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On the Structure of the Cytoplasm around the Blepharoplast in *Cycas revoluta.*

By

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With Plates XXVI & XXVII.

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The structure and nature of the astral rays around the centrosome have been studied and discussed by many investigators from both morphological and physiological points of view in fixed as well as living materials from the early days of cytological investigation to date. The blepharoplast has, in the whole structure, "exactly the appearance of a centrosome with its aster"[1], and its morphological nature in comparison with the centrosome was once seriously discussed. While the astral figure around the centrosome is visible in the living state of the cell[2], it has been reported by Hirase (1894) and Fujii (1899) that in *Ginkgo biloba* no radiation figure is observable around the blepharoplast in the fresh state of the cell. This difference between the cases of the centrosome and the blepharoplast led us to some closer comparative investigation of the structure of this vicinity of the cytoplasm in *Cycas revoluta* in the fixed and the living state of the cell. The results we obtained will be mentioned in the following few pages.

1) Coulter and Chamberlain (1917), p. 143.

2) On the botanical side the astral figure around the centrosome or around the localized poleplasma in the living state of the cell has been observed by Lauterborn in diatomes, and by Fitting in macrospore mother cells of *Isoëtes* (Lundegårdh, 1922, p. 285).

OBSERVATION.

1. Fixed Material.

The investigations were made with material sent from Kagoshima to our laboratory in Kyoto. Spermatozoids and eggs can well develop further in detached ovules from the plants. The material was fixed with FLEMMING's stronger solution or the Bonn modification of the solution soon after it reached us, and was imbedded in paraffin and sectioned with a microtome in the usual manner. For staining, FLEMMING's triple staining method was exclusively employed.

Two very large blepharoplasts stained red with safranin are found situated at both poles of the body cell (Fig. 1). These bodies or blepharoplasts contain many small vacuole-like bodies within. A beautiful system of rays is seen radiating from the blepharoplast as figured by IKENO (1898). With an objective of low magnifying power, the whole structure appears very much like a centrosome with its aster. In a high magnification made with ZEISS' apochromatic objective, 1.5 mm and a compensating ocular 12 or 18, the structure appears to be somewhat different in the two different fixations we employed. In the material fixed with FLEMMING's stronger solution the radiating figure is confined within a sphere, at the center of which the body of blepharoplast is situated (Fig. 2). The diameter of the sphere is about thrice as long as that of the blepharoplast. Outside this sphere the cytoplasm presents in an optical section a structure of double nets which reminds us of Fig. 12 in MEYER's "Analyse der Zelle," illustrating the double net structure of a jelly seen in the dark field illumination. Large nets consist of a row of small nets. In our Fig. 2 this structure can be most clearly seen in the part of the cytoplasm between the blepharoplast and the nucleus. The rays in the sphere run generally parallel two by two (Figs. 2 and 3). In some rays it is clear that these parallel rays are made up of a row of small nets or rings just in the same way as we have found them in the network of cytoplasm outside the sphere. A similar structure of rays

is shown in BÜTSCHLI's artificial aster (his Fig. 6, Taf. 1), which has been reproduced in our Fig. 6; especially clearly on the right hand side of the air bubble in the figure. In both cases this doubleness of the rays is most clearly seen close to the body of the blepharoplast or the air bubble. In the material fixed with the Bonn modification of FLEMMING's solution, the rays are much greater in number and much longer than those just mentioned above (Fig. 4). They are so long that some of them may reach the peripheral region of the body cell. The double net-like structure of the cytoplasm is not conspicuous in this fixation, but the double nature of rays is recognized in this case too.

It has not been studied whether the length of the rays is dependent upon the stages of development or different action of the fixing solution (cf. HERZFELD, 1927, p. 826). But it seems to be highly probable that it is due to the latter, because certain fixing solutions do not cause the production of any radiating figure at all, as was shown by Mr. NAGAO in our laboratory in his investigations with the same material in about the same stage[1].

For comparison, the body cells of *Ginkgo biloba* were examined. They were fixed with the Bonn modification of FLEMMING's solution. The general appearance of the fixed cytoplasm looks very much like that of *Cycas* fixed with the same fixative, the rays being very long (Fig. 5). The blepharoplasts are, however, much smaller than those of *Cycas* and closer observations were unobtainable.

