"The Cell Structure of the Cyanophyceæ.—Preliminary Paper." By HAROLD WAGER. Communicated by D. H. SCOTT, M.A., Ph.D., F.R.S. Received October 9, 1903.

The Cyanophyceæ form a group of plants which resemble the Algæ in some respects, but are sharply differentiated from them as regards the structure of their cell contents. During the last 20 years they have been the subject of numerous memoirs in which the problem of their cell structure has been attacked, but with very varying results, and much controversy has arisen concerning the exact nature of the central body or nucleus and the presence or absence of a chromatophore. On the one side, we have observers who deny the existence of a nucleus or chromatophore in these forms, on the other, those who consider that the central body is either a true nucleus or the representative of one, and that the colouring matter is contained in a definite chromatophore.

The investigation of the cytology of these organisms is certainly not an easy one, and it is not astonishing that so many different interpretations are given of the facts observed. But it seems to me that it is not an impossible task to arrive at more definite conclusions than has hitherto been done, although we need to know far more of the cytology of the lower organisms, both plant and animal, before we can determine with any certainty the exact nature of their cell structures or their relationship to other groups.

In attacking the problem it is necessary that the methods used should be as refined as apparatus and re-agents will allow, and it is especially important not only that the highest possible powers of the microscope should be used, but that the illumination should be as perfect as possible. For this purpose a good sub-stage condenser is necessary, and for the finer details of structure an oil immersion condenser has often been used. It is necessary also, in order to obtain good comparable results, that the species investigated should be in a sound and healthy state of development. The cells very soon become altered in character if kept under unfavourable conditions, and many of the contradictory accounts which have been given are probably due, in part, to want of care in this respect.

As regards the preservation and staining of these plants, the ordinary methods used in connection with cytological investigations are sufficient, but special care is required in their application, especially in the matter of staining and washing out. For fixing and preserving the material I have found corrosive sublimate, Flemming's chromosmium-acetic solution, Pereny's fixing fluid, Gram's solution of iodine and absolute alcohol the most useful. The various stains used included

Delafield's hæmatoxylin, Heidenhain's iron hæmatoxylin, methylene blue, fuchsin, methyl green and fuchsin, methyl green and eosin and picro-carmine. The specimens may be mounted whole either in balsam or glycerine or may be first of all sectioned in the microtome, by which certain details of structure can be shown up more clearly.

The Living Cell.—In the examination of the living cell under a high power objective it is not difficult to observe that the cell contents are differentiated into two parts, an outer peripheral layer in which the colouring matter is placed, and an inner central part which is colourless. The structure of these two regions is difficult to make out, but both may contain granules of varying sizes, and in some of the larger forms a distinct vesiculate structure can be observed. The outer coloured layer appears in many cases to encroach upon the central portion, which is thereby rendered irregular in outline, but in no case was a penetration of the coloured layer into the middle of the central region observed; in the younger cells near the ends of the filament the central portion is nearly always more regular in outline than in the older cells.

Cytoplasm.—In all forms which admit of a careful examination of stained specimens under high powers the structure of the cytoplasm appears to be vesiculate or reticulate, the meshes or alveoli being regularly arranged and somewhat drawn out in the radial and longitudinal directions, so as to give in transverse sections an appearance of trabeculæ radiating from the central body to the periphery of the cell (fig. 3). When granules are present these are found in or on the threads, never, so far as I can see, in the meshes of the network. In young vigorous cells the cytoplasm presents uniformly the structure just described, but in old and degenerating cells a very considerable amount of vacuolization is observable in which the central body often becomes involved, so that in such cases no distinction between central body and peripheral cytoplasm can be seen. These vacuolar spaces often contain a slightly coloured sap, probably produced by the breaking down of the colouring matters and their diffusion into the cytoplasm.

Colouring Matter.—It has not been possible to obtain any evidence of the existence of a differentiated chromatophore, such as obtains in the higher Algæ, either in fresh or stained specimens, and I am, therefore, quite unable to support Fischer's statement that the central body is surrounded by a cylindrical chromatophore. The colouring matter appears to be contained in minute granules distributed through the cytoplasm in such large numbers as to give the appearance of a uniform diffuse coloration to the whole of the peripheral layer. They appear in many cases to be arranged in regular rows which run slightly obliquely across the cell, and are so clearly defined in some forms as to give the impression that they are contained in definite

fibrils in the cytoplasm. But from a careful examination of the cells of *Phormidium retzii*, in which the coloured granules are sometimes easily seen, it appears to me that they are contained in the threads of the cytoplasmic reticulum only, and that the definite arrangement in rows is due to the uniform and regular distribution of the threads of the reticulum between the central body and the cell wall. Nevertheless the possibility of the existence of a filrillar structure in some forms is not excluded, and I am not prepared to state definitely that it does not occur.

