assumes a form and dimensions which are extremely variable, and permits the supposition that they become fragmented. In fact, many cyanoplasts are more or less thin in the median part and the two or three swollen extremities are somewhat far apart. It is not even improbable that there sometimes follows a multidivision, since one finds in the cytoplasm some tiny rotund colored bodies which are also azure blue and which probably represent the daughter cyanoplasts cut off from the mass of the adult cyanoplast, but in this regard my observations are incomplete. I could not follow this multiplication. Rather would I believe that the extremely variable form which the cyanoplasts take in this stage of their evolution is due to phenomena of degeneration and at the same time that the other bodies (corpicciuoli) to which I have referred represent young cyanoplasts formed by neoformation from the protoplast.

The fact remains that from the beginning in each cell one finds constantly a single cyanoplast which on becoming adult is accompanied by other spherical bodies of light color and of smaller dimensions.

Following the metamorphosis above referred to, we come to the coloring substance of the cyanoplasts, diffusing it into the cell cavity subsequent to the shrinkage and rupture of their involucre. This [the involucre] finally disappears completely and the cell sap, which at first was perfectly colorless, little by little, as the cyanoplast degenerates, takes on at first a pale blue color, then becomes intensely blue.

Origin and biologic significance. In the protoplast, as has been noted, one finds organs diverse as to constitution, structure, and functional value or worth, some of which arise from preexisting organs; others, on the contrary, originate directly from the protoplast by neoformation. Among these latter, as one may deduct from the history of their development above described, is the cyanoplast.

As to the appearance of the pigment, it may be said that it coincides generally with that of the cyanoplast. There are cases, however, in which the latter shows complete development independent of its pigment. Thus, for example, examining the flowers of the little plant which I found in an obscure corner of the greenhouse (under conditions of humidity and temperature differing from those of the majority of specimens examined by me) and in which only the apical parts of the pale petals showed azure blue color, I observed that in the margins, and especially toward the apex, some, among the numerous cyanoplasts, were completely developed and colorless (Pl. XVI, fig. 1); others, on the contrary, were developed but intensely azure blue in color; and, finally, still others showed diverse intensity of color.

In this case, then, the gradual transformation in the anthocyanine may be assisted by a substance already extant within the cyanoplast through metabolic processes which take place within the same.

Such a substance may be a chromogen of tannic nature the presence of which may be inherent within the young, colorless cyanoplast, since it may be colored in a particular manner by the use of salts of iron, osmic acid, and potassium bichromate.

Finally, so far as concerns the biologic significance of the bodies in question, I believe that they should be considered as specific organs on which depends the production of anthocyanine.

Facts analogous to those described for Billbergia nutans I have found in my study of many other plants, such, for example, as the following:

Liliaceae: Convallaria japonica Linn. (frutti).

Iridaceae: Iris fimbriata, Vent. (fiori).

Orchidaceae: Laelia anceps Lindl. (fiori).

Ranunculaceae: Aquilegia glandulosa Fisch. (fiori).

Ericaceae: Erica carnea Linn. (fiori).

Labiatae: Nepeta glechoma Benth. (fiori)*

Verbenaceae: Clerodendron Balfouri Hort. (fiori).

Caprifoliaceae: Weigela rosea Lindl., Weigela japonica Thunb. var. rosea (fiori).

Laelia anceps Lindl.

The petals and sepals in this species are of a vivid lilac rose color. The elongated lip has in front a reddish purple spot and is ornamented in the interior and in the throat with dark red stripes.

If one examines all these colored parts of the flower, one will find almost always that the pigment to which they owe their color is diffused in the cell sap ;in inquiring into its origin one should examine the sparse red spots of the gynostemium, which are of late formation.

Such spots are due to a group of epidermal cells each one of which contains a single cyanoplast (Pl. XVI, fig. 4). This cyanoplast is found immersed in the cytoplasm. In its adult stage it is of dimensions comparable to those of the nucleus, is reddish purple in color, has no definite position, and presents an involucre in which is included a colored substance which presents all the characteristic reactions of anthocyanine. In fact, this pigment is soluble in alcohol and especially in acidulated water, becomes green with nicotine and greenish yellow with alkali. It decolorizes easily with oxidizing substances.

On the other hand, the presence of an involucre in the cyanoplast may be demonstrated through the use of 50 per cent alcohol, $\frac{1}{2}$ per cent hydrochloric acid solution, 1 per cent solution of acetic acid, 1 per cent solution of chromic acid, and, above all, by oxygenated water or by osmic acid. By treating the sections with oxygenated water, one may set free from the cyanoplast its pigment and thus place easily in evidence its involucre which appears colorless as a vesicle which remains immersed in the cytoplasm. Treated with osmic acid, on the contrary, the involucre becomes blackish and vacuolized (Pl. XVII, fig. 9).

It is to be noted, moreover, that the above-named reagents do not act for a few minutes, but, if the action is prolonged, the involucre dissolves and disappears.

