THE EFFECT OF MOSAIC ON THE CONTENT OF THE PLANT CELL

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This paper is a report on a continuation of the studies on the cytology of mosaic plants. The studies in the first paper (1) were restricted to sugar cane, but the studies discussed in this paper were extended to include tobacco for the purposes of comparison. The greater part of this paper will be devoted to the chloroplasts, but some attention will be given to the mosaic patterns on the leaves and to other points.

The writer has already called attention to certain differences in the cells from old and young leaves of a cane plant infected with mosaic (1). It will be readily seen that the outside leaves of a sugar-cane plant are not only older than the inside leaves but that they have been subjected to the influence of sunlight and other external agencies for a much longer time; and it is well known that the older leaves of sugar cane and tobacco plants which are infected with mosaic, tend to lose their chlorotic character and become green after long exposure to sunlight.

In making these studies two plants of the same variety and age, one apparently healthy and the other infected with mosaic, were selected. Small pieces of tissue from corresponding parts of corresponding leaves were killed and fixed in weak Flemming solution, embedded in paraffine and stained by the triple-stain method. In the case of the diseased plant, the pieces were cut from both chlorotic and green areas and kept separate. This work was repeated several times but the drawings were made from two cane and two tobacco plants. In some cases plants with as many as thirteen leaves were selected while in other cases plants with as few as five leaves were selected. Sections were also made from the inner rolled leaves of the cane which had not been exposed to the direct action of the sun and in which it was impossible to distinguish the chlorotic areas.

Sugar cane and tobacco were selected for this work partly because they are important crops in Porto Rico and partly because they are so widely separated in the plant kingdom; one being monocotyledonous and the other dicotyledonous. In the sugar cane the leaves are rolled so that the young leaves are protected from the sunlight by the old leaves for a long time, while in the case of the tobacco the leaves are exposed to the direct action of the sun when very young. This is a very important point in making the comparison because of the fact that the sunlight appears to have some effect on the chloroplasts of the chlorotic cells.

Before entering into the discussion of the effect of mosaic on the contents of the cell we will briefly review a few of the well-known facts concerning the effects of mosaic on the plants. It is well known that when plants are severely infected with mosaic, they are usually dwarfed; that the leaves of mosaic tobacco plants are smaller than leaves of normal plants; that in the case of many species, the chlorotic areas of leaves of mosaic plants are slightly thinner than the leaves of healthy plants; in the case of tobacco and many other plants there is a decided modification of the leaf tissues of mosaic plants in that the palisade is replaced by mesophyll and that the mesophyll in general becomes more compact than the mesophyll of an apparently healthy leaf. The meager statements concerning cell contents are indefinite and contradictory. Thus far the writer has been unable to detect any differences between the tissues from green areas of mosaic plants and the tissues from apparently healthy plants. Therefore, for the sake of brevity, we will always refer to the green or normal in comparison with the chlorotic or diseased areas.

In general it may be said that the action of sunlight on diseased plants tends to reduce the symptoms of the disease so far as color is concerned. This has been noted by the other workers and the writer has made a careful study of this point on the two plants under consideration. In both cases leaves that are very chlorotic when young tend to become green with age. In fact this is so pronounced that in some cases it is almost impossible to select chlorotic areas in old leaves with any degrees of certainty. It is also true that the sunlight or some other factor tends to bring the choroplasts of cells in chlorotic areas to normal in appearance.

These studies have shown that in the chlorotic areas in leaves of sugar cane, the most striking cytological characters are the reduction of the chloroplasts in size and number, usually accompanied by an enlargement and deformity of the nuclei and occasionally by the presence of intra-cellular bodies. In the case of tobacco the chloroplasts respond to the disease the same as in the sugar cane but there is no such marked modification of the nuclei. However, the intra-cellular bodies are very frequent and readily detected.

The young leaves of the sugar cane before unrolling are uniformly white or nearly so, and it is impossible to say which areas are to become green and which are to remain white or chlorotic until they have been exposed to the light; but a cytological study of these leaves show that certain groups of cells already possess certain characters of chlorotic areas of later periods. The young leaves of diseased tobacco are usually almost white but develope the mosaic pattern with age. A cytological study of these young leaves reveals groups of cells which show the effects of the disease before the leaves show the mosaic pattern.

Kunkel (6) as a result of his studies on sugar cane says:

"The pattern shown by the leaves of most plants afflicted with mosaic is already present when the young leaves unfold and begin to turn green. For this reason it is in most cases not possible to determine how the chlorotic areas arise. In the case of sugar cane, however, the pattern shown by old leaves is usually not present on young ones and it is, therefore, possible to observe the chlorotic areas in process of formation. It has been observed that they begin as small spots scattered about over the leaf. The spots grow and finally include much tissue that was at first a normal green color. In their growth, the spots that happen to be close together coalesce and form chlorotic areas of various sizes and shapes. While the areas resulting from the fusion of two or more spots are quite irregular in shape, the spots themselves show considerable regularity."