2. *Living Material.*

Body cells in pollen tubes were observed in a medium of a 10% solution of cane sugar. In the living state there is found a large spherical or sometimes ellipsoidal nucleus at the center of the body cell, and a blepharoplast[2], which is round in the polar view and oval in the side view,

1) Unpublished.

2) HIRASE (1894) reports that in *Ginkgo biloba* the bodies soon disappear when mounted with water or a grape-sugar solution. In our case, however, no such case was met with, the bodies being quite distinct throughout the observations.

at each polar region of the cell. The nucleus assumes an irregular shape as soon as the cell begins to die. The blepharoplasts contain many round, but somewhat angular, and sometimes vacuole-like bodies within[1], which we have found in the fixed material too (Fig. 7). In the cytoplasm we find small granules or microsomes which, filling the interalveolar spaces, present a beautiful mesh-work arrangement in an optical cross-section (Fig. 8). When the cell is dying, the individual microsomes become obscure, but conversely the alveoli become distinctly observable. Near the blepharoplast the alveoli are much smaller, so that the microsomes are found much denser than where the alveoli are large (Fig. 7). But in the immediate vicinity of the blepharoplast we have generally an hyaline region of irregular shapes, partly filled with coarsely scattered masses of microsomes. There is no indication of the ray structure at all. In some instances, however, where no BROWNian movement was observed, a certain structure, which reminded us very much of that which was found in the material fixed with FLEMMING's stronger solution, could be found in this hyaline region. In these instances too, in an ordinary lighted room the hyaline part appeared quite structureless, but in a dark room, when we observed it very carefully, an alveolar structure, having no granules at all, could be seen in the hyaline part. The alveoli were of different sizes. At a certain distance from the blepharoplast, they were large and were surrounded by a row of small alveoli, a feature which closely resembles that observed in the fixed material. The nearer the alveoli are to the blepharoplast, the smaller they are in size, and the alveolar walls, continuing to those of the next alveoli, run convergently to the blepharoplast, thus presenting an appearance of rays. The general aspect is very much like the rays observed by IKENO (1898, p. 173), which he studied with fixed material. In another instance we came across a case which may explain this diversity in the results of observations just mentioned above. In this instance the hyaline region was entirely structureless at the beginning of the observation, as is normally the case. We did not attempt to make obser-

[1] In a certain condition the blepharoplasts appear quite homogeneous.

vations with the dark field illumination, but so far as the observations made in the ordinary way are concerned, the hyaline region around the blepharoplast appeared quite homogeneous. In this preparation BROWNian movement was observed in some of the microsomes. Observation continued for more than half an hour. At the end of the observation such an alveolar structure as mentioned above was noticed in the region around the blepharoplast. The contour of the nucleus was now irregular and the BROWNian movement of the microsomes had ceased and could no longer be seen. These facts show that the cell is dying and coagulation of the cytoplasm colloid is setting in, probably owing to the unfavourable conditions to which the cell was exposed during the observation. The observations were made under the microscope, an Osram lamp, 220V 100W, being used as the source of light. When we observe the spermatozoids of *Cycas revoluta* in diffused day light, they are active in the artificial medium for thirty or forty minutes, but when the Osram lamp is used as the light source, the spermatozoids soon die. From this fact we may perhaps rightly conclude that the coagulation phenomenon observed in the hyaline area is pathological, being diagonally opposite to the apparently similar phenomenon physiologically taking place in the formation of the aster in animal cells. It is a known fact that albumin may be coagulated by light (BECHHOLD, p. 144). The reasonable conclusion would be, therefore, that the hyaline area is a colloid, and the alveolar structure that gives an appearance of rays around the blepharoplast is merely a result of coagulation of hyaline plasma due to the unfavourable conditions to which it was exposed during observations, or of coagulation caused by fixing agents.

The observations mentioned above were made in Kyoto with material sent from Kagoshima. To see whether the results are the same even when very fresh material is used for observations, one of the writers (K.) went to Kagoshima last autumn, and made observations with material just taken from the plants. The results obtained were largely the same as formerly obtained in Kyoto. Some of the observations will be given below.

1). A 10% cane sugar solution was used as a medium. In this preparation the hyaline sphere was almost free from microsomes, but careful observations revealed the fact that the hyaline plasma is not crystal hyaline, but consists of many minute granules of different sizes. No indication of radiation figure was observed.