So far as concerns the development of the bodies in question, it is to be noted also that in this plant, as in the preceding one, they are not derived by division from preexisting bodies and are not transmitted from the mother cell to daughter cells but arise through neoplasia of the protoplast. In fact, they appear at first in the heart of the cytoplasm as very minute colorless spheres (Pl. XVI, fig. 3 d), in the interior of which later begins to form the red pigment (Pl. XVI, fig. 3 e).

Such spheres grow and in complete development may sometimes attain the dimensions of the nucleus.

Later there begins to appear on them phenomena of degeneration. The cyanoplasts, in fact, diminish in volume, while they present sparse vacuoles in their mass, among which one may often distinguish a single larger central one. Finally, the cyanoplast disappears and its pigment expands into the cavity of the cell, coloring the cell sap, which was at first perfectly colorless.

It is to be noted, finally, that in this species when the cyanoplast at first appears it is colorless and its pigment begins to appear in the interior later.

In such colorless cyanoplasts, treated with iron acetate, osmic acid, potassium bichromate, and with methylene blue, one is able to establish easily the presence of tannin.

Aquilegia glandulosa Fisch.

Examining the flowers in this species, one often is able to find in the violet-colored petals some cells in which the color is not diffused but is bounded instead, in one or more united spherical bodies. This presents a development and structure of the cyanoplast similar to those described above. Often in one or more points of their surface they show protuberances (Pl. XVII, fig. 7 d); their pigment shows all the reactions of anthocyanine.

Similar cellular bodies containing anthocyanine are found in the flowers of Nepeta glechoma.

Weigela rosea Lindl., W. japonica Thunb.

With an idea of getting an exact conception of the method by which the red color forms in the flowers of this species, I have studied many specimens and have found that they are not derived from preexisting substances soluble in the cell sap but have organized themselves into special spherical bodies immersed in the cytoplasm. These, being at first hardly visible, increase in size little by little and later, having reached a definite size (Pl. XVII, fig. 5), degenerate^t and dissolve completely, coloring the cell sap, which was at first colorless.

According to the data obtained from reactions which I got, the pigment of such body belongs to anthocyanine. It is contained with the membranous involucre the presence of which may be demonstrated by application of the usual methods described above, and particularly by using oxygenated water.

Convallaria japonica Linn.

In this species the cyanoplasts are found in process of formation in the epidermal and subepidermal cells of the adult fruit and, above all, in those of the ultimate strata of the seed coat in contact with the seed. Here the cyanoplasts in the adult stage are spherical, relatively small, immersed in the cytoplasm, and situated in an indeterminate position (Pl. XVIII, fig. 2).

They are at first present as very minute spheres azure blue in color (Pl. XVIII, fig. 1), or as colorless and refractile bodies which give all the reactions peculiar to tannin.

Such spheres grow, assume an intense coloration, in the end dissolve and color the cell sap, which for the first to this time has been perfectly colorless (Pl. XVIII, fig. 3).

Their azure blue pigment shows all the reactions of anthocyanine, since with acids it colors violet, with alkali it becomes greenish yellow, and with nicotine green; it dissolves, finally, in alcohol and in slightly acidulated water. Thus in this species one may demonstrate easily, treating the sections with oxygenated water, that the cyanoplasts possess an involucre which encloses the pigment alluded to.

Iris fimbriata Vent.

In Iris fimbriata one finds the cyanoplasts in the epidermal cells of the ovary and at the basal part of the petals in open flowers.

The cyanoplasts are here larger (Pl. XVII, fig. 6), in exact relation to the development of the cell, and they present an involucre which becomes evident whenever one uses methods above indicated.

It is a notable fact that the cyanoplasts in this species may appear in complete development, colorless, colored in varying degrees of intensity. In these colorless bodies, by the use of salts of iron, bichromate of potash, osmic acid, and methylene blue, the presence of tannin may be easily established, and, in others, applying suitable reagents, one may demonstrate the fact that their pigment belongs to anthocyanine.

Clerodendron Balfourii Hort.

The pigment to which is due the coloration of the red corolla of this species is, generally, in open flowers, dissolved in the cell sap.

However, he who examines many flowers will conclude that the origin of this pigment is like that of anthocyanine hitherto alluded to.

In fact, in not infrequent cases is to be found, immersed in the cytoplasm, a red spherical body (Pl. XVIII, fig. 7), in the cell in which the cell sap is perfectly colorless. This body represents the cyanoplast.

It originates by neoformation from the protoplasm, like the cyanoplasts of the preceding species, and toward the end of its evolution, like these, dissolving, the intense red color spreading through the cell sap (Pl. XVIII, fig. 9).

In each cell one generally finds a single cyanoplast. They show an involucre the cavity of which is occupied by the coloring substance. The involucre is made evident by treating ^tthe sections with oxygenated water or with osmic acid, and the coloring substance presents all the reactions characteristic of anthocyanine, since with water and alcohol it dissolves easily, with alkali it assumes a greenish yellow color, with nicotine in dilute aqueous solution it becomes green, and, finally, it decolorizes easily with sulphurous anhydride or with oxygenated water.

