

# The Nucleus of the Yeast-Plant.

BY

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With Plates **XXIX** and **XXX**.

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## LITERATURE.

THE following interesting observation was made by Nägeli in 1844 concerning the nuclei in the cells of Fungi: 'Structures resembling nuclei may be detected here and there in the cells of the Fungi. The fermentation-fungus in the must of Wine and in Yeast often exhibits a little nucleus of whitish mucus, lying on the membrane, regularly in each cell.'

This is the first reference made to the presence of a nucleus in the Yeast-cell. It may perhaps be doubted if Nägeli had before him the body known to recent observers as the nucleus; but it is interesting to find that under certain conditions the nucleus can be observed in the fresh condition of the cell, and is always in the position indicated by Nägeli.

Five years later the nucleus of the Yeast-plant was made the subject of investigation by Schleiden ('49), who applied reagents to determine its presence. In his work he states that, on treating Yeast with ether, alcohol, or potash, one

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finds rounded delicate cells with a thin but clear cell-wall, containing clear contents with more or less delicate granules which singly or in groups occur on the inner surface of the cell-wall, and (almost?) always a large, round flat body (a cytoblast).

Brücke ('61) objected to Nägeli's observations, and says that, without wishing to maintain that Nägeli had not true nuclei before him, he is able, with improved apparatus and by observations on living material as well as on material treated with iodine and acetic acid, to state that no nucleus is visible, and remarks that nobody is justified in taking bodies of varying size and number, such as often occur, for nuclei.

Schmitz ('79), in his valuable paper on the nuclei of the Thallophytes, was able to show however, by the use of haematoxylin, that the Yeast-cell possesses a body which stains more deeply than the rest of the protoplasm. It occurs about the middle of the cell in the protoplasm near the vacuole, and he regards it as a nucleus.

Strasburger also ('84, '87) was able, by means of the same staining fluid, to demonstrate the nucleus described by Schmitz. He says that on fixing with picric acid and staining with haematoxylin, a rounded nucleus is to be seen near the centre, stained more deeply than the remainder of the cell-contents. In the living unstained condition a nucleus is not visible.

Zalewski ('85) found that the presence of a nucleus can be very easily rendered evident by placing the Yeast-cells in pure water for some time and staining with haematoxylin. The nucleus is oval in shape and possesses a small nucleolus in the centre. It stains more deeply than the other part of the protoplasm, which forms a dense layer round the nucleus. In budding cells the nucleus could not be discovered, nor in cells in which spore-formation was taking place; in these cases it was probably undergoing division, as a nucleus was observed in the fully formed spores and budded cells.

Objections to these observations were brought forward by Krasser ('85), who was able to observe granular structures

in Yeast-cells after staining, but could not recognize a nucleus, and was not able therefore to confirm the statements of previous observers. He points out that there is no specific staining reaction for the nucleus. The absence of a definite nucleus in the Yeast-cell is supported by the very rapid growth of the organism. The Yeast-cell possesses nuclein, but this is distributed through the protoplasm.

Both Hansen ('86) and Zacharias ('87) succeeded in demonstrating the presence of the body described as a nucleus by Schmitz.

Zimmermann ('87) states in his text-book (*Morphology and Physiology of the Plant-cell*) that he observed in a preparation stained with haematoxylin a deeply stained body which might be regarded as a nucleus, but he does not think this has been satisfactorily demonstrated. In a more recent communication, however ('93), he gives, in an account of the literature of the subject, this observation as supporting the view that the Yeast-cell contains a nucleus; and in a footnote referring to the doubt expressed by Krasser that Zimmermann had not been able to satisfy himself that a nucleus was present, he expressly states that in his figure given on p. 23 he regards the deeply stained body as a nucleus and the lighter body as a vacuole.

The evidence in favour of the presence of a nucleus is so far perhaps not very convincing or satisfactory, and Raum ('91), after an examination of a large number of various kinds of Yeast, came to the conclusion that it was not possible to state definitely that a nucleus is present in the Yeast-cell. On staining, with methylene-blue and Bismarck-brown, cover-glass preparations which had been allowed to dry and had been fixed by heat or corrosive sublimate, he found that the protoplasm stained brown and that a number of granules present in it stained black; whilst on staining with eosin and methylene-blue, the granules became dark violet. The number of granules varies in the different species and also their arrangement. They are not always present. Whether they are composed of nuclein is doubtful, but the author

finds that nuclein is present in Yeast-cells and was able to prepare and stain it. The granules do not appear to be concerned in any way with the process of budding or spore-formation.

In opposition to these observations of Raum and Krasser we have those of Moeller ('92), who shows that each Yeast-cell possesses one nucleus. This is a homogeneous body which possesses neither membrane nor nucleolus, and is capable of changing its shape. Its position in the cell varies; it may be central, or parietal, or at one of the poles of the cell. In the process of budding a portion of the nucleus makes its way from the parent cell through the opening into the bud. This then breaks off and rounds itself off as the nucleus of the newly formed cell. In order to observe the nucleus satisfactorily, the author makes use of cover-glass preparations fixed in a solution of iodine in potassium iodide. They are left in this solution for about twenty-four hours, then passed through water and dilute alcohol into absolute alcohol. Before staining they are soaked in saturated solution of picric acid for some time, washed in water, and stained in an alkaline solution of haematoxylin, or in one of the aniline dyes, fuchsin, methylene-blue, &c. The preparations are finally washed in water, dried, and mounted. From an examination of the spores, Moeller comes to the conclusion that they do not possess either a nucleus or a nuclear membrane, and that in consequence they are not true spores.

Mann ('92) observed in actively budding Yeast-cells, after staining in Ehrlich's acid haematoxylin, erythrosin, or eosin, a deeply stained granule which he supposes to be either a nucleolus or a nuclear chromosome.

Krasser however ('93) states again that the cells of Yeast contain nuclein, but that it is not contained in the body described as a nucleus by Moeller. It appears to be distributed in a finely divided form throughout the whole of the protoplasm. It is easily demonstrated macrochemically, but he finds considerable difficulty in demonstrating it by microchemical tests. He does not accept Moeller's view therefore

that the body described by him can be regarded as a nucleus, and in fact he failed to discover it in all Yeast-cells.

Moeller ('93) in a later communication points out that a better method of demonstrating the nucleus consists in boiling the cells in distilled water for one or two minutes, and then staining with haematoxylin by Heidenhain's method. In yet another communication to a different journal ('93 *a*) he gives an account of further observations which he has made to show that Krasser's contention that a true nucleus is not present is not the correct one, and reiterates his statement that the vegetative cells contain a distinct nucleus. He also finds that the spores contain a similar body. In the formation of the spore-nuclei, the mother-nucleus increases in size and becomes elongated and constricted. The ends separate from one another to opposite poles of the cell, the connecting thread breaks through and disappears, and two daughter-nuclei are thus formed. The division is a direct one. In contradiction to his former statement he finds that true spores are formed in the mother-cell: but whether the *Saccharomycetes* belong to the *Ascomycetes*, and to the group *Exoasci*, appears not yet proved; at present they must be regarded as *genera incertae sedis*.

Hieronimus ('93) introduced another difficulty into the solution of this vexed question by his discovery in Yeast-cells of a structure which he regards as similar to that which he has described for the *Cyanophyceae*. The protoplasm is full of angular granules which have a strong affinity for stains, and are so arranged that they form a thread interwoven in such a manner as to form a regular spiral or ball, which he calls the central thread. Sometimes this ball is located at one side of the cell, at others it expands and appears to pervade the whole of the cell; sometimes it is found separated into two portions, one at each pole of the cell, connected together by a single row of these granules. During the process of budding a portion of this thread passes into the daughter-cell.

Dr. A. Gortz, however, as quoted by Zimmermann ('93),

was unable to confirm the observations of Hieronymus, although he used the same methods and the same appliances, but was able to demonstrate the nucleus by the use of Merkel's solution and subsequent staining with fuchsin and methylene-blue. He did not complete his investigations, however, owing to the appearance of Janssens' paper.