2). A 20% cane sugar solution was used as a medium. Near the blepharoplast microsomes were scattered irregularly or in more or less polygonal shapes in the hyaline plasma, but no indication of radiation figure could be seen. 30 minutes after the preparation was made, microsomes appeared as relatively dark drops imbedded in the hyaline matrix.

3). A 30% cane sugar solution was used. The blepharoplast was found surrounded with minute granules of different sizes. 45 minutes after the first observation was made, the general aspect was the same as that observed at the end of the observation of case 2, microsomes appearing as relatively dark drops.

In short there was not a single case, where the radiation figure was observed in the quite healthy state.

For comparison, body cells of *Ginkgo biloba* were observed in the same artificial medium as in the case of *Cycas revoluta*. The general structure of the body cell was the same as that of *Cycas*. The blepharoplasts contain many light-refracting bodies in them. Microsomes are found in the region around the blepharoplast and larger granules at the region near the nucleus, a larger amount of the latter existing at the polar side of the nucleus and a smaller amount towards the equatorial region, where they are minimum. The region around the blepharoplast is so full of microsomes that BROWNIAN movement can not be observed, and no structure that may indicate rays is found there at all.

CONCLUSION.

According to the micro-dissection studies of CHAMBERS (1917), the formation of the aster of the centrosome is a reversal phenomenon from the sol to the gel state of the cytoplasm. The "sphere" or the hyaloplasm-sphere around the centrosome "is a liquid region free of granules

occupying the center of the aster." "The increase in size of the sphere is apparently due to the accumulation of liquid flowing into the sphere from all parts of the cytoplasm. The aster rays appear to be channels in which the centripetal flow occurs. The cytoplasm between the rays is in the gel state to which the rigidity of the aster is due." "A periodical reversal of the sol to the gel state and *vice versa* has been demonstrated in the cell protoplasm during division."

In *Cycas revoluta*, in the healthy condition of living material, we find a hyaline region of irregular shape having the blepharoplast in its center, but nothing in this region nor in the cytoplasm outside it that may indicate the radiation figure of the passage of liquid or rays[1]. In fixed material a beautiful system of rays is found in this hyaline area of the cytoplasm in *Cycas* as well as in *Ginkgo*. These rays are, however, an artificial product caused by fixatives. According to IKENO (1898), in a very young stage, the aster is not visible even in fixed material. The hyaline plasma around the blepharoplast is not hyaline in the strict sense, but full of minute granules of different sizes down to the limits of microscopical vision. When the cell is brought to an unhealthy condition, there probably occurs a certain change in the colloidal state of hyaline plasma, which may be taken as an analogous phenomenon to what takes place on coagulation of the cytoplasm by fixation. When the cell presents any sign of the radiation figure, it is no longer healthy. This forms a contrast to the aster in animal cells, which is reversible from its gel to the sol state or *vice versa* in the periodic recurrence of the nuclear division.

It is the generally accepted view that the centrosome may have a close relation to the mechanism of mitosis in some way or other. But as to the question how, then, the centrosome acts upon the mitosis, there are different views. Among them, those based on chemical points of view, such as put forward by CARNOY (1885),[2] BÜTSCHLI (1892),[2] and

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[1] In his book "Zelle und Cytoplasma," LUNDEGÅRDH writes: "Als feine Ströme betrachtet auch CHAMBERLAIN (1909) die Strahlungen um die Blepharoplaste bei *Dioon edule*." Unfortunately, however, the original paper is not within our reach at present, and has not been consulted here.

[2] WILSON (1919), p. 110.

BERNSTEIN (1912) are noteworthy. The fact demonstrated by CHAMBERS (1917) in the micro-dissection studies that the aster is most rigid near its center and is less rigid towards its periphery seems to conform with these chemical views.

While the astral structure in animal cells is seen, and can be touched with micro-dissection needles in cells in the living state, there is no such structure at all around the blepharoplast. This structural difference between the cytoplasm around the centrosome and that around the blepharoplast, seems to show that the physiological functions of these two bodies are different, one from the other.

SUMMARY.

A comparative study in the fresh and fixed state of the cytoplasmic structure around the blepharoplast was carried out in *Cycas revoluta*, and for comparison parallel observations were also made in *Ginkgo biloba*.