Erica carnea Linn.

In order to study the cyanoplasts of this species, it is necessary to examine specially the calyx which, like the corolla, is colored rose violet. Such coloration is due to a pigment which behaves like anthocyanine and which forms in the cyanoplasts. They are relatively very large and are generally found in numbers greater than one per cell (Pl. XVIII, fig. 4). Their structure and their development resemble perfectly those of the cyanoplasts of the preceding species. In the ultimate stages of their evolution they show vacuolization; finally, they dissolve completely and cause coloration of the cell sap, which at first was colorless (Pl. XVIII, fig. 6).

Besides the cyanoplasts, one should note in this species the presence of little spheres which are refractile to light (Pl. XVIII, fig. 4), which one finds singly (rarely more than one) per cell. They are in the epidermis as well as in the parenchyma of the leaves, of the sepals, and of the petals.

These little spheres turn brown on treatment with osmic acid, stain with Sudan III, with Scarlet R, and with tincture of Alkanna. They do not present the reactions of proteinaceous substances. As a consequence, they should be referred to as elaiospheres.

Likewise in this species, in rare instances, one may find some colorless cyanoplasts, which show all the reactions of tannin.

Conclusions.

From the ²assembly of facts above presented I believe that one may draw the following conclusions, limited naturally to those cases which I studied:

(1) The anthocyanine develops autonomously.

(2) Anthocyanine does not form in ordinary vacuoles, nor is it derived from a preexisting substance in solution in the cell sap, but is formed, on the contrary, within a special organ which I have designated cyanoplast.

(3) The cyanoplast originates directly from the protoplast by neofor-
mation .

(4) The cyanoplast is void of proteinaceous substance and shows an involucre the chemical nature of which is unknown and in which may be found tannin substances.

(5) Anthocyanine may be derived from substances belonging to the group of tannins, since the presence of such substances is found in cyanoplasts in which anthocyanine develops.

(6) Certain substances extant within the involucre of the cyanoplast may become transformed in the anthocyanine by means of special metabolic processes.

(7) External agents may suspend the transformation in the anthocyanine of the substances resident in the cyanoplast and then it remains colorless.

(8) The cyanoplast presents a definite development terminating in degeneration and the spreading of its pigment through the cell cavity.

(9) The color of the anthocyanine varies (red, violet, or azure blue), first depending on the influence of the cell sap. One ought not here to consider all these red, violet, and azure blue pigments as constituting a single compound which colors according to the degree of acidity of the cell sap, but, according to the results of the chemical research of other authors, it may be asserted that anthocyanines are different in their properties.

Botanical Institute of Pavia, July, 1911.