Janssens ('93) states that not only do the cells of Yeast contain a nucleus, but that in the process of budding and spore-formation it divides karyokinetically, and the author was able to observe some stages of this, including the spindle-figure. He examined various Yeasts, including *S. Cerevisiae* and *S. Ludwigii*, to which he paid special attention. The nucleus possesses a nuclear membrane and a nucleolus. The latter is about one-third the diameter of the nucleus, and is homogeneous. In spore-formation the nuclear wall disappears from around the nucleolus. The first karyokinesis takes place longitudinally in *S. Ludwigii*, transversely in *S. Cerevisiae* I. The diaster and equatorial plate are easily seen. The second karyokinesis completes itself perpendicularly to the first, and the two spindles have a perpendicular direction to one another. The spores contain one nucleus, which becomes especially clear if the cells are placed during germination in water to which a little wort has been added.

Dangeard ('93 and '94), from a study of material which had been fixed by alcohol and stained with haematoxylin, also affirms that a distinct nucleus is present. The nucleus is placed in the layer of protoplasm surrounding the central vacuole. It is spherical in shape, possesses a thin membrane, and a large nucleolus which takes up the stain strongly. The process of division of the cell takes place by the formation of a bud on that side of the cell diametrically opposite to the nucleus. The nucleus passes to the point of attachment of the young bud and undergoes division which, according to the author, is in most cases direct. One-half then passes into the daughter-cell. No membrane is visible round the nucleus while this is taking place, but as soon as it comes into the daughter cell it develops the ordinary structure.

Eisenschitz ('95) raises a very interesting question: he finds that on cultivating Yeast for a day or two in beerwort coloured with methyl-green, congo-red, &c., the granules in the cell become stained. The granules are partly within and partly outside the vacuoles. The granules are of different chemical nature. The author thinks that the granules and vacuoles may be regarded as the preliminary stages of a nucleus.

Macallum ('95) is inclined to regard the existence of a nucleus in the Yeast-cell, in its usual condition, as extremely doubtful, and supports Krasser's view that nuclein is disseminated through the cytoplasm. On repeating Moeller's experiments, and using this observer's method, he found that now and then a structure such as is described by Moeller is present in *S. Cerevisiae*; but on comparing these preparations with others made by hardening and fixing in corrosive sublimate and staining with haematoxylin and eosin, he finds that this body is stained by the latter but not the former, and after fixation with Flemming's fluid appears to have no particular affinity for any dye. In *S. Ludwigii* there is in the great majority of cells a corpuscle which corresponds with the nucleus of Moeller, but which behaves towards stains in a similar manner to the above. A substance like chromatin in its reaction to staining fluids appears to be disseminated through the protoplasm. The distribution of assimilated iron-compounds in these cells confirms these results. In *S. Cerevisiae* the assimilated iron is, like the substance which absorbs haematoxylin, distributed through the protoplasm, and sometimes in the latter in the form of granules. In *S. Ludwigii* it is chiefly found at the periphery of each large vesicle when there are only a few of the latter; but when they are numerous, the cytoplasm gives a uniform reaction for iron corresponding in its depth to that given with haematoxylin. There appears to be present also a substance which constitutes corpuscles of a nucleolar nature, which stain with eosin, and give a marked reaction for iron, but do not stain with haematoxylin. The

final conclusion arrived at by the author is that 'there is no nucleus, although such an organ may occur in other stages' which he has not been able to observe.

Henneguy ('96) describes some observations made by him in 1886 on a red Yeast which had appeared accidentally among his cultures. This Yeast, which he examined in the fresh state and after being stained, exhibited very strongly a nucleus surrounded by a nuclear membrane and possessing a nucleolus.

Crato ('96) shows that in an elongated Wine-Yeast which he examined, physodes are present with the protoplasmic network. On staining with iodine, a compact body which stains yellow is seen to be present; this may be the nucleus.

Buscalioni ('96), in his observations on *Saccharomyces guttulatus*, describes the division of the nucleus. The resting nucleus is a homogeneous body which divides directly by constriction whilst budding takes place. The two daughter-nuclei remain connected together by a thin filament until one of them has passed into the bud. A similar method of division is followed in the formation of spores. The latter differs slightly from the former, and may be regarded as a much reduced form of karyokinesis; the former is a simple process of fragmentation.

My own observations ('97) showed that in *S. Cerevisiae* the nuclear body can be easily demonstrated by careful staining with haematoxylin, Hartog's double stain of nigrosin and carmine, or aniline-water solution of gentian-violet. It appears to consist, in the majority of cases, of a homogeneous substance, spherical in shape, placed between the cell-wall and the vacuole. The process of budding in a Yeast-cell is accompanied by the division of this nuclear body into two. The division is a direct one, and does not take place in the mother-cell, but in the neck joining it to the daughter-cell. When about to divide, the nucleus places itself just at the opening of this neck, and proceeds to make its way through it into the daughter-cell, until about half of it has



passed through, when it divides completely, and the two nuclei thus formed separate from each other towards the opposite sides of their respective cells. In the process of spore-formation the nucleus divides into four, each becoming the nucleus of a spore.

Janssens and Leblanc ('98) state that all cells of Yeast contain a nucleus which possesses a membrane, caryoplasm, and a nucleinated nucleolus. At the commencement of fermentation the nucleus is vacuolized, and presents the appearance of a vacuole containing a little sphere animated by Brownian movements. In the process of budding the nucleus divides indirectly in some cases, directly in others. In *S. Cerevisiae* the nucleolus is divided into two in the mother-cell in the neighbourhood of the bud. In the cells which are about to form spores one finds two nuclei. These use together, and the result is practically a fertilized egg-cell. This nucleus then divides by a reduced process of karyokinesis. This division is again repeated, and four nuclei are formed, each of which forms the nucleus of a spore.

Bouin ('98) states that the Yeast-cell contains in its normal condition a distinct nucleus. During fermentation this nucleus loses its clearness, and by putting out prolongations more or less clearly defined, it comes into close relation with the cytoplasm of the cell. Under the influence of an exaggerated concentration of the nutritive solution, or a reduction in the mineral elements, or by an increased temperature, the cells increase in size and become plurinucleate. This explains the observations of Hieronymus and others. The granules observed by these authors represent the nucleus which has become divided by a series of divisions not followed by cellular divisions. In the process of budding the nucleus divides more often directly than indirectly, but sometimes during budding, and always in the formation of spores, the division partakes of the indirect method.

In a recent paper, Macallum ('98) described a new method for the detection of combined phosphorus in tissues, and

pointed out that 'the method has resulted in demonstrating the presence of masked phosphorus in the chromatin of all animal and vegetable cells, in nucleoli . . . pyrenoids of *Protophyta*, &c. . . . It also shows that in non-nucleated organisms like the *Cyanophyceae* and *Saccharomyces*, the phosphorus-holding substance, or nucleo-proteid, although sometimes in the form of granules or spherules which have been taken for nuclei, is frequently dissolved in the cytoplasm.'

Errera ('98) states that he has been led to the following conclusions by a study of the cells of *Saccharomyces Cerevisiae*, part of which merely confirm former researches:—

1. A relatively large nuclear body exists in each adult cell.
2. Young cells contain no such body; a little later the old nuclear body divides, and one of its two daughters wanders through the narrow connecting-channel into the young cell.
3. After the division is complete, the two cells are still kept together by a mucilaginous neck-shaped pedicel, which appears not to have been noticed hitherto.
4. Carbohydrates are stored up in Yeast in the form of glycogen, which accumulates or disappears from the vacuoles very rapidly, according to conditions of nutrition and growth.

The evidence in favour of a nucleus in the Yeast-cell, as shown by these investigations, is very considerable, but it is very evident that its exact nature has not yet been determined. This is due, partly to its small size, partly, as we shall see later, to the difficulty of interpreting various structures which occur in the cell, and partly to the fact that the nuclear apparatus differs materially in structure from the nucleus of the higher plants.

#### METHODS.

##### *Fixing and hardening.*

Various methods of fixing and hardening have been tried, including the chrom-osmium-acetic mixture; chromic acid; solution of picric acid in absolute alcohol; picric and osmic

acid, and osmic acid alone; all of which give fairly good preparations: but the best results have been obtained by a saturated solution of corrosive sublimate, which should act for at least twelve hours, and by Gram's solution of iodine, which was used by Moeller, and subsequently by other observers, and which I have found to be of immense value in this work. The solution should remain on the Yeast for twenty-four hours.