Further on in this paper he says:

"While a part of this enlargement was due to the growth of the leaf, most of it resulted from the spread of chlorosis in the normal green tissues bordering the spots."

Our studies indicate that the chlorotic areas in sugar cane are quite definitely determined before the formation of chlorophyll which enables the observer to detect them with the unaided eye. The areas which are to be chlorotic can certainly be detected in properly prepared microscope sections. These areas increase in size with the growth of the leaves, but so far as the writer has been able to determine they do not encroach on the surrounding green tissues and do not coalesce. The increase in size appears to be by cell division and growth and not by infection of suronnding cells.

Kunkel also states that—

"It has been observed, however, that new spots sometimes arise in the normal green tissues as leaves unroll from the spindle."

My study of sections show that groups of cells so small that they cannot be seen with the unaided eye frequently occur in green areas of young leaves. It is very probable that groups of this kind enlarge with the growth of the leaf and give the appearance of new spots.

Kunkel also says—

"It has frequently been observed that the central areas of some spots are a normal green color and that only the borders are markedly chlorotic. Sometimes a small longated chlorotic spot lies in the center of the green area."

The writer has observed the same character and has also been able to trace them by means of sections back into young leaves.

It will be readily seen from this discussion that the patterns cannot be detected on the young leaves previous to the formation of chlorophyll. Exposure to sunlight intensifies the patterns for a time but eventually the chlorotic areas undergo a slow formation of chlorophyll until it is practically impossible to detect the patterns in the very old leaves. This has been observed by Kunkel who says:

"In other varieties, the light, greenish areas, instead of deteriorating as the leaf ages, have a tendency to recover. They may become so green that it is difficult to distinguish them from areas of normal green color."

The presence of the great masses of trichomes makes it very difficult to study the early stages of the mosaic patterns on the tobacco. However, the writer is inclined to believe that they develope in the same manner as in the sugar cane; and it is certainly true that their later history is the same.

CYTOLOGICAL CHARACTER

The references to the chloroplasts in the early literature are brief and unimportant. Koning **(5)**, in his study of tobacco mosaic (1899) found that chloroplasts were disorganized. Ivanowski **(4)**, in his studies on tobacco mosaic (1903) reports that the chloroplasts in the chlorotic cells were yellowish and scarcely reacted to the starch test but later they contained as much starch as they could hold. Westerdyk **(9)** made a study of the tomato mosaic and reports (1910) that the chloroplasts of the chlorotic areas were slightly smaller. Melchers **(8)**, made a study of the mosaic of tomatoes and reported (1913), that no difference was detected in the number and size of the chloroplasts in the yellow and green areas. Matz **(7)**, made a study of the mosaic disease of the sugar cane and writes (1922) as follows:

"In the diseased tissues the chloroplasts are few and are evidently misshaped and broken up. In stained sections they look like mere ink spots, one or two in a cell. This destruction of chloroplasts is a symptom of sugar-cane mosaic and it fixes the seat of the disease more definitely. Apparently the cell walls and other cell contents are not affected, but the chloroplasts are gradually destroyed."

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'It was seen that the breaking up of the chloroplasts begins with a reduction in their size. The chloroplasts in the healthy or green parts of the same leaf were normal in their size and numbers, while in the discolored or pale-green striped chloroplasts in all stages of reduction were noted.''

Dickson **(2)**, made a study of a considerable number of species of plants infected with mosaic and reported (1922) that the cells of the chlorotic areas contained less chlorophyll because of the fewer chloroplasts, less chlorophyll per plastid, the beaking down of the plastids or the general coalescence and degeneration of the plastids.

As a result of cytological studies on the mosaic of sugar cane, the writer of this paper wrote **(1)** as follows:

''A study of the cells of these leaves shows that the chloroplasts are more numerous in the dark-green than in the light areas. Therefore, it appears that in the case of the light-green areas the formation of the chloroplasts is inhibited but that with the exposure to sunlight this inhibition is gradually overcome.''

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''The chloroplasts in the white or yellow areas were fewer in number and smaller than in the green areas.''

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''The small chloroplasts of the mosaic cells are usually spoken of as having undergone a process of degeneration. The writer has studied the chloroplasts in the very young unrolled leaves and is satisfied that these chloroplasts are undeveloped rather than disintegrated. They have never reached normal development. Furthermore a study in a series of leaves from those just unrolling to the outermost on a plant shows that the chloroplasts of both normal and mosaic cells increase in size after being exposed to the light. These changes which are apparently due to age or light are so pronounced that there is very little difference in size of the chloroplasts in a leaf of the same age from a healthy plant. The writer is very doubtful if disintegration ever occurs in the case of primary infection (*i.e.*, infection from the seed).''