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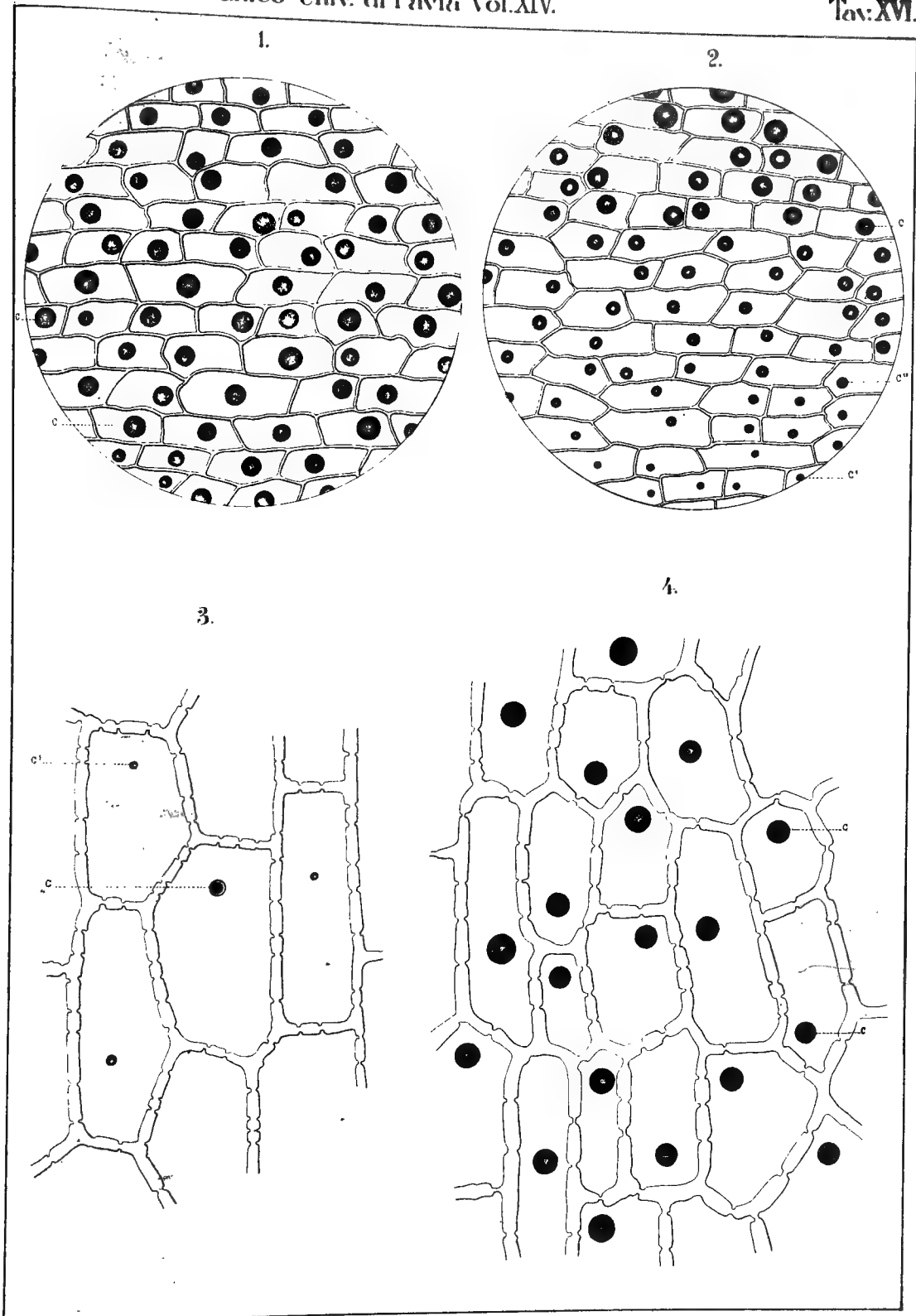
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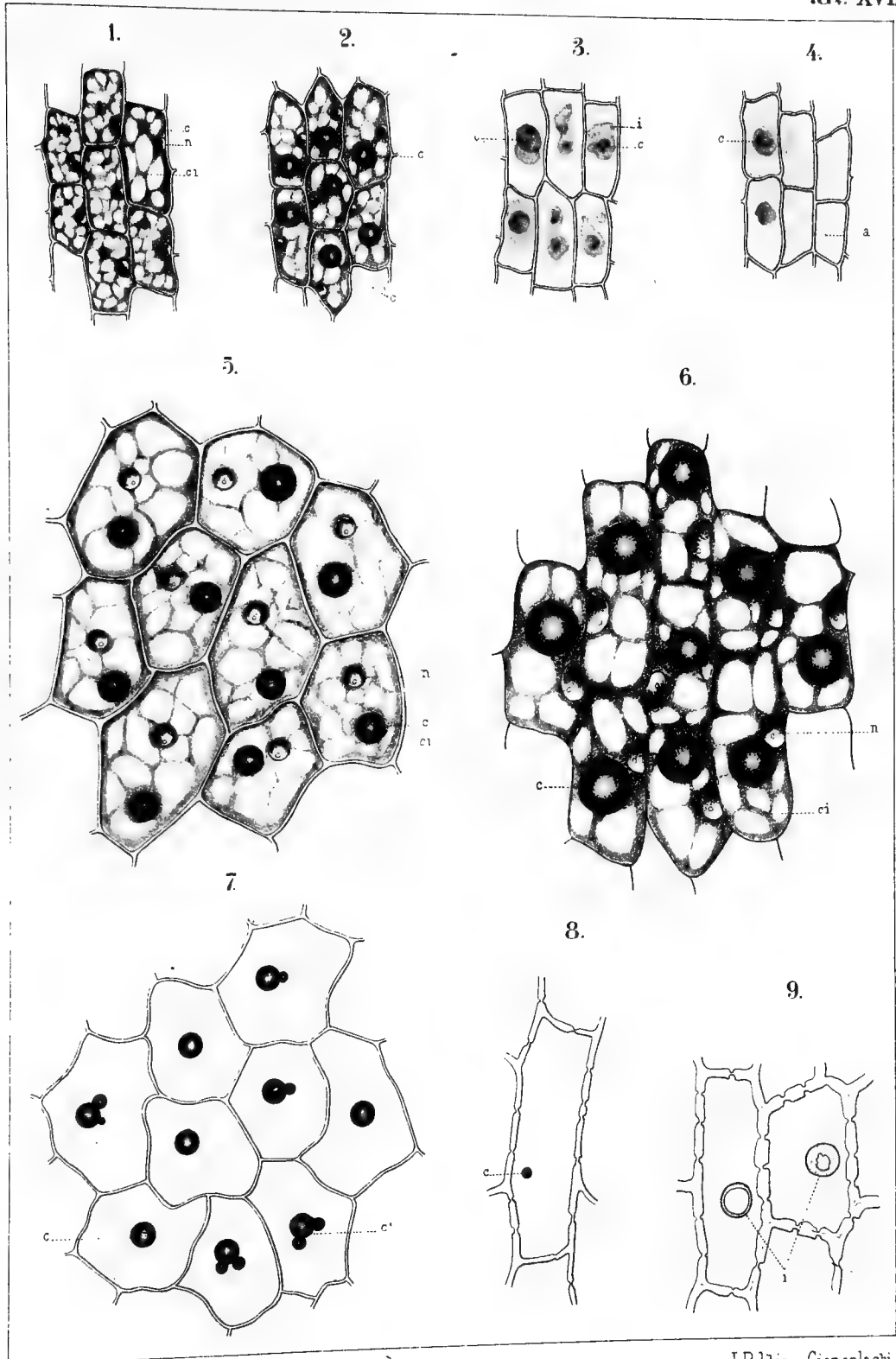