The Yeast-cells may be fixed *en masse* in a small bottle, or cover-glass preparations may be made. Lindner ('97) observed that Yeast-cells behave in the same manner towards dyes as do Bacteria: like them they may be dried on a cover-glass and stained with various aniline-dyes. The spores also behave in a similar manner to the resting spores of Bacteria, and may be stained very easily with fuchsin.

It has been found by Janssens and Leblanc, and by myself, that completely drying up the living Yeast-cells on a cover-glass produces much contraction and disintegration of the contents. Janssens and Leblanc have found, nevertheless, that the liquid on the cover-glass may be almost completely evaporated without the Yeast-cells becoming quite dry, and that they stick sufficiently firmly to the cover-glass to allow the subsequent operations of hardening and staining to be carried out.

The method of fixing cover-glass preparations by heat, as practised by some observers, is not a good one, as has been already pointed out by others, but I should not say with Janssens and Leblanc that it is *absolument condamnable*. I have found it useful in certain cases, and have occasionally obtained very good preparations.

The method employed by me, however, is different from either of these. I first fix and harden the cells before making cover-glass preparations of them. I found that, even with the partial drying up, as practised by Janssens and Leblanc, the Yeast-cells showed signs of contraction of their contents; and further, that in the process of hardening and staining,

a large proportion of them were lost owing to their loose attachment to the cover-glass. The method I adopt obviates these difficulties, and in practice it is found that the cells may be completely dried up on the cover-glass without showing any signs of disintegration, if they have previously been well fixed and hardened. The method of procedure is a simple one. They are first of all placed in the fixing solution, either corrosive sublimate or preferably a solution of iodine in potassium iodide. They are then washed in water, 30% alcohol, 70% alcohol, and finally in methylated alcohol, which is constantly changed until all the iodine is washed out. Cover-glass preparations may then be made. A small quantity of the alcohol with Yeast-cells is placed on a cover-glass or slip. The alcohol is allowed to evaporate until the cells are nearly dry; then a drop of water is added and the Yeast-cells are thoroughly mixed up in it and spread out in a thin layer. When they have settled down the water is drained off, and they are then allowed to dry up completely. The cover or slip, with its layer of cells, is placed in water again for a few seconds and then stained.

#### *Staining and Mounting.*

Nearly all the methods of staining in vogue for nuclear work have been tried with more or less success.

*Fuchsin and methyl-green.* This is a very useful combination. It is prepared by adding an aqueous solution of methyl-green to an aqueous solution of acid fuchsin until a deep violet liquid is obtained. A drop of it placed on quite damp or wet blotting-paper should show a deep violet central spot surrounded by a narrow irregular blue or green ring. Cover-glass preparations stained in this for two minutes, then washed in water for ten seconds or so and mounted in dilute glycerin, show the nuclear body red, the cytoplasm blue-pink, and the vacuole and its contents blue, nearly the same colour as the protoplasm. The nuclear body may be perfectly easily made visible even in the most refractory specimens by this method, and especially in the

following manner, by which permanent preparations can be made. The cover-glass preparation is stained for two hours, washed in water, then in 70% alcohol, then again in water and in 70% alcohol, and so on until on examination under the microscope the protoplasm appears clear. It is then washed quickly in methylated alcohol and absolute alcohol, cleared in xylol and mounted in Canada-balsam. The nuclear body is coloured red and perfectly differentiated from the colourless protoplasm.

*Methyl-green and eosin.* By this combination the vacuole and its contents are stained green or blue, the protoplasm and nuclear body pink. The stain is allowed to act for one-half to two minutes; the preparation is then washed in water and examined in dilute glycerin. Permanent preparations may be made by drying up completely after washing in water, then clearing in xylol and mounting in balsam.

The successful application of these two methods depends to a large extent upon the judgment of the investigator in determining the right moment at which to stop the washing out in water or alcohol.

*Haematoxylin.* A dilute solution of Delafield's haematoxylin in water, allowed to act on Yeast-cells for several hours, which are then washed in water and 2% alum solution, generally shows up the nuclear body quite clearly. The preparation may be washed in alcohol, cleared in xylol, and mounted in balsam. Good preparation can also be obtained by Heidenhain's iron-alum method. The cover-glass preparations are first of all mordanted in a 2.5% solution of iron-alum in water for about three hours. They are then well washed in water and stained in a .5% solution of haematoxylin in distilled water for six to twelve hours, then they should be washed well in water, and soaked again in the iron-alum solution. In this the stained portions turn black, but are then gradually decolourized by a prolonged stay in the solution. After about two or three days, or sometimes more, the stain is found to have nearly disappeared from all parts of the cell except the nuclear body, but sometimes

a very prolonged stay in the solution is necessary in order to obtain a good differentiation. The preparations may be decolourized more quickly and effectively perhaps in alum solution.

*Safranin.* A solution in water to which a 3.5% solution of aniline in water has been added may be used. The preparations remain in this for two or three hours; they are then washed in water, well washed in alcohol and acid alcohol, cleared in xylol and mounted in balsam. The nuclear body stains but slightly, the same as the protoplasm, but in good preparations the vacuole and its contents are coloured bright red.

*Gentian-violet.* A solution of this in aniline-water is very useful. Preparations should be stained for about half an hour; then washed in water and thoroughly washed out in 70% alcohol, and finally mounted in balsam. In good preparations the nucleus stains pale reddish blue, the granules and vacuolar contents a deeper reddish colour. It is a very intense stain, and when carefully used gives good results. The combination of this stain with safranin and orange has not in my hands been productive of good results.

*Fuchsin and methylene-blue.* Stain first in carbol-fuchsin, then wash out in water and dilute alcohol, or very dilute solution of sulphuric acid, and subsequently stain in a dilute aqueous solution of methylene-blue. The nuclear body and spores are red, the protoplasm blue. This is not a very good combination for the study of Yeast-cells.

*Carbol-fuchsin.* Janssens and Leblanc give this as a good stain; but I have found that the combination of fuchsin and methyl-green, or fuchsin and methylene-blue, is far more effective. By itself the fuchsin stains somewhat diffusely. It is however an excellent stain for ripe spores when used hot, as in the staining of spores of Bacteria: preparations should be mounted in balsam.

*Carmine and Nigrosin.* This is used according to the method given by Hartog ('95) and gives fairly good results. It is necessary, however, to be very careful to wash out the

carmine very thoroughly as well as the nigrosin. A long stay in the alcoholic solution of acetic acid is generally necessary. This method is best adapted to the staining of cells which are to be cut by the microtome. I have also been able to obtain useful preparations showing the division of the nucleus in spore-formation by means of it.

*Regina-violet.* An aqueous solution is used. The cells are stained for about two minutes, then washed in water and 50% of alcohol, and mounted in dilute glycerin. Nuclear body, vacuole, and protoplasm stain reddish, but the nuclear body and vacuole are differentiated from the cytoplasm by being rather more deeply stained.

*Microtome-sections.*

The fixed and hardened Yeast-cells may be stained, according to the method of Hartog, in carmine and nigrosin. The methylated spirit in which they are preserved is poured off and replaced by a very dilute solution of acetic acid nigrosin in 50% spirit. After remaining in this a short time, the liquid is drawn off and replaced by Mayer's carmine; the liquid is well shaken and allowed to remain for several hours. The carmine is then poured off, and the Yeast-cells are washed several times in 30% alcohol, which is then replaced by acetic nigrosin in very dilute solution. They should remain in this until sufficiently differentiated, which may be ascertained by occasionally placing a few cells under the microscope. When this has taken place the stain is drawn off, 30% alcohol added, then 50%, and they are gradually brought into absolute alcohol, in which they remain for an hour or so. The absolute alcohol is replaced by carbolized xylol, and the bottle or tube containing the stained cells is then placed on a water-bath, and pieces of hard paraffin wax added until all the xylol is evaporated and the cells are left in pure paraffin. They are then well shaken to ensure thorough penetration, and allowed to settle in a mass at the bottom of the tube or bottle. When this has taken place, the tube is plunged into cold water. The paraffin solidifies, and the

embedded Yeast-cells may now be got at by breaking the tube carefully. The paraffin-block thus obtained is trimmed, fastened to the microtome, and cut in the ordinary way. The ribbon is fastened to the slide by cement, the paraffin melted; the slide soaked in xylol, and finally mounted in balsam. In this way sections of the Yeast-cell are obtained in which the nuclear body can be very distinctly seen. Instead of carmine and nigrosin, haematoxylin may be used, according to Heidenhain's method.