Since the writing of the above paper, Eckerson **(3)**, published the results of her studies on tomato mosaic in which she describes a flagellate organism found in the chloroplasts. In this paper she repeatedly refers to the ''dissolution'' and ''liquification'' of the chloroplasts.

In the light of these recent studies on mosaic it seemed advisable to extend my own studies; to repeat the studies on the sugar cane and to make a comparison with the tobacco. These two species were available in great abundance and since they are widely separated in the plant kingdom they appeared to be especially satisfactory for this purpose.

The plants for these studies were carefully selected. Healthy and diseased plants of sugar cane or tobacco of the same variety, age and approximately of the same size having been selected, the studies were made on corresponding tissues under as nearly the same conditions as possible. The plants were grown within less than 100 yards of the laboratory which facilitated the immediate study of fresh tissues and the immediate killing and fixing of tissues for sectioning.

In the study of the sugar cane it must be remembered that there are two types of parenchyma cells in the leaves: (1) The single layer of cells which form a sheath around the fibro-vascular bundle and which we will refer to as the sheathing cells are very regular in size and shape. They are tubular, more or less cylindrical and placed lengthwise with the fibo-vascular bundle; and (2) the ordinary mesophyll cells which are very irregular in both size and form.

In the series of cross sections of leaves of healthy plants from the inside outward (Figs. 1 *a* to 1 *e*), beginning with leaves that are of an age corresponding to diseased plants in which the chlorotic areas can be detected without error, it will be readily seen that there is no very great variation in character of cells, nuclei or chloroplasts. Cross sections from corresponding parts of diseased plants (Figs. 2 *a* to 2 *e*) show that the nuclei are more or less enlarged or irregular in form and that there is a tendency for the chloroplasts to be slightly more numerous and larger in the outer leaves which are older and have had a longer exposure to light than in the inner leaves.

These characters are much more prominent in longitudinal sections than in cross sections as will be readily seen by a study of the figures 4–6 which were drawn from sections made from the same leaves as those from which figures 1 and 2 were drawn. The nuclei and chloroplasts in the sheathing cells in leaves from the inside of a healthy plant are practically the same (Figs. 3 *a*–3 *e*). An examination of sections from corresponding cells of a diseased plant (Figs. 4 *a*–4 *e*) show that the nuclei in the sheathing cells are slightly larger than in the corresponding cells of a healthy plant. Also the chloroplasts in the sheathing cells of the inner leaf are few and small but increase in size and number from the inner to the outer leaves. The same facts are brought out by a study of the nuclei and chloroplasts of the ordinary parenchyma cells. The nuclei and choroplasts are very uniform in cells of leaves of a healthy plant (Figs. 5 *a*–5*e*) regardless of age; but in corresponding cells of the diseased plants the nuclei are very generally larger and irregular in form, while the chloroplasts tend to increase in number and size from inner to outer leaves. A considerable number of plants were used in making these studies, but all the drawings 1 to 6 were made from two plants.

A more detailed study of the nuclei and chloroplasts of these same cells emphasizes the points previously referred to. The nuclei (Fig.

7) of the sheathing cells of a healthy plant are very regular and uniform in size while those from the sheathing cells of a diseased plant (Fig. 8, *a* to *e*) tend to be slightly larger and occasionally elongated. In the ordinary mesophyll cells of a healthy plant the nuclei (Fig. 9) are quite regular as compared with the nuclei (Fig. 10, *a* to *e*) from the corresponding cells of a diseased plant; many (but not all) of which are very irregular in size and shape.

The chloroplasts in the sheathing cells (Fig. 11) of a healthy plant are large and the markings well defined as compared with the chloroplasts (Fig. 12, *a* to *e*) from the corresponding cells of a diseased plant which are small and with poorly defined markings. The chloroplasts (Fig. 13) in the ordinary mesophyll cells of a healthy plant are large and regular as compared with the chloroplasts (Fig. 14, *a* to *e*) from the corresponding cells of a diseased plant. However, as previously stated, the chloroplasts in the chlorotic areas in the older leaves of a diseased plant are very nearly or quite normal in appearance. No disintegration of the chloroplasts were observed in any case except in the very old leaves which were decaying from old age or in tissues which were decaying as a result of the attacks of fungi.