These coloured granules appear to contain both the colouring matters which are known to occur in the Cyanophyceæ, chlorophyll and anthocyan. Hegler considers them as special organs of the cell, and suggests that they should be called *Cyanoplasts*, but it seems to me that they may very well be compared to the "grana" which are found in the chromatophores of such forms as Euglena, diatoms, &c.,* the difference between them being that in the Cyanophyceæ the "grana" are free in the cytoplasm, in other green plants they are contained in specialised portions of it. This probably indicates that so far as the colouring matter is concerned, we have in the Cyanophyceæ not only a simpler, but also a more rudimentary type of structure than in those forms with a specialised chromatophore or chlorophyll grain.

The Central Body.—The central, colourless portion of the cell is considered by many observers to represent the nucleus of the higher plants, by others to be merely a colourless portion of the cytoplasm, or a slightly differentiated portion of it. Whatever it may be it certainly differs in appearance in the living state from the nucleus of the Algæ and higher plants, and, in fact, one of the arguments which might be adduced against its nuclear nature is that in the fresh condition it is so very easily seen, whilst the nuclei of some higher forms are very difficult to make out in the living condition. The question of the presence of a nucleus in the Cyanophyceæ is probably one of the most controverted questions of recent years. So long ago as 1879 Schmitz announced the discovery, in the cell of a species of Gleocapsa, of a homogeneous, deeply stainable, central mass, which he took to be the nucleus. He came to the conclusion later, however, that this could not be regarded as a true nucleus, but only as a chromatic granule. Scott† and Zacharias,‡ working independently in 1887, came to the conclusion that the central body represents the nucleus of the higher plants. Zacharias was able to make out in some forms which he examined a network-like structure, and demonstrated in it the presence of nuclein, whilst Scott figured structures with a network and

* See Arthur Meyer, 'Bot. Cent.,' 1882.
† 'Journ. Linn. Soc. Bot.,' 1887.
‡ 'Bot. Zeit.,' 1887.

division stages remarkably like some of the stages in mitotic nuclear division in the higher plants. In a later paper* Zacharias states that although the central body contains nuclein it cannot be regarded as a true nucleus. Bütschli,† in the same year, on the other hand, comes to a very definite conclusion that the central body is not only nuclear in nature, but that it is the homologue of the nucleus of higher forms. Fischer‡ controverted Bütschli's observations, and stated that the appearances figured by that author are due to a plasmolyzed condition of the cells. In a later paper§ he withdrew this statement, but he still maintained that the central body is not of the nature of a nucleus. Deinega‖ was unable to come to any definite conclusion as to the presence or absence of a nucleus. Zukal¶ considered certain granules in the central body to be the nuclei. Hieronymus** considered it to be an open nucleus, and to have a peculiar structure. Palla†† says the central body is homogeneous, and contains no granules. Nadson‡‡ says it is of a nuclear nature. Macallum§§ finds that it contains chromatin, but states that it is not a nucleus, and that there is nothing resembling a nucleus in the Cyanophyceæ. Hegler‖‖ comes definitely to the conclusion that, notwithstanding the absence of a membrane and nucleolus, the central body is a nucleus, and divides by a process of karyokinesis similar to that which occurs in higher plants. Massart,¶¶ on the other hand, in 1901, basing his conclusions upon facts observed after staining the cell *intra vitam* with methylene blue, states that not only does it not present the characters of the nucleus of the higher plants, but that it cannot even be regarded as a nucleus of a simpler type.

It is obvious from these various contradictory statements that a considerable amount of caution is necessary in arriving at any conclusion as to the nature of the central body; but I have very little doubt from my own observations that it is of the nature of a nucleus, that it possesses certain of the characteristics of the nuclei of the higher plants, but not all, and that it can very fairly be regarded as a nucleus of a simple or rudimentary type.

* 'Bot. Zeit.,' 1890.

† 'Ueber den Bau der Bacterien, etc.,' Leipzig, 1890.

‡ 'Die plasmolyse der Bakterien,' 1891.

§ 'Untersuchungen über den Bau der Cyanophyceen und Bacterien,' Jena, 1897.