#### *Other Methods of Mounting.*

Instead of being embedded in paraffin, the stained Yeast-cells may be mounted directly in balsam from the carbolized xylol. Care must be taken, however, to thoroughly break up the mass of cells with a brush on the slide in order to get an even regular layer. But perhaps the best method of doing this is to allow the cells, after carefully mixing them up with a brush on the slide, to dry up completely. By this means a single layer of cells only is obtained, if reasonable care be used. The slide can then be placed in xylol and mounted in balsam in the ordinary way.

Another method which is very convenient is to allow the xylol to evaporate from the bottle, leaving the mass of Yeast-cells perfectly dry. Small quantities of this dried stained Yeast can be handed out to students, or sent by post in small packets without coming to any harm. It may be very easily examined. A small quantity is crushed up in water and either examined at once, or the water may be drained off by means of blotting-paper and dilute glycerin added. If a permanent preparation is desired, the cells which have been spread out in water on the cover or slip are allowed to dry up completely; xylol is then added, and finally the preparation is mounted in balsam. This apparently rough treatment has very little, if any, effect upon the nuclear body; but for the investigation of the more delicate structure of the Yeast-cell it is not to be recommended.



SPECIES EXAMINED.

The species of Yeast examined include—

1. *Saccharomyces Cerevisiae*—obtained from Leeds breweries.
2. Compressed Yeast—obtained from various agents in Leeds.
3. *S. Cerevisiae*—Hansen I.
4. *S. Ludwigii*.
5. *S. pastorianus*.
6. A red Yeast found in the air of the Laboratory and cultivated on gelatine.
7. *S. Mycoderma*.

I am indebted to the kindness of Professor E. Chr. Hansen for Nos. 3, 4, and 5, and I take this opportunity of tendering him my thanks for the specimens he was good enough to send me.

In order to make observations upon Yeast at different stages of fermentation, it was obtained fresh from a brewery; the wort was drained off, and cultures started in Pasteur's solution. In several series of investigations the Yeast was examined at the end of every hour, and specimens were fixed and hardened at the end of 1, 2, 3, 12, 16, 24, 38, 49, and 72 hours. Observations were also made upon Yeasts kept in sugar-solutions of various strength and in distilled water, all of which afforded useful information.

GENERAL STRUCTURE OF THE YEAST-CELL.

The contents of the Yeast-cell vary according to the conditions under which it is placed. In fresh actively growing Yeast the cell-contents are generally clear and homogeneous, with perhaps one or more bright refringent granules.

In young Yeast-cells and cells in an early state of fermentation—three or four hours in Pasteur's fluid—a vacuole or vacuoles can be seen. Each vacuole contains at least one refringent particle which is in a state of movement, and

in many cells there are two or more moving particles present. As fermentation proceeds these vacuoles disappear, and the protoplasm for a time appears homogeneous and clear; but as the culture-solution becomes exhausted the contents become more granular, large brightly refractive fat-globules appear in it, the protoplasm contracts away from the cell-wall, the cell-membrane loses its turgescient appearance, and the whole cell presents an appearance of disintegration.

Compressed Yeast-cells nearly always contain numerous brightly refractive granules. These are sometimes distributed regularly through the whole of the protoplasm; sometimes they are located only around the vacuole, or more or less densely grouped together on one side of the cell. These are the granules which Hieronymus regards as of the nature of a nucleus, and are called by him the central thread. They increase in number when the cells are placed in 5% sugar-solution, and sometimes almost completely fill the cell. The vacuoles may, as in other Yeast-cells, contain one or two brightly refractive granules which exhibit a Brownian movement (see Figs. 33-40).

#### THE NUCLEAR APPARATUS.

By the term nuclear apparatus is meant that portion of the Yeast-cell which appears to be set apart to perform the function of the nucleus.

According to Schmitz, Hansen, Strasburger, Moeller and others, the Yeast-plant possesses a nucleus of a simple structure consisting of a spherical homogeneous body placed on one side or near the centre of the cell. This body, which I propose for the present to call the nuclear body, can be very easily made visible by staining in methyl-green and fuchsin or in haematoxylin. To stain in methyl-green and fuchsin, a small quantity of fresh brewer's Yeast which has been fixed and hardened according to the method of Moeller by means of iodine-solution, is spread thinly over a cover-

glass or glass-slip and allowed to dry. A drop of the methyl-green and fuchsin mixture is then placed upon it and allowed to remain for two or three minutes. This is then washed off in water and the preparation examined in dilute glycerin under a one-sixth inch objective. The nuclear body will be seen coloured red and beautifully differentiated from the rest of the protoplasm, which remains colourless, or only slightly stained pink-blue. It is a perfectly homogeneous body even when observed under the highest powers of the microscope; but it is sometimes surrounded more or less completely by granules, which are stained blue or blue-pink, and these give it, especially when seen with inferior glasses or illumination, a granular appearance.

By means of haematoxylin it can perhaps be seen just as easily, but the preparation takes a longer time. I have found the following to be a good method. A cover-glass (or slip) preparation is taken prepared as above, and soaked for half an hour in a 2.5 % solution of alum. It is then well washed in water and stained for half an hour in a .5 % aqueous solution of haematoxylin, and again well washed in water. It is now decolourized for half an hour or longer in the alum-solution and examined in dilute glycerin, or it may be passed through alcohols of various strengths and mounted in balsam. The nuclear body is by this method stained reddish blue or sometimes blue-red, and is beautifully differentiated from the protoplasm which remains very lightly stained. It is more clearly seen in dilute glycerin than in balsam.

Every cell of the Yeast-plant, except quite young buds, contains one of these nuclear bodies; very rarely are two to be found, except during budding or spore-formation. It is found in vigorously active Yeast, which has been fermenting for twelve hours, on one side of the cell, in close contact with the cell-wall; in a few cells it may be seen in a more central position, but very rarely exactly in the centre of the cell.

In cells which are stained very lightly, the nuclear body

appears to be surrounded on all sides by a more deeply stained membrane in close contact with it (Fig. 4). This seems in good preparations to be finely granular in nature, but it is not sufficiently definite to allow any positive statements to be made concerning it. It may perhaps be only a slightly denser portion of the nuclear body. The nuclear body is sometimes surrounded by granules which radiate into the surrounding protoplasm, giving it a star-shaped appearance which is described by Bouin ('98) as a nucleus.

On the whole the nuclear body appears to resemble the nucleolus of the higher plants more than anything else, and should probably be compared to it in function.

When stained as above described, or with the carmine-nigrosin combination, the nuclear bodies of different cells generally appear to be fairly uniformly stained and present a similar appearance in all; but in preparations stained with gentian-violet (see page 512), a difference in the affinity for the stain is observable in the nuclear bodies of various cells. In some cells the nuclear body is deeply stained; in others only faintly stained. It is generally clearly defined; but in badly stained or insufficiently washed-out preparations it may appear irregular in outline, as already described by Moeller and Bouin. This is due to the granular substance often found around the nucleus, sometimes in close contact with it, but which is not to be regarded as a part of the nuclear body, and in well-stained preparations is sharply defined from it.

The nuclear body can also be fairly easily rendered visible by allowing a dilute solution of iodine to run in gently under the cover-glass. The protoplasm stains first and the nuclear body is then visible as a pale unstained spherical body on one side of the vacuole. As the protoplasm becomes more deeply stained the nucleus becomes clearer.

If fresh Yeast be placed in Gram's solution of iodine for twenty-four hours, washed in water and placed in 30% alcohol, and then in 70% alcohol, the nuclear body can be very easily seen as being slightly more refractive than the rest of the protoplasm. It can also be very easily seen in specimens

preserved in methylated spirit; and most of the ordinary reagents used for fixing render it as a rule more or less visible<sup>1</sup>.

In fresh Yeast it can in some cases be made out by careful examination under the one-twelfth inch objective, especially in compressed Yeast. As seen in the fresh condition, it is a pale slightly refringent spherical body. Its presence is often masked in compressed Yeast by the granules around it, but its position may be indicated by a slight flattening or indentation of the vacuole on that side on which it is placed.