The above studies were followed by the study of cross sections of leaves well within the spiral which had not been exposed to the direct light of the sun. These leaves were white and there was no indication of the formation of chlorophyll (Figs. 15–16). By carefully selecting severely diseased plants it was possible to find many groups of cells with enlarged or deformed nuclei. It appears that these groups of cells are destined to grow into the chlorotic areas. These cells no doubt increase in size and divide but the writer is very doubtful if the surrounding cells become chlorotic. It will be readily seen that it is difficult to secure evidence for or against this opinion but the study of spots on leaves that were showing the green failed to show any evidence that the spots were increasing in size except with the growth of the leaf. The writer is in disagreement with Kunkel on this point.

Groups of apparently normal cells so small that they cannot be detected by the unaided eye can be found within groups of diseased cells of the chlorotic areas. This no doubt accounts for the small island of green tissue surrounded by chlorotic tissue as observed by Kunkel and the writer. Likewise, groups of abnormal cells so small that they cannot be detected by the unaided eye are frequently found in sections made from apparently normal tissue.

Similar studies on healthy and diseased plants of tobacco do not show any great variation in the nuclei, but the history of the chloroplasts is exactly the same as in the sugar cane. The chloroplasts in the mesophyll cells of a healthy plant are large and regular (Fig 18–19) as compared with the chloroplasts from corresponding cells of the chlorotic areas of a diseased plants (Figs. 20 and 22.) In the study of a series of sections from young to old leaves of a diseased plant, there is a pronounced tendency for the cloroplasts to become normal in appearance with age and exposure to light and possibly other agencies.

The tendency of leaves of diseased plants of both sugar cane and tobacco to become more or less uniformly green with age and exposure to sun light is no doubt due to tendency of the chloroplasts in the cells of the chlorotic areas to increase in number and size. This could not possibly be the case if the chloroplasts were undergoing disintegration.

The intra-cellular bodies previously described by Kunkel are rare in sugar cane but are quite common in diseased tobacco (Figs. 23–25). They appear to be the same as the bodies described and figured by Iwanowsky in his studies on mosaic of tobacco. The writer is inclined to believe these bodies to be the result, rather than the cause of the disease although it is difficult to point out any very definite reasons for this opinion. Circles (Fig. 23) and some other points noted by Iwanowsky can be found occasionally in both diseased tobacco and diseased sugar cane but there does not appear to be any good reason for believing that they have anything to do with the disease. Small moving bodies are very abundant in the cells of mosaic tobacco plants but these same bodies can be found in healthy plants, although they are not so abundant. These same or similar bodies can also be found in diseased and in healthy sugar cane but are much less abundant than in tobacco. The writer failed to find any flagellate bodies attacking the chloroplasts as were described by Eckerson as attacking chloroplasts of the tomato. However, this phase of the question is worthy of further investigation.

SUMMARY

1. The mosaic pattern is indistinct on the very young leaves; becomes prominent with the growth of the leaves; then becomes less distinct with age.

2. The chlorotic areas tend to become green with age. This is probably due to the action of the sunlight on the chloroplasts.

3. The chloroplasts in the chlorotic areas are fewer in number and smaller than in the green areas. There is no evidence of disintegration.

4. The tendency of the chlorotic areas to become green with age is due to increase in number and size of the chloroplasts.

5. The chlorotic areas can be detected by cytological studies before the unfolding of the leaves.

6. The chlorotic areas increase in size by cell division and cell growth and not by the infection of surrounding cells or by a disintegration of the chloroplasts of the surrounding cells.

7. The formation of apparently new chlorotic areas in leaves is probably due to very small infected areas which increase in size with the general growth of the cells.

8. Green areas in a chlorotic area can be detected by cytological studies before they can be detected by the unaided eye.

9. The small size and number of chloroplasts is common to both sugar cane and tobacco when infected with mosaic.

10. The nuclei of diseased cane cells are usually enlarged or deformed, but this is not true of tobacco.

LITERATURE

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EXPLANATION OF PLATES

Figs. 1*a* to 1*e*.—Cross sections from a series of normal leaves showing sheath cells of the fibro-vascular bundles and ordinary parenchyma cells. 1*a* is the youngest and 1*e* the oldest leaves in the series. Leaves *b* and *d* omitted.

Figs. 2*a* to 2*e*.—Cross sections from a series of mosaic leaves showing sheath cells of the fibro-vascular bundles and ordinary parenchyma cells. 2*a* is the youngest and 2*e* the oldest leaves in the series. Leaves *b* and *d* omitted. Note the reduced number and size of the chloroplasts and the abnormal nuclei as compared with Fig. 1*a* to 1*e*. Note also the chloroplasts tend to become normal in appearance with age.

Figs. 3*a* to 3*e*.—Longitudinal sections of sheath cells of the fibro-vascular bundles from the same leaves as Figs. 1*a* to 1*e*.