‖ 'Bulletin Soc. Impér. des Nat.,' Moscou, 1891.

¶ 'Sitzungsb. Kaiserl. Akad. in Wien,' 1892.

** 'Cohn's Beitz. z. Biol. d. Pflanzen,' 1892.

†† 'Jahrb. f. Wiss. Bot.,' 1893.

‡‡ 'Ueber den Bau des Cyanophyceen-protoplastes,' 1895.

§§ 'Trans. Canadian Institute,' 1899.

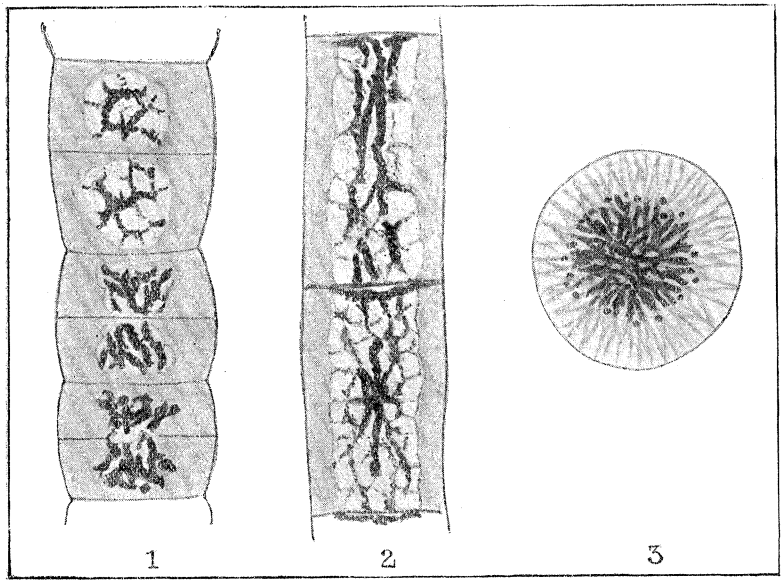
‖‖ 'Jahrb. f. Wiss. Bot.,' 1901.

¶¶ 'Sur le Protoplasme des Schizophytes,' Brussels, 1901.

If the cells of any of the larger species of Cyanophyceæ, with somewhat elongate cells, be stained with hæmatoxylin, according to the method of Heidenhain, or with a solution of fuchsin, the substance of the central body becomes clearly defined from the peripheral cytoplasm. It does not possess a definite membrane, but in certain cells, generally near the ends of the filaments, it is found inside a vacuolar space in the centre of the cell, so that it is clearly separated from the cytoplasm by the delicate vacuolar layer or membrane (fig. 1). In the older cells this vacuolar space seems to disappear, but in Tolypothrix, even in the oldest cells of the filament, it can still be recognised (fig. 2). The central body is usually, in young cells, surrounded on all sides by the peripheral coloured layer, but in some forms, and especially in older cells, it extends from one end of the cell to the other, and is in close contact with the transverse walls (fig. 2), whilst the peripheral coloured cytoplasm forms a cylinder around it. The central body appears in such cases to be intimately concerned in the formation of the cell wall, and is in close connection with the cyanophycin granules which are often found in contact with it in certain species, and which are usually regarded as of the nature of reserve food substances. It seems not unlikely that the central body is concerned in the formation of these granules. In one case which I examined, the central body was almost entirely free from chromatin, whilst the cyanophycin granules, with which it was in close contact, were, contrary to their usual character, stained deeply in hæmatoxylin, the central body being only very slightly stained. I have also obtained a distinct reaction for phosphorus in granules, which by their position and general appearance were cyanophycin granules, whilst the central body gave only a very slight indication of it.

The substance of the central body consists of a more or less regular granular network (figs. 1, 2, 3). The granules are small, and usually uniform in size, but here and there may sometimes occur a granule or granules larger than the rest. The ground substance of the net work upon which the granules are placed stains only slightly in nuclear stains, and appears to correspond to the linin or plastin network of the nuclei of higher forms. The granules themselves stain deeply in nearly all the nuclear stains; they resist the action of artificial digestive fluid, and give a distinct, and in some forms a strong reaction for phosphorus when treated according to the method devised by Macallum. They probably, therefore, correspond to the "central substance" of Zacharias, and to those portions of the central body which are found by Macallum to contain masked iron and phosphorus. From a consideration of these facts it is difficult to escape the conclusion arrived at by Macallum that "there can be . . . very little doubt that a chromatin-like substance is present in the central body." And when we further consider the structure of this central body, its

granular network, its definite position, and its delimitation from the rest of the protoplasm inside a vacuolar space, it seems difficult to escape the further conclusion that it is at least to be regarded as a simple type of nucleus.