The relation of Hieronymus' granules to the nuclear body is interesting. They may be easily observed if ordinary compressed Yeast be placed in very dilute sugar-solution and examined with a high power. Nearly all the cells will then be found to contain them. There may be only a few present, as in Fig. 33, or many, as in Figs. 34-40. In some cases they are grouped closely around the nucleus, as if connected in some way with it (Fig. 34), probably for purposes of nutrition. Sometimes the granules are found only on one side of it, sometimes on two sides or all round (Figs. 34 and 36), except in the region of the cell-wall and the vacuole. In addition to the granules around the nucleus we find a few or many in the protoplasm around the vacuole. In other cases the granules are not found grouped in this way round the nuclear body, but are distributed more or less regularly through the cell (Figs. 38, 39). In cells which had been kept in dilute sugar-solution for some hours, the granules were more commonly found grouped around the nucleus. After about twelve hours in a warm place in dilute sugar-solution, the granules increase in number, the protoplasm becomes vacuolar, and the nucleus takes up a position more in the centre of the cell, where it is surrounded by the granules on all sides (Figs. 39, 40). Sometimes the granules seem to

<sup>1</sup> Cells of *S. Cerevisiae* placed in a solution of alkanin for twenty-four hours or longer also show the nuclear body quite clearly, stained light red. The same body also gives, in cells hardened in alcohol, a definite reaction for phosphorus when treated according to the method described by Macallum ('98).

show the appearance of a coiled thread. This appearance is often observed just before the sporulation of a Yeast-cell.

On staining some of these cells on the slide with fuchsin, the granules can be seen stained fairly clearly, and in the midst of them the nuclear body faintly stained and rather difficult to make out. Some of Hieronymus' figures give one a very good idea of the appearance of these granules when stained, except that only in very few cases could any appearance of the nature of a coiled thread be seen. I have not yet been able to ascertain exactly what these granules are, but from the fact that some of them disappear on soaking in ether, and that they become coloured red in alkanin, they are probably of an oily nature; the others are probably proteid granules.

In addition to the nuclear body, there is present in the Yeast-cell in all species which I have examined, another structure which seems to be part of the nuclear apparatus. In young actively-growing cells this is represented by a vacuole containing a stainable substance, sometimes in the form of granules, sometimes in the form of a network, sometimes an irregularly shaped mass attached to the wall of the vacuole by fine threads (Fig. 1, &c.). In older cells it is represented by a more or less deeply stained granular network in which a small vacuole or vacuoles is sometimes visible (Figs. 22-27). This vacuole is taken for the nucleus by Janssens and Leblanc, who describe the nucleus of the Yeast-plant as consisting of a membrane, caryoplasm, and a nucleinated nucleolus. But according to them its structure is not always the same: in some cells the nucleus is a homogeneous body, but at the commencement of fermentation it presents in the fresh cell the aspect of a vacuole containing a spherule animated by Brownian movements. The moving spherule is regarded by the authors as the nucleolus, and is the same thing as the crystalloid of Hieronymus. In other words, the vacuole which can easily be seen in most Yeast-cells, without any special preparation, at the beginning of fermentation, is regarded by these observers as the nucleus.

The moving particle or nucleolus stains in the ordinary nuclear stains, and is supported to the wall by a caryoplasm of delicate threads.

These observations of the authors mentioned are in so far correct that, as previously stated, a vacuole with the structure they describe occurs in young cells; but whether it should be regarded as a nucleus or not is a question for further consideration. The presence of the nuclear body described by previous observers seems to have escaped their notice in the younger cells, although they have apparently seen it in older cells, for the body in these cells which they describe as the nucleolus is doubtless in many cases the *nuclear body* of previous observers. But as I have shown, by appropriate staining both a nuclear body as well as a vacuole can be seen in all cells which contain the latter, except in quite young buds; and when the nuclear body is seen through the vacuole we get an appearance which recalls at once the structure of the nucleus in higher plants (Fig. 7).

That there is reason for regarding the vacuole as possessing some of the attributes of a nucleus will be seen in what follows; for both Eisenhitz ('95) and Macallum ('95) had given indications of such a possibility in their memoirs; and I was able also to show ('97) that in addition to the nuclear body, there is a granular network present in the cell in close contact with it which resists the action of digestive fluids and is coloured intensely by nuclear stains.

In order to see the exact relation of the nuclear body to the vacuole it is necessary to examine Yeasts at different stages of fermentation, for there are two kinds of vacuoles, if we may speak of them as vacuoles—nuclear vacuoles, as I propose for the present to call them, and glycogen-vacuoles. The former are visible most clearly in Yeast-cells during the first few hours of fermentation; the latter are gradually formed as fermentation proceeds, and are generally of such a size as to completely fill the cell, leaving the nuclear body and a thin lining layer of protoplasm on the wall of the cell.

Yeast-cells taken three hours after the commencement

of fermentation, and stained according to the method already described, in methyl-green and eosin, for a few seconds, washed in water and examined in dilute glycerin, showed the following structure in different cases:—

1. A small vacuole (nuclear vacuole) containing granules and a delicate network stained green or blue, and a few granules which remain unstained; a layer of granular protoplasm stained pink, and a nuclear body in close contact with the vacuole, but *never* inside it, also stained pink or reddish blue (Figs. 1-4).

2. Small cells stained intense green all through, with a nuclear body (green), vacuole and granular contents (green), and homogeneous protoplasm (green) (Fig. 31).

3. Small cells with numerous vacuoles in a homogeneous protoplasm, and a nuclear body, all stained intense green, or in some cases with vacuoles and nuclear body green, protoplasm blue. Sometimes the nuclear body was found in the midst of the vacuoles, sometimes on one side of them (Figs. 28-30).

4. Cells with nuclear body blue, vacuole and contents blue, protoplasm pink.

5. Small cells with apparently no nuclear body, but with protoplasm and vacuole stained intense green. The absence of a nuclear body is only apparent however, for on carefully washing out the stain it is brought into view, and in methyl-green and fuchsin by sufficient washing out it is always visible, stained red.

6. Cells in which the vacuole is surrounded more or less completely by granules, which are stained blue. The vacuole contains very little stainable substance in most cases (Fig. 10).

7. Cells here and there with nuclear body blue, a vacuole present but not well marked, pink protoplasm, and a number of granules (blue) scattered through the protoplasm.

8. Cells in which a vacuole is not visible, but in its place a more or less regular granular network in contact with the nuclear body (Fig. 9).



Staining in methyl-green and fuchsin for a few seconds produces the same effect, but the nuclear body stains red, and the vacuole and its contents are not so clearly differentiated. Nevertheless, with careful staining good results are obtained. In successful preparations the nuclear body is bright red, the vacuole and its network deep blue, the protoplasm faintly stained blue. In some respects this is a more useful combination than methyl-green and eosin.

In aniline-water-safranin, the vacuolar network stains bright red, the nuclear body and the protoplasm light red.

In Delafield's haematoxylin—a solution which had been kept a long time—the nuclear body stains light red, the vacuolar network and granules deeper red. By the method of Heidenhain the nuclear body stains deep blue or black, the vacuole and contents lighter blue or black, the protoplasm remaining colourless or only faintly stained.

The nuclear body is always in close contact with the vacuole, and appears to be very intimately connected with it. Even when from whatever cause any contraction of the vacuole takes place, the nuclear body always remains in close contact with it, and one is never able to see any divisions between the two (Fig. 27). Granules inside the vacuole are often seen in contact with the nuclear body, and in some cases appear as if about to become absorbed into it. It seems likely that as the cells become older the contents of the vacuole may in part become absorbed into the nuclear body.

The appearance of the vacuole varies in different cells. In some cells it is large and contains very little stainable matter; in other cells it is small and often contains a dense mass of stainable substance. The stainable substance in the vacuoles is partly in the form of a network, partly in the form of granules. In some cells the network is distinctly granular, in others it consists of very fine, delicate threads. In some vacuoles there is sometimes a large, sometimes a small, central portion which stains deeply and is surrounded by delicate threads connecting it to the membrane of the

vacuole; this has the appearance of a nucleolus, but is not to be distinguished by its staining properties from the other substance in the vacuole, and its shape is also irregular in many cases, although it often is distinctly spherical. The network structure of many or most of the vacuoles recalls very distinctly the structure of the nucleus in the higher plants in the resting stage (Fig. 4), and its reaction towards stain is distinctly comparable to this also, although somewhat masked by the deeply stainable character of the protoplasm. The contents of the vacuole seem to contain a considerable amount of chromatin, as shown by its reaction towards stains, especially methyl-green, and its insolubility in digestive fluid. In some cells all the chromatin-substance appears to reside in the vacuole, in others it is diffused through the protoplasm, and in some cells it appears in the nuclear body. The first condition is found in young, actively-growing cells three or four hours after fermentation.