Figs. 4*a* to 4*e*.—Longitudinal sections of sheath cells of the fibro-vascular bundles from the same leaves as Figs. 2*a* to 2*e*.

Figs. 5*a* to 5*e*.—Ordinary parenchyma cells from same leaves as Figs. 1*a* to 1*e*.

Figs. 6*a* to 6*e*.—Ordinary parenchyma cells from same leaves as Figs. 2*a* to 2*e*.

Fig. 7.—Four nuclei from sheath cells of normal leaves. From same leaves as Figs. 1*a* to 1*e*.

Figs. 8*a* to 8*e*.—Four nuclei from sheath cells of mosaic leaves. From same leaves as Figs. 2*a* to 2*e*.

Fig. 9.—These nuclei from ordinary parenchyma cells or normal leaves. From same leaves as Figs. 1*a* to 1*e*.

Fig. 10.—Eight nuclei from ordinary parenchyma cells of mosaic leaves. From same leaves as Figs. 2*a* to 2*e*.

Fig. 11.—Chloroplasts from sheath cells of normal leaves. Same as Figs. 1 and 3.

Figs. 12*a* to 12*e*.—Chloroplasts from sheath cells of normal leaves. Same as Figs. 2 and 4.—(*a* inside leaf. *e* outside leaf.)

Fig. 13.—Chloroplasts from ordinary cells of normal leaves. Same as Figs. 1 and 5.

Figs. 14*a* to 14*e*.—Chloroplasts from ordinary cells of mosaic leaves. Same as Figs. 2 and 4. (*a* inside leaf. *e* outside leaf.)

Figs. 15–16.—Sections through leaves of mosaic plants which had not been exposed to the sun. No chlorophyll had been developed but many of the nuclei were abnormal.

Fig. 17.—Cross section of normal leaf of tobacco.

Figs. 18 and 19.—Cells from cross section of normal leaf of tobacco figured in 17.

Fig. 20.—Cross section of mosaic leaf of tobacco. Same age as 17. Note the abnormal chloroplasts.

Figs. 21 and 22.—Cells from cross section of mosaic leaf of tobacco, figured in 20. Note the abnormal chloroplasts and the intra-cellular bodies.

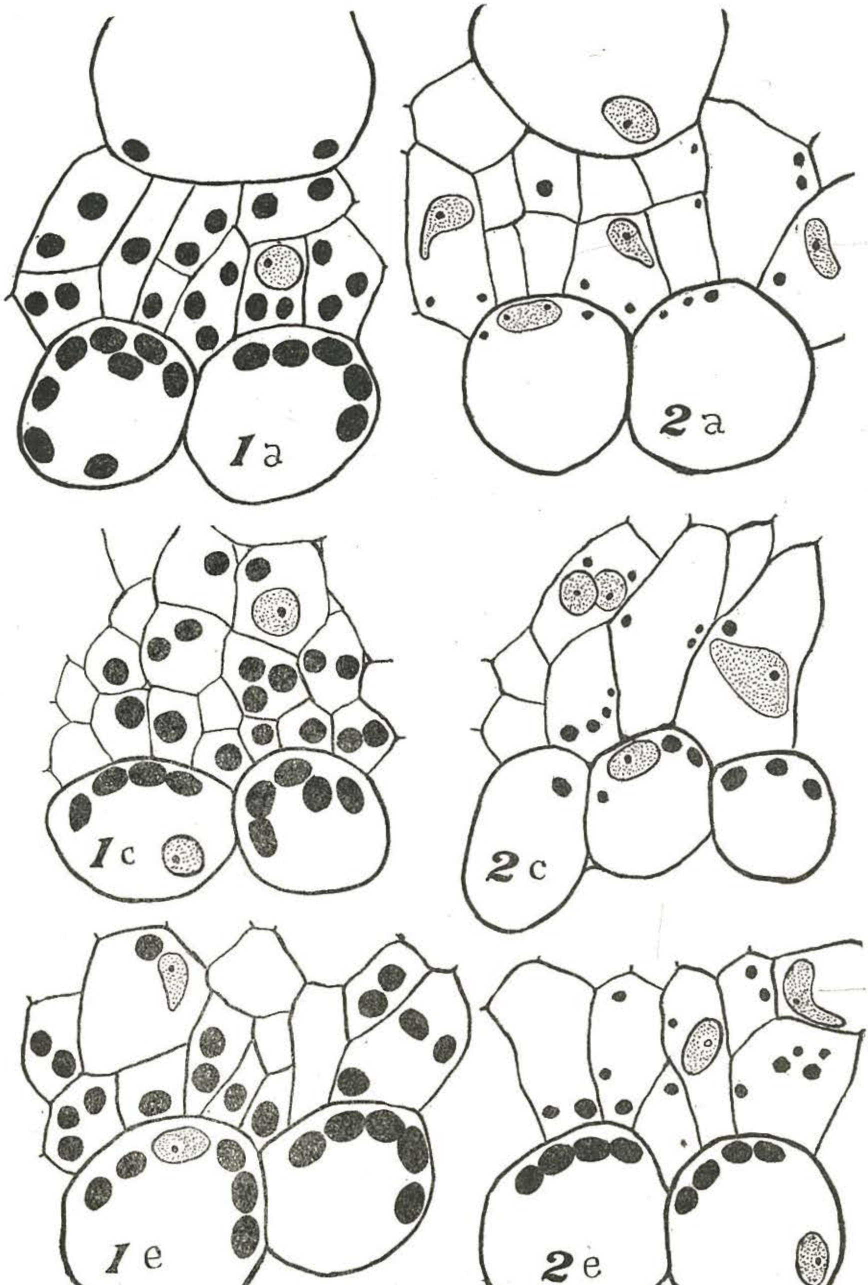
Fig. 23.—Cells from cross section of mosaic tobacco leaf, figured in 21. Note the intra-cellular body in one cell and the rings in the other.