FIGS. 1 and 2.—*Tolypothrix lanata*: (1) Young, (2) Old cells.
FIG. 3.—*Oscillaria limosa*: transverse microtome section.

Cell Division.—The division of the cell is brought about by the formation of a transverse wall which grows inwards from the lateral wall and divides the cytoplasms and nucleus into two equal or nearly equal parts (fig. 1). The division of the nucleus is direct, but it simulates the mitotic figures seen in the higher plants, and recalls some of the features of nuclear division in Euglena. It may possibly represent a very rudimentary form of mitosis. As the cell grows in length the chromatin network becomes drawn out in a longitudinal direction and presents the appearance of a number of chromosomes standing side by side. In some cases these threads appear to be actually separate from one another, but on closer examination this appears not to be the case; they are always found to be more or less connected together and retain generally the features of the network as seen in the resting stage. As the new transverse cell wall grows it comes into contact with the nucleus, which thereby becomes constricted as shown in the lowest cell in fig. 1, and the chromatin network then becomes divided into two parts, thread by thread, until in the last stage only one thread may be left, which may persist for some

time connecting the two daughter nuclei together until complete separation takes place (see fig. 2). In some cases, shortly after the division is completed, delicate threads may be observed connecting the daughter nuclei to the new cell wall, and we get then a resemblance to the diaster stage of ordinary karyokinetic division. This is the only indication of the existence of anything like a spindle figure which I have observed in the Cyanophyceæ, and it is not by any means a well-marked or constant feature of the division.

The division of the cell appears to go on independently of the nuclear division. It is quite common to find, long before the first division is complete, several new cell walls in various stages of development in other parts of the cell.

Theoretical.—From a careful consideration of the observations contained in this paper it seems clear that, although we cannot regard the central body of the Cyanophyceæ as a nucleus of a normal type similar to the nuclei of the higher plants, it certainly appears to possess a sufficient number of the characteristics of such nuclei to warrant its recognition as a nucleus of a simple or rudimentary type. If we take the chief chemical and morphological characters of the nuclei of higher organisms and compare them with those of the central body, we shall find that it has as much claim to be regarded as a nucleus as some of the structures which are accepted as nuclei in the protozoa. Out of some twelve main characters, chemical and morphological, which are found attaching to the nuclei of higher plants, we have seven, and possibly nine, occurring in the nuclei of the Cyanophyceæ.

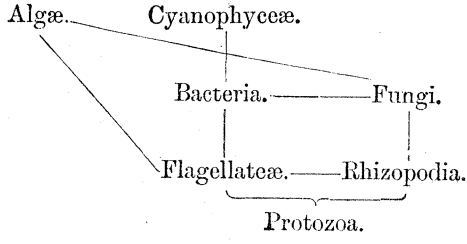
These are: (1) the presence of a nuclear network; (2) its reaction towards nuclear stains; (3) its behaviour towards digestive fluid; (4) the presence of phosphorus; (5) the presence of masked iron; (6) the amitotic division, which resembles, in some respects, the division in Euglena; and (7) the presence of chromatin granules on a linin framework. It differs from the nucleus of the higher plants in the absence of a true mitosis with spindle fibres, and in the absence of a nuclear membrane and nucleolus; but under certain conditions the deeply-stained substance of the central body is found condensed into a deeply-stained granule suspended by delicate fibres in the centre of the cell, and in young cells the central body is often limited towards the cytoplasm by a vacuolar membrane, so that the presence, under certain conditions, of a body resembling a nucleolus, and at least a rudimentary nuclear membrane, is not excluded.