The nuclear vacuole may persist but a short time as such. At quite an early stage in the fermentation we find several cells in which a distinct vacuole is not to be seen, but only a granular network in contact with the nuclear body; and as fermentation proceeds still further, the vacuole disappears from nearly all the cells, leaving only this irregular granular network in contact with the nuclear body. On staining in methyl-green and eosin, both the nuclear body and the granular network around it are now found to stain intensely green or blue, apparently indicating that a portion of the green-staining substance has been taken up into the nuclear body (Figs. 21-27). The nuclear body at this stage is generally found closely pressed to the cell-wall by the mass of glycogen which has appeared in the cell as a result of an abundant supply of nutriment (Fig. 25).

With methyl-green and fuchsin, the nuclear body at this stage still stains red, but with a slight tinge of blue in most cases, and the granular substance in close proximity to it stains blue. The other contents of the cell stain pink. The granules are sometimes placed in a more or less regular

group on one side of the nuclear body, sometimes they surround it on all sides, and occasionally they are found distributed through the protoplasm (Figs. 21-27). In these older cells of Yeast, where the nucleus is restricted to the cell-wall by the large glycogen-vacuole, the nuclear body is sometimes surrounded by a vacuolar network in which granules may or may not occur (Fig. 26). This vacuolar network is sometimes very regularly placed around the nuclear body, which then looks as if surrounded by a halo, and has occasionally given rise to a false interpretation of its structure. This was especially well seen in some specimens of *S. Cerevisiae*, Hansen I, which had been sent to me in corrosive sublimate solution by Dr. Hansen (Fig. 26).

On treating fresh Yeast with digestive fluid (pepsin-glycerin) for twenty-four hours, a reduction in the stainable cell-contents is observed. In many cells a somewhat large, irregular granular mass is the only portion which stains deeply; in others, two or sometimes three such masses are observed, all connected together by deeply-stained granular strands. I was at first much puzzled by this, as no nuclear body was visible; but on repeating the experiment with a more careful staining, I found the nuclear body reduced in size and masked by the more deeply-stained granules around it. The granular substance in contact with the nuclear body varies much in size and shape, and is sometimes at some distance away from it, but is always connected with it by means of deeply-stained strands. The nuclear body stains much less deeply than this granular mass, and there seems to be no doubt that the latter consists of the much contracted and in part disintegrated nuclear vacuole. In older cells in which the nuclear apparatus had become restricted to the wall of the cell by the glycogen-vacuole, the whole mass—nuclear body and granules—stained the same green-blue colour in methyl-green and eosin and of the same intensity, an indication that at this stage in the development of the cell a considerable portion of the chromatin is taken up into the nuclear body.

In *S. Ludwiggii* and *S. pastorianus* the structure of the

nuclear apparatus is in the main similar to that of *S. Cerevisiae* (Figs. 41-53). The nuclear vacuole seems to persist for a longer time however, as it does in compressed Yeast. The nuclear body is in close contact with the vacuole as a rule, but occasionally it may be separated some distance from it (Fig. 50). One often finds that a definite generally curved row of granules extends from the nuclear vacuole through the protoplasm to one or both ends of the cell (Figs. 43, 49, 50). In addition to the chromatin-vacuole, there are one or two large vacuoles present normally in a cell in the resting condition. As in *S. Cerevisiae*, there may be two or more small vacuoles present in young cells in place of one (Fig. 44, 48).

#### ORIGIN OF THE VACUOLE.

Young cells often contain numerous vacuoles surrounding the nuclear body (Figs. 28, 29, 30, 32). Some of these vacuoles are very small and are nearly filled up completely by a granule which stains an intense green, and thus appears to be of the nature of chromatin (Fig. 28). Under a low power these small vacuoles present the appearance of granules merely, and would be easily mistaken for the ordinary granules of a Yeast-cell; but under a high power their vacuolar nature can be easily made out in well-stained specimens. It is difficult to escape the conclusion that the vacuoles arise in some way in connexion with the granules. As the cell grows the vacuoles gradually fuse together to form the single vacuole in close contact with the nuclear body.

I have observed the same phenomenon in young Fungus-hyphae, probably *Mucor*, which constantly occur among the Yeast-cells in my cultures. In these hyphae the nuclei appear to be homogeneous in structure, and indeed stain in precisely the same manner as the nuclei of the Yeast-cells near them. In all the younger hyphae large vacuoles can be seen containing a substance staining green just as occurs in Yeast-cells, and these large vacuoles arise by the fusion of numerous smaller ones.

The single nuclear vacuole of the Yeast-cell thus has its origin in many cases from a fusion of numerous small vacuoles, and these smaller vacuoles are developed in all probability from the granules in the protoplasm.

There are some small cells however which contain only one vacuole. This, as we shall see later, probably arises by a division of the parent vacuole in the mother-cell.

#### GLYCOGEN-VACUOLES.

Errera ('82, '85, '98), Clautriau ('95), and others have shown that the Yeast-cell contains glycogen. It is not equally abundant in all stages of growth. In the earlier stages of fermentation it is more abundant than in the later stages. As fermentation proceeds the glycogen is used up, and finally in old cultures practically no glycogen is to be seen, a few cells here and there only exhibiting the characteristic reaction when treated with iodine.

In the majority of cases the glycogen is located in a large vacuole in each cell which appears shortly after fermentation has commenced ; but during the first two or three hours the glycogen when present is mainly diffused through the protoplasm. In the glycogen-containing cells when stained with iodine, the nucleus is generally visible as a transparent, colourless or slightly greenish refractive body, sometimes spherical, sometimes flattened against the cell-wall (Figs. 23-25). Sometimes the entire cell-contents, with the exception of a thin lining-layer of protoplasm on the cell-wall, consist of this substance, which according to Errera ('85) doubtless plays the same part as starch in the higher plants.

So far as can be observed at present, the glycogen is located in a special glycogen-vacuole. It never appears, or only to a slight extent, in the nuclear vacuole. If a cell containing glycogen be stained for a few seconds in acetic methyl-green, the nuclear body and lining-layer of protoplasm become stained. If now a solution of iodine be added and the cells mounted in dilute glycerine, a beautiful double colouration

will be observed, differentiating the protoplasm and nuclear body and producing a clear definition of the glycogen-vacuole.

#### GENERAL CONSIDERATIONS.

We have thus seen that there are two structures in the Yeast-cell which together appear to represent the nucleus of the higher plants—the nuclear body and the nuclear vacuole. One of these—the nuclear body—is a permanent constituent of the cell; the other is not. The nuclear vacuole, when it is present, possesses some of the attributes of the chromatin-network of the nucleus of the higher plants, and in many cases presents a remarkable resemblance to it. The chromatin may be, and often is, very abundant. But the fact that the vacuole containing it may disappear, leaving the chromatin more or less completely disseminated through the protoplasm without the formation of chromosomes, except perhaps during the divisions leading to the formation of spores, seems to indicate that we are dealing with a body of much simpler construction than the nucleus of the higher plants; and it may be that this nuclear vacuole represents merely a store of chromatin-material for the use of the cell.

In the vegetative cells of other Fungi we appear to have at certain stages a similar structure. Vuillemin (*Études biologiques sur les Champignons*, p. 7) has shown that, in the hyphae of *Entomophthora glaeospora*, one finds here and there, in contact with a nucleus, clear spheres surrounded by a delicate membrane, which are not to be regarded as vacuoles but as being of a nuclear nature; and I have observed in the young hyphae of a Fungus, probably *Mucor*, a similar occurrence of chromatin-containing vacuoles. A careful examination of the vegetative cells of other members of this group of plants may possibly show that it is not uncommon.