In fixed material rays are found radiating from the blepharoplast. The general appearance of the ray figure is different with different fixatives.

In living material there is found no ray structure at all around the blepharoplast. The cytoplasm of the body cell is alveolar in structure, microsomes being arranged in interalveolar spaces. Near the blepharoplast the alveoli are smaller down to the limit where the term alveoli is now to be substituted by aggregation of microsomes, generally leaving an apparently hyaline area of irregular shape free from microsomes in the immediate vicinity of the blepharoplast.

In dying body cells very careful observations have revealed the fact that there appears an alveolar structure in the hyaline area. The alveoli are smaller near the blepharoplast and larger the further away from it they are. The walls of the alveoli make an appearance of a ray-figure, continuing from one to the next.

A comparison of the cytoplasmic structure around the blepharoplast observed by us and the centrosome studied by CHAMBERS has been attempted and the structural difference between them is pointed out.

In conclusion we wish to express our sincere thanks to Professor YOSHIMURA, Director of the Imperial College of Agriculture and Forestry of Kagoshima and Professor KAWAGOE of the same College for their kindness in placing material at our disposal and also in giving liberty to use their laboratory during the visit of one of us to Kagoshima.

September, 1928.

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EXPLANATION OF PLATES.

Plate XXVI.

Fig. 1. Body cell of *Cycas revoluta*. Fixed with FLEMMING's stronger solution. Microphotographed with ZEISS' apochrom. obj. 4 mm × comp. oc. 8. Magnification: about × 345.

Fig. 2. The upper blepharoplast of the same is shown. Microphotographed with ZEISS' apochrom. imm. 1.5 mm × comp. oc. 18. Magnification: about × 1750.

Fig. 3. The same (Fig. 2) enlarged: × 1.5.

Fig. 4. Blepharoplast of *Cycas revoluta*. Fixed with the Bonn modification of FLEMMING's solution. Microphotographed with ZEISS' apochrom. imm. 1.5 mm × comp. oc. 12. Magnification: about × 1450.

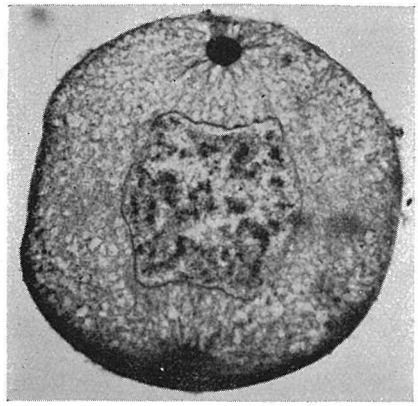
Fig. 5. Blepharoplast of *Ginkgo biloba*. Fixed with the Bonn modification of FLEMMING's solution. Microphotographed with ZEISS' apochrom. imm. 1.5 mm × comp. oc. 12. Magnification: about × 1150.

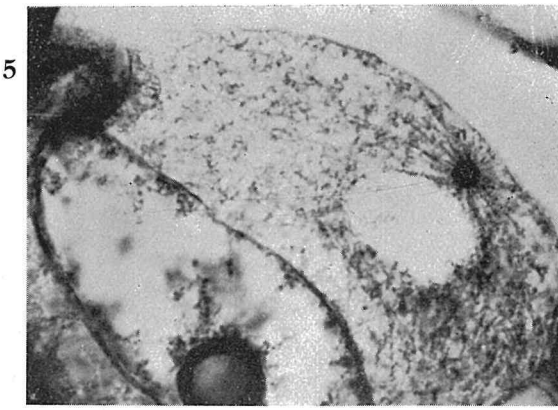
Fig. 6. Reproduced from BÜTSCHLI's Atlas, Fig. 6, Taf. I.