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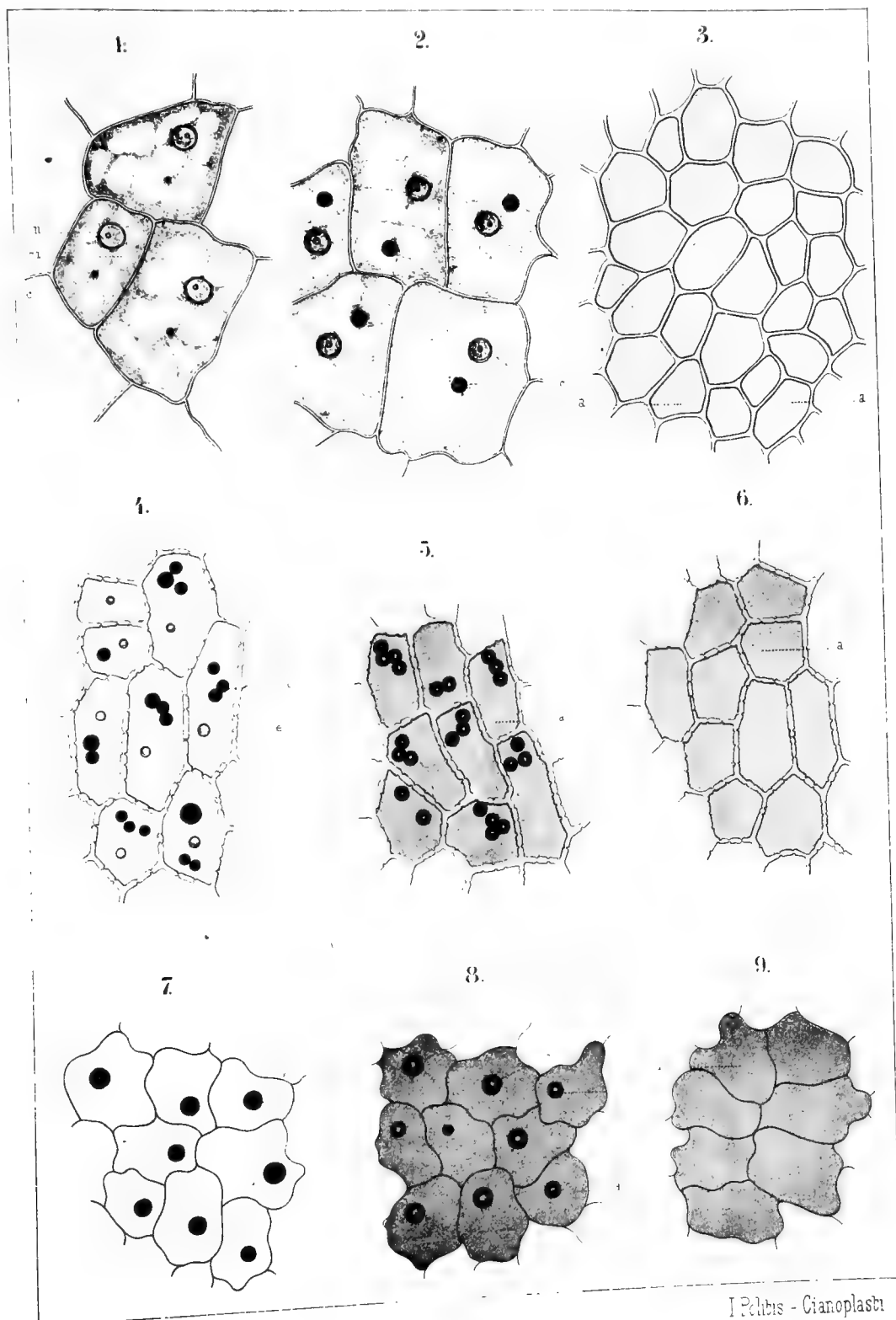
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I Pelibus - Cianoplasti