Fig. 24.—Cell from cross section of mosaic of leaf of tobacco. Note the intra-cellular bodies and the abnormal chloroplasts.

Fig. 25.—Plant hair from mosaic tobacco. Note the intra-cellular body in the outer cell.

Fig. 26.—Nuclei and intra-cellular bodies from cross section of mosaic leaf of tobacco, figured in Fig. 20.

PLATE I



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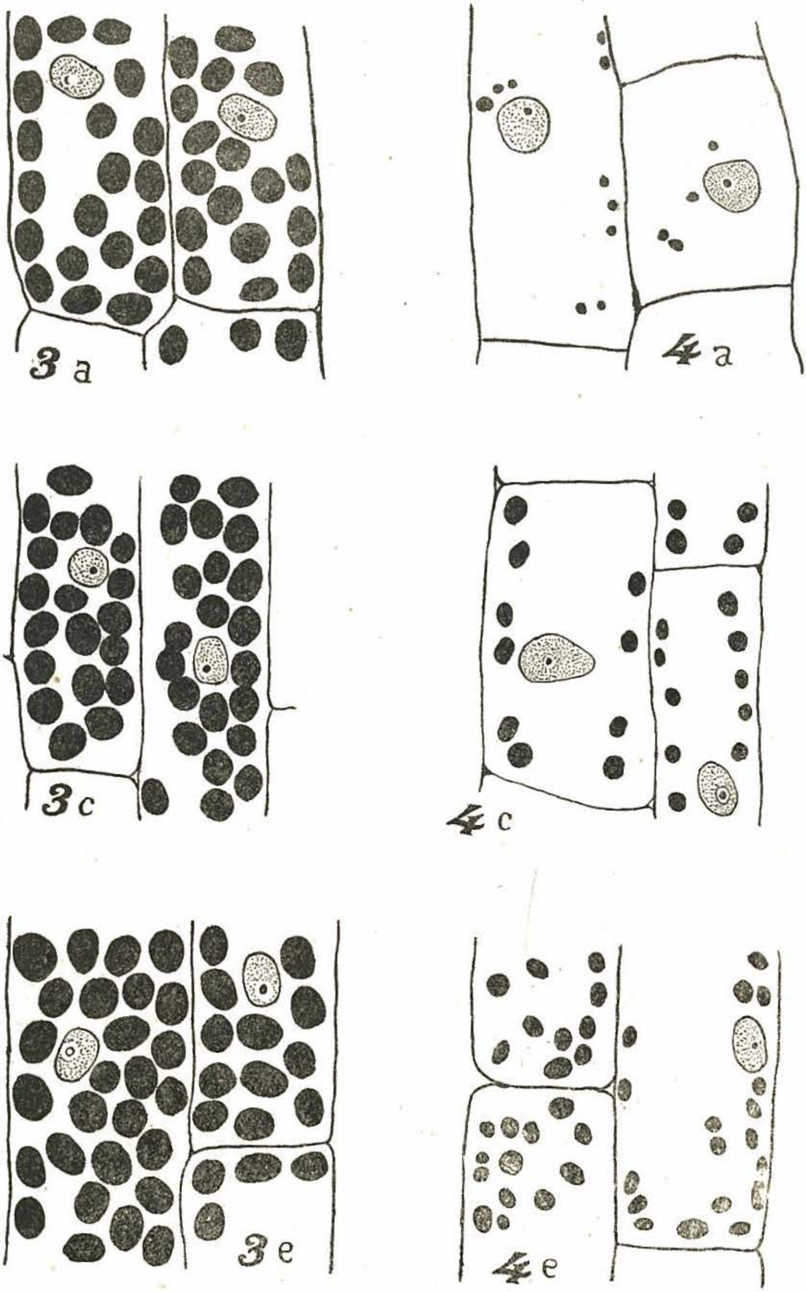