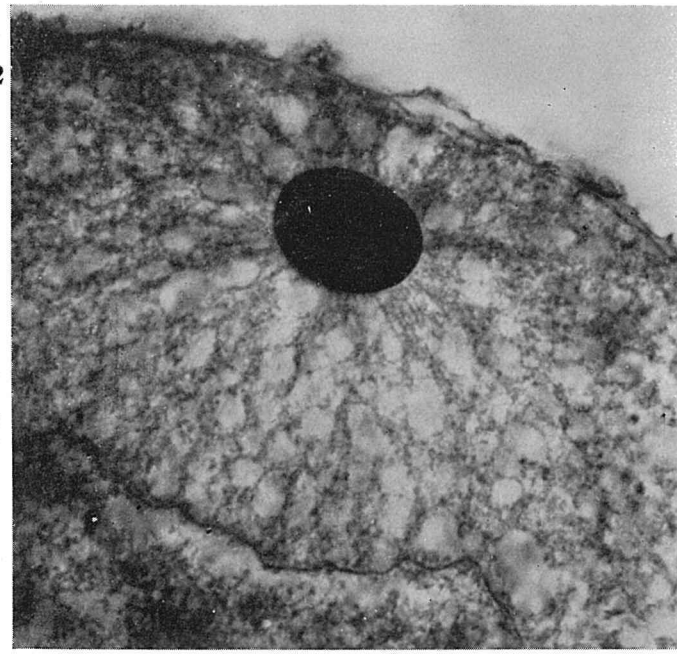
Plate XXVII.

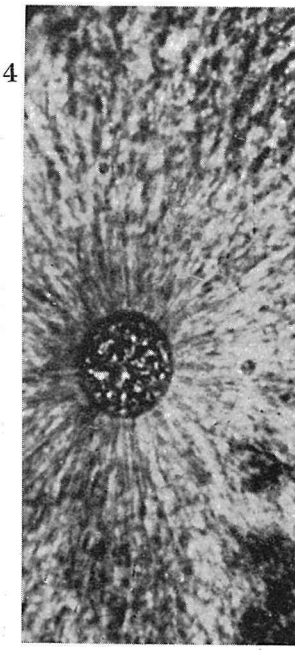
Fig. 7. Blepharoplast of *Cycas revoluta* in fresh material. Microphotographed with ZEISS' apochrom. imm. 2 mm × comp. oc. 18. In this preparation the large bulk of the hyaline area around the blepharoplast is full of minute granules of microscopically visible sizes.

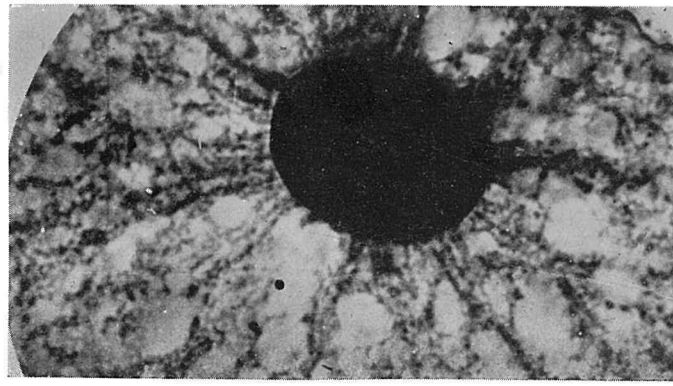
Fig. 8. Cytoplasm of fresh body cell of *Cycas revoluta* showing its alveolar structure. Microphotographed with ZEISS' apochrom. imm. 2 mm × comp. oc. 12.

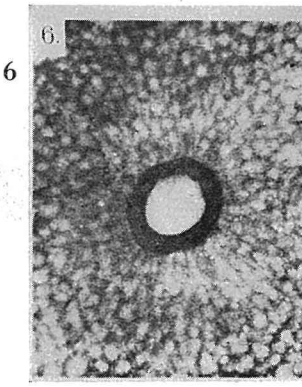












Phot. Kuwada & Maeda.

Kuwada & Maeda : Cytoplasm Structure round Blepharoplast.