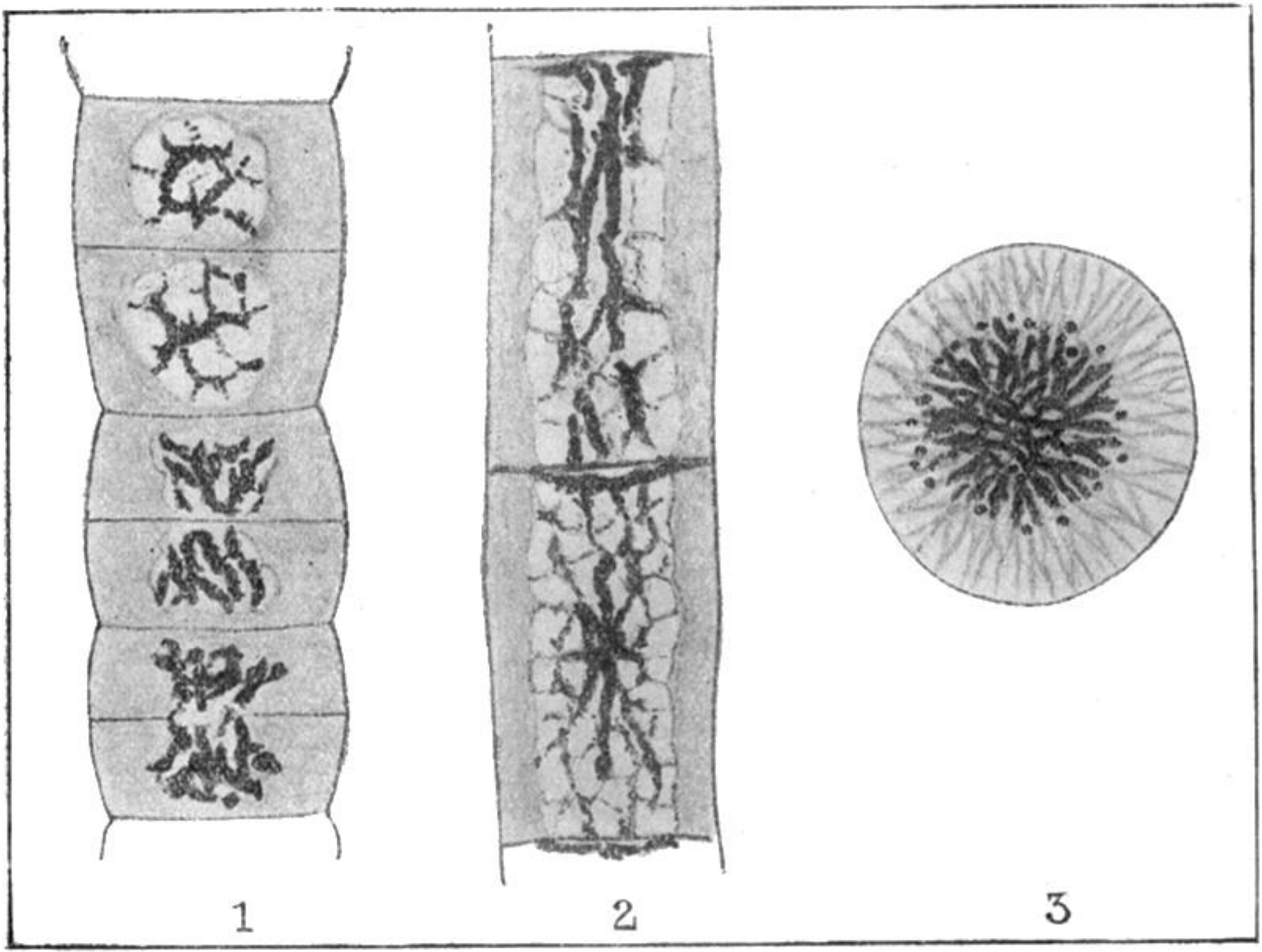
If it is not a nucleus then it can only be (1) a specialised portion of the cytoplasm; (2) a body of the nature of a pyrenoid; or (3) a special organ of the cell of which we know not the function.

There seems to me to be no doubt that it is among these plants and in the bacteria and protozoa that we must seek for those types of nuclei which will give us a clue to the most rudimentary condition

of the nucleus; and if we conceive that, in a very rudimentary stage of cell development, the chromatin was diffused through the cytoplasm either in the form of granules or in a liquid condition, then it is not improbable that the accumulation of the chromatin in a single chromatin granule, as in some protozoa, or in a granular network inside a vacuole as in the Cyanophyceæ, would indicate a developmental stage in the evolution of the nucleus.

Relations of the Cyanophyceæ to other Groups.—So far as the structure of the cell is concerned, the Cyanophyceæ do not appear to have any very definite characteristics which connect them with any known group of plants, and certainly not with the Algæ. They may possibly be allied to the bacteria with which they appear to have some features in common, but so far as our present knowledge goes their position with respect to other groups is as indicated in the following diagram :—





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