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ON A SPECIAL CELLULAR BODY FOUND IN TWO ORCHIDS. Translation of:
Sopra uno speciale corpo cellulare trovato in due orchidee. ^{a/}
Atti Ist. Bot. Univ. Pavia, II, 14: 377-383. pl. xix. 1914.

In two species of orchids, Coelogyne ~~Cristata~~ Lindl., and Eria stellata Lindl., a fact was noted which confirms the object of the present study.

Coelogyne ~~Cristata~~ Lindl.

In the leaves of the perigonium and in the gynostemium of this species there is to be noted in each cell of the epidermal tissue and of that immediately adjacent thereto a granulose protoplasm in which may be found immersed a very large spherical nucleus, abundant leucoplasts, which are round or oval, and generally active (with amilacei inclusions), situated preferably roundabout the nucleus, and, finally, a body which, from its chemical constitution, is different from that as yet found in the plant cell.

Such a body is distinguished by its physical property and particularly by the special and characteristic refractility with which it is endowed. In the living cell it appears spherical, colorless, of homogeneous aspect, and of considerable dimensions, so much so that in its complete development it may attain to dimensions slightly inferior to those of the nucleus.

It is found in the cytoplasm now in the midst of the cell, now near the cell wall, without relation either to the nucleus or to the leucoplasts. There is ordinarily one per cell, rarely, however, two or more. Such bodies are found even in the epidermis of the floral axis.

^{a/} See also: Rendiconti dell' Acc. dei Lincei, anno 1911.

Microchemical examination. The studies were made on fresh material. Thin pieces of epidermal tissue were immersed in different reagents and then examined under the microscope.

The bodies presented the following reactions:

In absolute alcohol, in ether, in chloroform, after a few moments of immersion, they dissolved.

In water at ordinary temperature they vacuolized slowly but did not disappear.

They dissolved in 10 per cent sodium chloride, in 10 per cent solution of potassium nitrate, in alkali, and in very dilute acids.

With solutions of potassium iodide they stain intensely brown, while at the same time they vacuolize and, after the appearance of numerous vacuoles, disappear.

With the use of Millon's reagent (cold), the body assumes a rose color.

Applying contemporaneously to the sections concentrated solutions of sugar and sulphuric acid (Raspail's reagent), it dissolves instantly. As a consequence, one does not succeed in obtaining a clear reaction.

Immersing the sections in a drop of nitric acid, they take on a yellowish color.

Sudan III, Scarlet R, and tincture of Alkanna, no color results.

With iron chloride and iron acetate in aqueous solution, they take on a blackish blue color after a short immersion.

With bichromate of potash in concentrated solution, they acquire a distinctly reddish brown color.

With osmic acid, they turn black after a short time.

With ammonium carbonate, potassium carbonate, and sodium carbonate, they stain yellow, and, with the progress of the action of the reagent, there

is often observed in its interior some granules or numerous little spheres due to a precipitation. These granules dissolve in water and absorb many aniline dyes.

With alkaloids (caffeine, chinine, nicotine, morphine, cocaine, atropine, strychnine, and codeine), in 2 per cent solutions, one obtains sometimes a scant precipitation similar to that obtained with the carbonates.

Methylene blue in aqueous solution is absorbed with avidity and communicates to the body, after a short time, a beautiful color.

Examining the reactions alluded to, we reach the following conclusions:

In Sudan III, Scarlet R, and tincture of Alkanna, we did not get the characteristic color for oleose substances.

The reactions obtained with a solution of iodide of potassium, with Millon's reagent, and with Trommer's reagent are the same as those which in the microchemical examination serve to show proteinaceous substances.

Osmic acid, ferric salts, bichromate of potash, alkaline carbonates, alkaloids, and methylene blue behave precisely as in the presence of tannin.

From this complex of reactions one may conclude that within the body under examination there exist proteinaceous and tannic substances, while oleose substances are lacking.

Colorization. In some works by Pfeffer (1, 2) a/ may be found references to the absorption of aniline dyes by the living cell. It is there to be noted also that a color substance incapable of osmosis may be contained in the cell sap because of the presence of diverse bodies. From these are noted to date fluoroglucin and tannin. This latter, according to Pfeffer, may be determined by any of all of the absorbable aniline dyes except rosolic acid.

Having established by means of many reagents the presence of tannin in the body under examination, I believed it worth while to check the observations of Pfeffer, using, in addition to the colors used by him, some others.

a/ Reference is made by number to "Literature cited," near end of translation.

The stains which I used are the following: Aniline red, gentian violet, dahlia violet, aniline blue, malachite green, iodine green, methyl green, thionine, methylene blue, safranine, neutral red, nigrosine, chrysoidine, vesuvine, Bismarck brown, Congo red, and light green.

Of these staining materials, aniline red, gentian violet, dahlia violet, malachite green, iodine green, methyl green, thionine, methylene blue, safranine, neutral red, vesuvine, Bismarck brown, and light green were able to determine in a very short time, by use of the most dilute solutions and without any previous fixation, the presence of tannin in the body in question.

Nigrosine, on the contrary, Congo red, and aniline blue were not absorbed even in concentrated solutions.

Development. The body in question does not participate in the phenomena of kariokinesis in the cells of the floral axis during their course of development. It appears first in some and then in all the cells as a very minute, little sphere, extremely refractile to light, immersed in the cytoplasm and enveloped frequently by a fine, granulose substance of tannic character.

Such spheres grow with the growth of the cells in which they occur and, arriving at completed development, reach dimensions slightly inferior to those of the nucleus.