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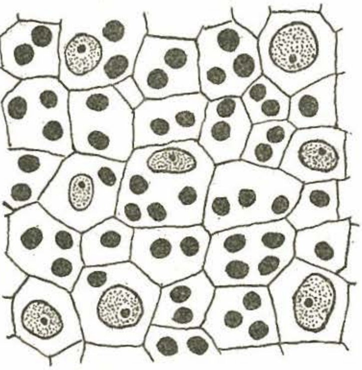

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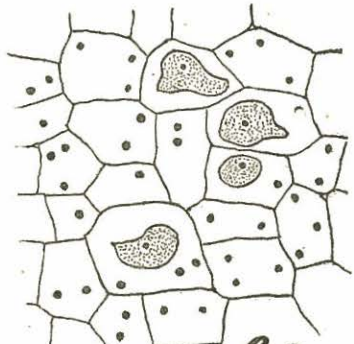

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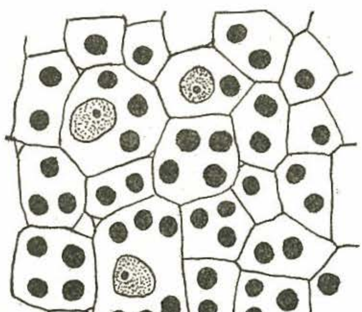

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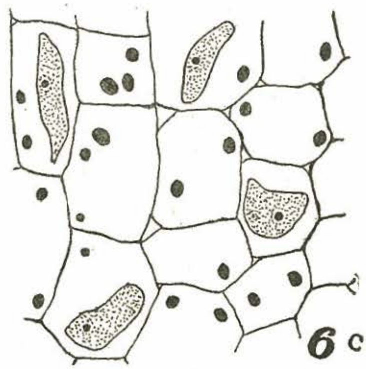

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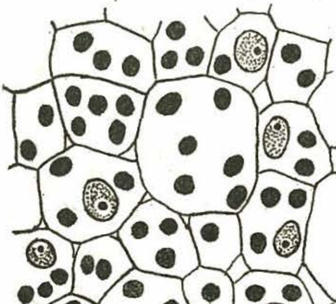

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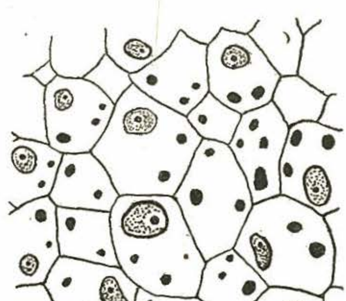