The structure which we have called the nuclear body resembles in many ways in its reactions the nucleolus of the higher plants; and the fact that it may under certain con-

ditions contain chromatin-substance, recalls the structure observed in such cells as those of *Spirogyra* and perhaps the young cells at the apex of the root in *Phaseolus*, in which much if not all of the chromatin resides in the nucleolus.

We may I think therefore fairly conclude that the nuclear apparatus of the Yeast-plant consists of (1) a nucleolus, of homogeneous structure, the nucleus of the majority of previous observers; and (2) a store of chromatin which may occur either (a) in a network enclosed in a vacuole in close contact with the nucleolus, or (b) in a network in direct contact with the nucleolus, or (c) disseminated through the protoplasm. The chromatin is under certain conditions taken up into the nucleolus, viz. in spore-formation, or in the later stages of fermentation when it seems to be very abundant in the cell.

Further, if we regard it as a simple form of nucleus it may be either (1) a primitive structure representing an early stage in the phylogeny of the nucleus; or (2) a degenerate nucleus such as might be the result of the degradation of the Yeast-Fungi from higher forms, as is usually supposed to be the case; or (3) a special adaptation to the conditions under which the Yeast-plant lives and its rapid vegetative reproduction by budding.

The latter alternative is supported to some extent by the phenomena which occur during spore-formation in the Hymenomycetes. In the basidia, when the spores are formed, four nuclei are present, each possessing the normal structure of a nucleus. But before they pass into the spores everything seems to disappear except the nucleoli, which are left free in the protoplasm at the base of the sterigmata. This separation of the nucleolus from the rest of the nucleus is probably a special adaptation due to the necessity of passing through the narrow neck of the sterigma into the spore; and one can readily understand how valuable such an adaptation would be to the Yeast-cell with its very rapid vegetative reproduction, and how likely it is that such a condition of its nucleus should become a more or less permanent one as long as vegetative reproduction is unchecked.

As to the first alternative, the importance of the study of the cytology of the Yeast-Fungi and other low forms of Fungi as an aid in the elucidation of the very fascinating problem of the phylogeny of the nucleus need not be enlarged upon. There is in these lower forms a wide field of research open, which in the hands of a skilful chemist and cytologist may be very fruitful of results.

The comparison which might be made between the nuclear apparatus of the Yeast-plant and that of some of the Infusoria is an obvious one, but it seems to be only a superficial resemblance. The terms 'nucleus' and 'nucleolus' were rejected for the macro- and micro-nucleus of the Infusoria because they possessed neither the structure nor the physiological signification of the nucleolus and nucleus of ordinary cells (see *Traité de Zool. Concrète*, Delage and Herouard, 1896, p. 410, tome i). On the other hand, as we have seen, the nuclear vacuole and nucleolus of the Yeast-plant can be very definitely compared to the nucleus and nucleolus of ordinary cells.

The structure of the simple nuclear apparatus of the Yeast-cell may possibly afford some clue to the structure of the protoplast of the Bacteria and Cyanophyceae. The comparison, which may possibly be made of the central body of the latter with the nuclear body of Yeast, is at once apparent; and the appearances often presented by the larger Bacteria when compared with such elongated forms as *S. mycoderma*, *S. pastorianus*, *S. Ludwigii*, are very striking.

#### BUDDING.

In the process of bud-formation, both the vacuole, when it is present, and the nucleolus take part. In those cases where the nuclear vacuole is not present, the granular network, or group of granules which represents it, takes part in the process. The former is found chiefly during the earlier stages of fermentation, the latter during the later stages.

When the bud first appears on the mother-cell the nucleolus is found exactly on the opposite side of the cell with the



vacuole between it and the bud (Fig. 10). At first the young bud contains protoplasm only, but as development proceeds the nuclear vacuole begins to pass into it (Fig. 11). Then the nucleolus makes its way to the base of the opening of the mother-cell into the bud and at once begins to divide (Figs. 12-15). The vacuole at the same time divides. The products of division are unequal, the smaller portion is found in the daughter-cell, the larger portion in the mother-cell; but both portions remain connected together for some time by a granular thread (Figs. 14-16, 18). The division of the nucleolus takes place in a very simple fashion either in the mother-cell or, as is more commonly the case, in the neck connecting it to the daughter-cell (Figs. 16-21). In the former case it becomes elongated, and constricted in the middle, finally separating into two equal or nearly equal portions, one of which then makes its way through the narrow neck into the daughter-cell (Figs. 19-21, 52). In the latter case the nucleolus puts out a projection into the narrow opening between the two cells which makes its way into the daughter-cell. When about half of it has passed through, division takes place and two equal portions are formed, one in the mother-cell, the other in the daughter-cell (Figs. 16, 17, 53). This method is the one more commonly found in *S. Cerevisiae*; the other occurs more commonly in *S. Ludwigii* and *S. pastorianus*, and occasionally in *S. Cerevisiae* also. As soon as the two nuclear bodies are separated from one another they move away to opposite ends of their respective cells (Fig. 18); the granular thread between the two vacuoles is broken, and the division of the nuclear apparatus is complete.

When the nuclear vacuole is not present, the granular network in contact with the nucleolus undergoes a division into two more or less equal portions either in the mother-cell (Fig. 21) or in the neck joining it to the daughter-cell (Fig. 17); but in either case a granular thread is drawn out between them, and remains until all connexion between the two cells ceases.

The granules which thus pass into the young bud seem to develop in some way into the small vacuoles often found in young cells, by the fusion of which the single large vacuole is formed.

#### SPORE-FORMATION.

The following is a very easy method of obtaining spores. Fresh compressed Yeast is placed in a dilute sugar-solution (about 5%) in an ordinary glass tumbler, and left to ferment at the ordinary summer temperature of a room. The surface of the liquid soon becomes covered with a scum, due to the fermentation at once set up, part of which is left sticking to the sides of the glass as the liquid evaporates. In the course of forty-eight hours the cells in the scum on the side of the glass begin to sporulate, and at the end of three days a large number of cells are obtained with spores in all stages of development. The method is a simple one, and has never failed to produce spores in large numbers. Spores are also easily obtained when a thin layer of compressed Yeast is spread over blotting-paper which has been soaked in 5% sugar-solution, and kept moist in a shallow dish covered by a glass plate. The sugar-solution seems to induce the formation of spores more freely than the plaster slabs soaked in water.

The spores can be easily demonstrated by staining cover-glass preparations in fuchsin as recommended for the spores of Bacteria. This stains only the ripe spores. The immature spores can be made visible by subsequent staining in a dilute aqueous solution of methylene-blue. In successful preparations the ripe spores are red, unripe spores blue, and the protoplasm light red.

The changes which take place in the cell, leading up to the formation of the spores, can be observed in fresh living cells, but it is necessary to stain them very carefully to observe the details of the process. The two best stains for the purpose are the combination carmine and nigrosin, and the solution of gentian-violet in aniline-water. Other stains have been found

useful, but these two have given me the most satisfactory results. It is not easy to get satisfactory preparations, however, owing to the difficulty of washing out the stain so that the cells are neither too deeply nor too lightly stained.

In the process of spore-formation as seen in the living cell, the protoplasm first of all becomes filled with bright refractive granules, most of them exhibiting a Brownian movement (Figs. 34-40). The nuclear body can generally be very easily seen, and one vacuole only is present in most cells. Then the vacuole disappears, its place being taken by two or more smaller ones, which are still further subdivided until finally the protoplasm appears structureless, or in favourable specimens exhibits the foam-structure described by Butschli. The nucleolus at the same time moves towards the centre of the cell and is surrounded on all sides by the bright granules. A condensation of the protoplasm towards the centre or one side of the cell now takes place, and gradually the spores are separated out by a division of this protoplasm into two or more rounded masses, each of which becomes surrounded by a membrane and gradually ripens into a spore.

From an examination of well-stained specimens, the changes which take place in the nuclear apparatus have been followed. Shortly after the Yeast-cells have been placed in the sugar-solution, the nuclear vacuole which contains a deeply-stained sphere or network of chromatin-like substance—the nucleolus of Janssens and Leblanc—begins to divide, first of all into two, then probably by further division into numerous smaller portions, until finally a delicate foam-structure of the protoplasm is produced and the chromatin-substance becomes distributed through the protoplasm. The nucleolus is found near the centre or one side of the cell at this stage and is slightly less deeply stained than the protoplasm (Fig. 54).