Later, namely, when the ^{flowers} begin to wither, one often observes in these bodies noticeable degenerative phenomena; in fact, they lose their rotund form, their homogeneous aspect, and assume irregular, varied forms, while there appears in them numerous vacuoles which give them a spongy appearance.

Among these vacuoles, which are situated at the periphery, there often appear in the form of a ball those which expand as if an internal pressure were to burst them. Not infrequently one finds two vacuoles nearly touching

each other and, where there is a fracture of the partition separating them, it dissolves, thus resulting in the formation of a single vacuole. Finally, following the fusion of many of these vacuoles, there forms a single large one bounded by a thick wall.

Often, aside from the formation of the vacuole, one notices appearing from one or more points of the surface of the body a refractile substance of homogeneous appearance. If, now, one uses a solution of iodide of potassium, one may be able to distinguish two substances which behave diversely; one which colors intensely brown within and another which assumes a yellow color peripherally. In the central part there sometimes appear granules of variable dimensions.

Summarizing, we observe the following: That the body in question possesses a round form, homogeneous structure, and is colored brown in a solution of potassium iodide. On degenerating, it vacuolizes, its form becoming irregular. With reagents herein above referred to one can observe two distinct substances.

Eria stellata Lindl.

In the leaves of this species, in each cell of the epidermis and of the parenchyma, is to be noted in the cytoplasm as well as in the nucleus and in the leucoplasts, which are roundabout it, a colorless spherical body, strongly refractile to light, and presenting the following reactions:

Treated with a solution of potassium iodide, it stains yellow or brownish, according to the degree of the concentration of the solution.

With concentrated or dilute alkali, it dissolves and disappears rapidly.

With sulphuric acid and with other mineral acids in concentration, it becomes invisible after a short immersion.

In water at ordinary temperature, it vacuolizes but does not disappear.

In saline solutions of sodium chloride, potassium sulphate, and ammonium chloride, after 24 hours, it appears as a vacuole bounded by a thick wall.

This is not soluble in alcohol, nor in acetic acid, sulphuric acid, nor dilute hydrochloric acid.

With Millon's reagent heated over so little, one obtains a more decided reaction; in fact, immersing the tangential sections taken from the ventral or dorsal surfaces of the leaf blades in a drop of the reagent, the body, retaining its original form, assumes a reddish color which, after slow warming of the preparation, becomes intensely brick red.

With especially rich coloring substance (quinoline, Sudan III, Scarlet R, and sulphate Nilblau), one obtains no color reaction. One obtains, on the contrary, an important reaction by using 1 per cent osmic acid. Treating sections similar to the preceding with this reagent, a great number of the bodies in question turned entirely black, while some showed a black interior part due to tannic substance and an external peripheral colorless part which is refractile. This, with solutions of potassium iodide, stains brownish yellow, shows all the other special reactions of proteinaceous substance, and is, therefore, to be considered as forming a plasmatic involucre in which tannic substance is included. Such an involucre, in preparations fixed in alcohol and then treated with a mixture of fuchsine and methyl green, appears granulose and distinctly red in color.

The body under examination, moreover, because of the tannin which it contains, turns reddish brown with bichromate of potash in concentrated solution, bluish black in iron acetate and iron chloride, brown in 1 per cent chromic acid, yellow when treated with alkali and with alkaline carbonate. It, in fact, absorbs many of the aniline colors without any previous fixation.

From the sum of these observations it is evident that this body consists of two substances and that one of these is proteinaceous and the other tannic in character.

Finally, it is to be noted that in the neighborhood of this body may frequently be found numerous spherical corpuscles, isolated or in groups, endowed with Brownian movement. They are almost invisible in indifferent solutions, and may be observed in sections treated with proper reagents.

With osmic acid, in fact, they turn black; with bichromate of potash and chromic acid they become intensely brown; with iron acetate and iron chloride they take on an obscure blue color; they show, moreover, all the reactions of tannin, and it is here, probably, that they will be found related to the body under examination and which represents the products of its activity.

Origin and Morphologic and Biologic Significance of the Cellular Bodies under Examination.

The protoplast in the course of its functional activity, as has been noted, produces often by neoplasia the more important bodies to which belong, according to the results of the history of their development above described, also the body in question.

As far as concerns its morphologic significance, its method of multiplication, chemical composition, and the morphologic characters which this body presents, the idea that it may have some connection with chromatophores is to be excluded; for, I believe that especially because of the presence of tannin, it should be considered related to cyanoplasts (3) and because of the presence of the vesicle of tannin by which it is to be distinguished by its reaction from proteinaceous substances which show more evidently, and because of the special biologic function which it may perform, it should be considered related to cyanoplasts.

So far as concerns this latter, I have sought to study if this body, having for its location the epidermal cell and containing tannin, has not the function of protecting the organs in which it is found, from the ravages of snails (as supposed by Stahl regarding some plants containing tannic substances), but the results which I obtained were completely negative. Moreover, the snails would eat without damage to themselves the leaves of Eria stellata and the floral axes of Coelogyne cristata which were given them.

Conclusions.