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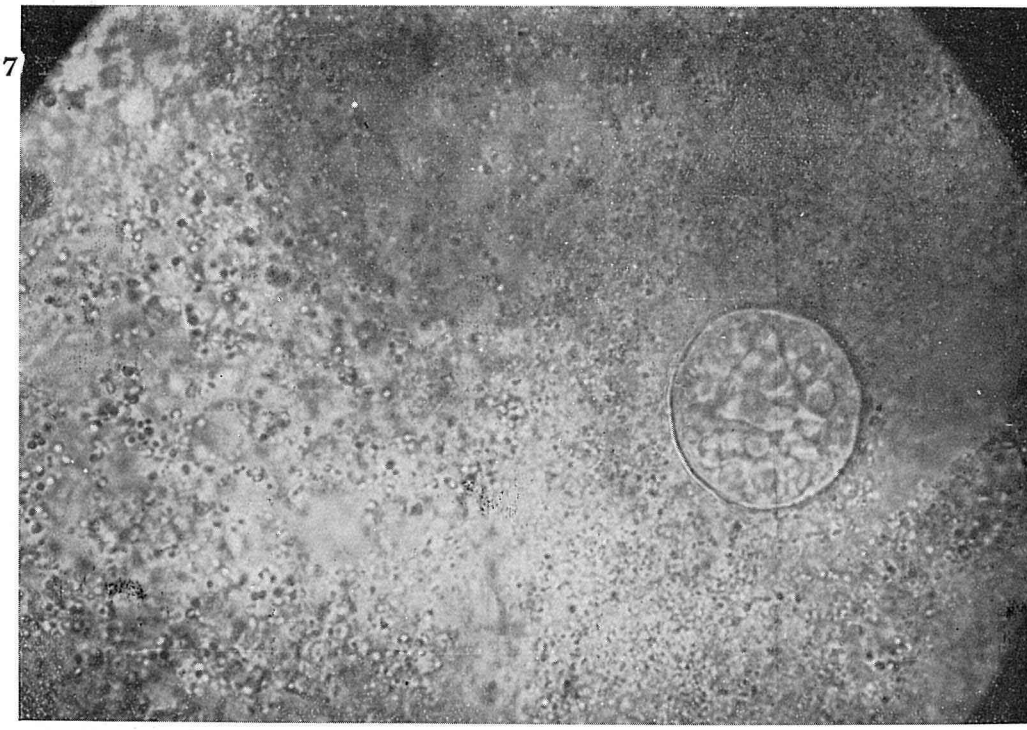

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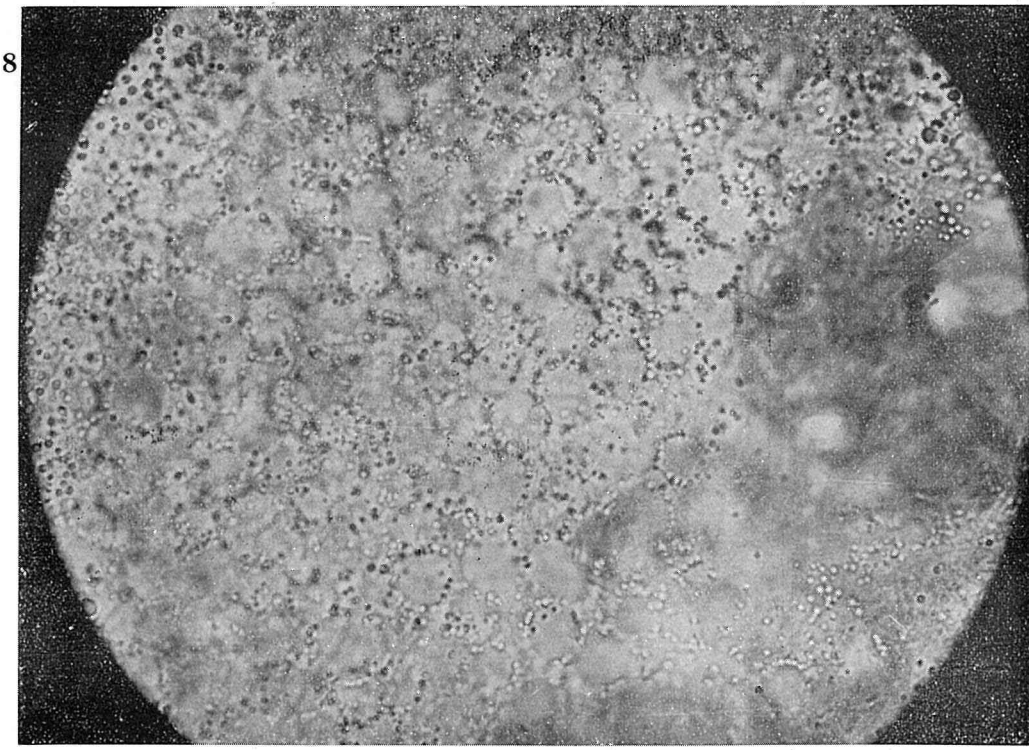

Phot. KUWADA & MAEDA.

KUWADA, & MAEDA : Cytoplasm Structure round Blepharoplast.