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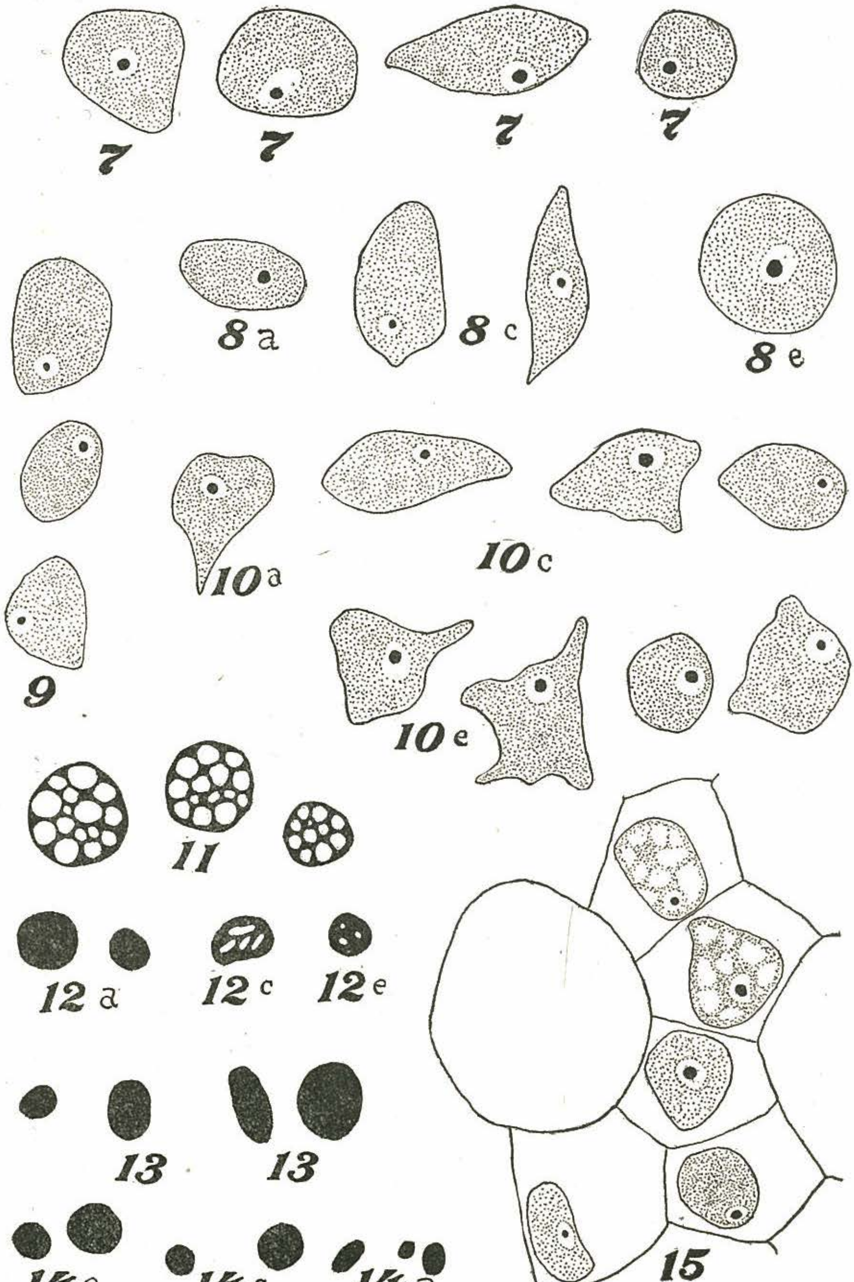
PLATE IV



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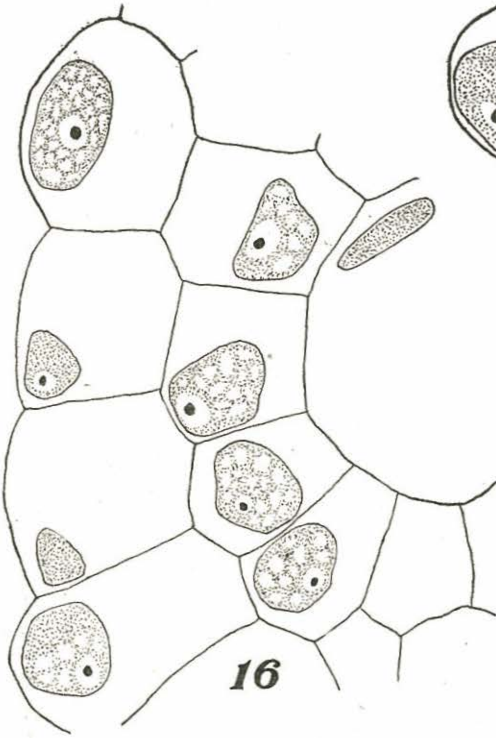

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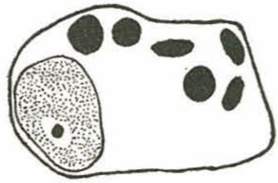

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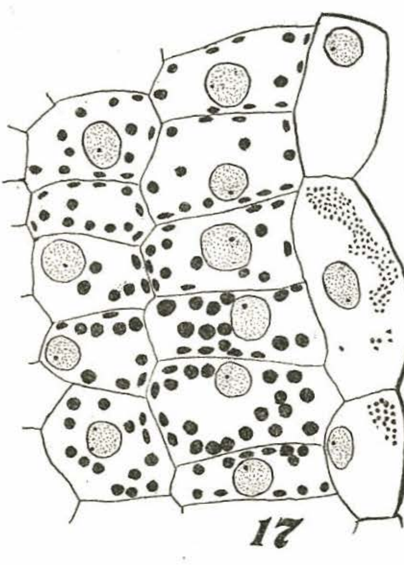

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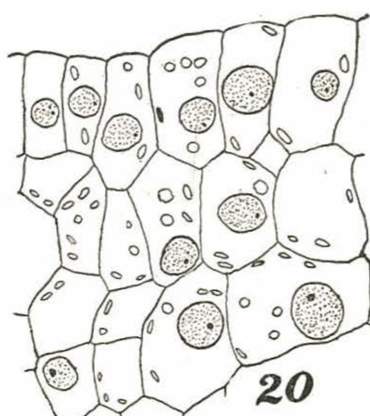

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PLATE VI