In Eria stellata and on Coelogyne cristata there was revealed the presence of a body which up to that time had not been described by any previous author. It forms by neoformation from the protoplasm and shows reactions of the proteinaceous substances and also those of tannic nature. It does not disappear during the life of the cell in which it is found. It is not used as an organ of defense against the ravages of snails.

What biologic action it may have I do not now know.

Botanical Institute of Pavia, October, 1911.

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PLATE LEGEND.

PLATE XIX.

c = special cellular body; n = nucleus; l = leucoplast; ci = cytoplasm.

Fig. 1. Epidermal cells of the perianth of Coelogyne Cristata Lindl.

" . . 2. Cells of the lower epidermis of an adult leaf of Eria stellata Lindl.

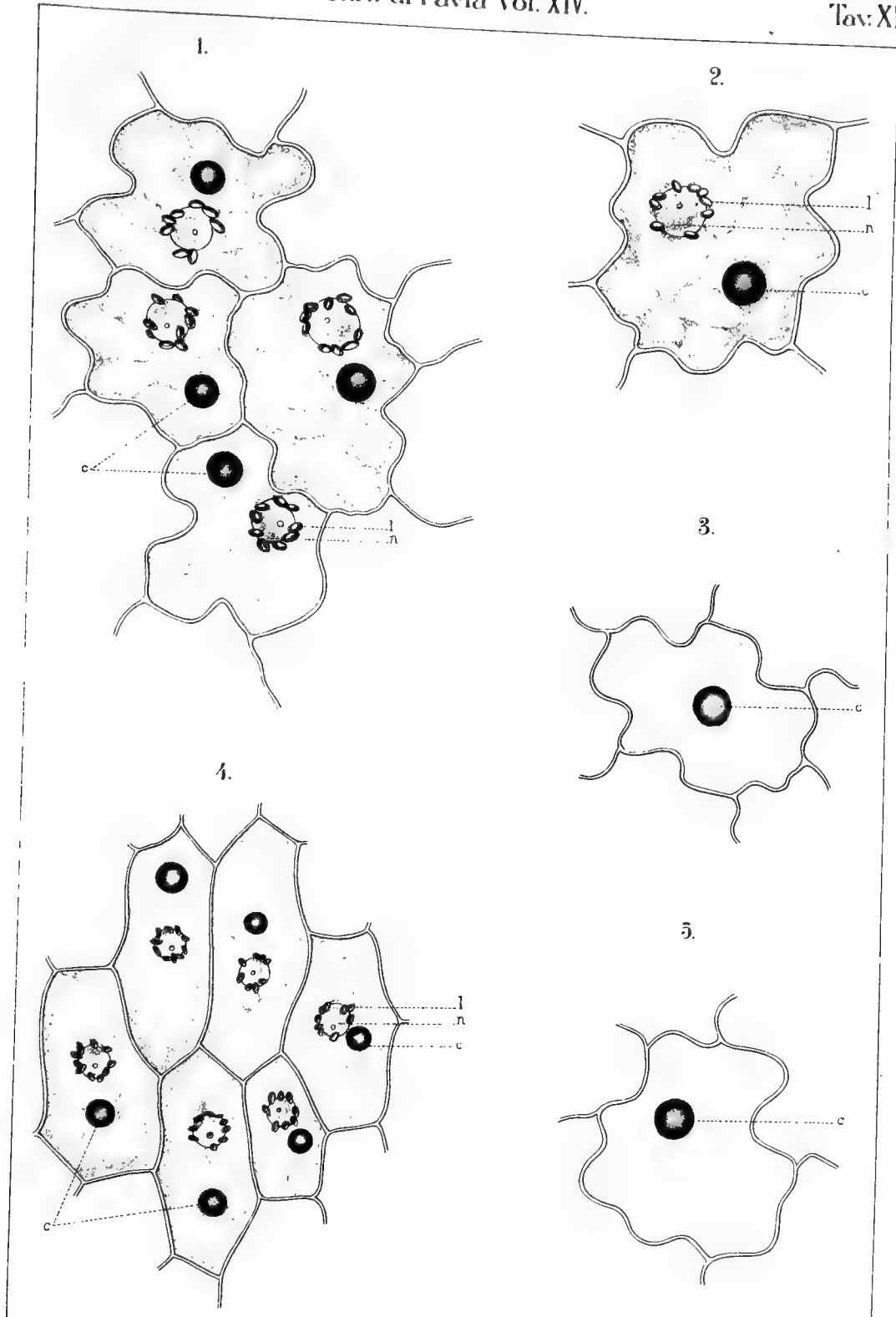
" 3, 4, 5. Three epidermal cells of the perianth of Coelogyne Cristata.

The special cellular body is represented stained (without preventive fixation) with light green (fig. 3), with Methylene blue (fig. 4), with safranine (fig. 5).

Note:- The figures were drawn with No. 4 ocular and No. 9 Koristka objective.

March, 1923

Translated by H. B. Humphrey.



L'Autore del.

Lit Tacchinardi e Ferrari Pavia

I. Politis - Speciale corpo cellulare