At this stage the nucleolus is very clearly seen, but in the earlier stages it is visible only after very careful staining. This has led Janssens and Leblanc to make, I think, a very curious but perhaps natural mistake. The primary division of the vacuole into two is described by them as the division

of the nucleus. At a later stage these two nuclei disappear, but in their place a single large nucleus is found. From this they come to the conclusion that the two nuclei fuse together again, and that the process may be regarded as a conjugation of two nuclei, transforming the cell into an egg, '*Ces deux noyaux, en se conjuguant, transforment cette cellule en un œuf.*' As I have shown above, their first two nuclei are produced by division of the vacuole, their single large nucleus found at a later stage is probably the nucleolus.

In the cell at this stage the protoplasm is uniformly stained rather more deeply than the nucleolus. In preparations stained with gentian-violet, the nuclear body is light blue, the protoplasm reddish blue, with a number of minute granules scattered through it.

This stage gradually passes into one in which the peripheral layer of the protoplasm loses its capacity for stains, leaving the central portion more deeply stained than before (Fig. 55). Numerous deeply-stained granules at the same time appear, chiefly around the outside of the deeply-stained central protoplasmic mass (Figs. 56, 57). This stage appears to correspond with that stage in the living condition of the cell which has been described as a condensation of the protoplasm either towards the centre or to one side of the cell. At the same time the nucleolus itself undergoes a change. Its central portion becomes more deeply stained than its peripheral portion (Fig. 56), presenting an appearance strikingly similar to that which I have observed constantly in the nucleoli of sections, stained with gentian-violet, of the root-apex of *Phaseolus*, just previous to the formation of the chromosomes. This stage is succeeded by one in which the central deeply-stained mass of protoplasm decreases in size, and at the same time the central deeply-stained granular mass in the nucleolus becomes larger (Figs. 57, 58). We are I think justified therefore in concluding that the increase in the stainable material of the nucleolus is due to the absorption of stainable substance from the surrounding protoplasm.

The nucleolus now begins to divide. Its outline becomes slightly irregular, and the deeply stained granular mass becomes more prominent. Then an elongation of the nucleolus takes place (Fig. 60), and we have gradually formed a long row of granules surrounded by a lightly stained blue substance, derived from the nuclear body, stretching across the cell either in a longitudinal or a transverse direction (Figs. 61-63). These granules gradually become separated into two groups by constriction, but they remain connected together for some time by a less deeply stained substance drawn out between them (Figs. 64, 65, &c.). Finally complete separation is effected and two daughter-nucleoli are produced (Fig. 68). Each one then divides again in the same manner, but in such a way that the line of division of one is perpendicular to that of the other, so that the two dividing nuclei often present the aspect of a cross (Figs. 69, 70, 72). Further divisions may take place leading to the formation of as many as eight nuclei (Fig. 71), but in most cells only four are produced (Figs. 73-75). Each of the four nucleoli thus formed becomes surrounded by protoplasm and a thin cell-membrane, and thus constitutes a spore lying free in the remainder of the protoplasm (Figs. 76-78). The spores are at first very small, but they gradually increase in size at the expense of the surrounding protoplasm, a thick cell-wall being produced around each, until finally they completely fill the mother-cell, the wall of which at this stage is in consequence not easily visible. They are then mature and enter upon a resting-stage.

The process of nuclear division just described may perhaps be regarded as a case of direct division in which the chromatin-substance is previously taken up into the nucleolus and separated out in the form of granules, which ultimately divide into two equal or nearly equal groups. But it may possibly be regarded as a very simple case of karyokinesis, if we look upon the granules as chromosomes, and the lightly stained substance which surrounds them during division as of the nature of a spindle-figure. The difficulty of observing all

the details of the division is, however, so great that one must be very cautious in attempting an explanation of the facts observed.

#### SUMMARY.

It may be useful to give here a short summary of the conclusions at which we have arrived as a result of this investigation.

1. All cells of Yeast contain a nuclear apparatus.
2. In the earlier stages of fermentation this consists of a nucleolus in close contact with a vacuole which contains a granular chromatin-network, and exhibits a structure in many cases like the chromatin-network of the nuclei of higher plants.
3. In the later stages of fermentation the chromatin-containing vacuole may disappear, its place being taken by a granular network or a number of chromatin-granules, which may be disseminated through the protoplasm or grouped around the nucleolus.
4. The nucleolus is present in all cells. It appears to be a perfectly homogeneous body, which may, however, at times appear granular owing to the granules around it.
5. In young cells numerous chromatin-vacuoles are often found. These appear to fuse together to form the single vacuole which occurs in cells during the early and sometimes later stages of fermentation.
6. In the process of budding, the division of the nuclear apparatus does not exhibit any definite stages of karyokinesis. It must, I think, be regarded as a direct division of the nucleolus into two equal or nearly equal parts, accompanied by division of the chromatin-vacuole, network, or granules.
7. The nucleolus divides either in the neck joining the bud to the mother-cell, or more rarely in the mother-cell itself, one of the products of division passing subsequently into the bud.
8. In spore-formation, the chromatin disseminated through the protoplasm becomes absorbed more or less completely

into the nucleolus, which then divides by elongation and constriction into two. During the division deeply stained granules (chromosomes?) appear surrounded by a less deeply stained substance, which remains for a time connecting the two daughter-nucleoli together. This may perhaps indicate a simple intermediate stage of karyokinesis.

9. Subsequent divisions take place resulting in the formation of four (sometimes more) nucleoli. Each nucleolus becomes surrounded by protoplasm and a delicate membrane, and thus the spores are formed standing free in the remainder of the protoplasm.

10. The spores are at first very small, but they soon increase in size; the surrounding protoplasm becomes used up; the spore-membranes increase in thickness until at last in the mature condition they completely fill the mother-cell.

11. In *S. Ludwigi* and *S. pastorianus* the structure of the nuclear apparatus is similar to that in *S. Cerevisiae*, and its division during the process of budding appears to be also the same.

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## EXPLANATION OF FIGURES IN PLATES

### XXIX AND XXX.

Illustrating Mr. Wager's Paper on the Nucleus of the Yeast-Plant.

All the figures have been drawn freehand, with the aid of Zeiss's apochromatic 2 mm. aperture 1.4, and oculars 8 ( $\times 1000$ ), 12 ( $\times 1500$ ), and 18 ( $\times 2250$ ). In most cases the outlines of the cells, &c., have been drawn with the aid of the camera lucida. Figs. 1-3, 5-21, 28-32, and 41-53 have been drawn from preparations stained in methyl-green and eosin; Fig. 4, safranin; Figs. 22-27, methyl-green and fuchsin; Figs. 33-40 from living cells; Figs. 54-57, 60, 62, and 63, gentian-violet; Figs. 58, 59, 61, 64, 65, 68, and 72-78, carmine and nigrosin; Figs. 66, 67, and 69-71, Heidenhain's haematoxylin.

#### *Saccharomyces Cerevisias.*

Figs. 1-5, 7-12, 14-18, and 27-32, after three hours in Pasteur's solution.

Fig. 1. Cell showing nuclear body and vacuole with network and one deeply stained granule.

Fig. 2. Ditto showing three deeply stained granules in the vacuole.

Fig. 3. Ditto showing a vacuole not much larger than the nuclear body.

Fig. 4. Cell containing a vacuole which shows the nuclear-like network very clearly.

Fig. 5. Cell with vacuole full of deeply stained substance, partly enclosing the nucleolus.

Fig. 6. Cell of compressed Yeast—the vacuole contains two deeply stained granules and delicate threads. Both nuclear body and vacuole are to some extent surrounded by unstained granules—Hieronymus' granules. Two hours in sugar-solution.

Fig. 7. Cell showing nucleolus as seen through a vacuole. The appearance is presented of a nucleus with nuclear membrane, &c.

Fig. 8. Cell showing nucleolus, in part surrounded by a dense mass of granules.

Fig. 9. Shows the nucleolus in contact with what appears to be a very definite chromatin-network. Whether this is contained in a vacuole or not, could not be made out clearly.

Fig. 10. Shows the position of the nucleolus at the time when the cell begins to bud. The vacuole in this case contains very little, if any, stainable substance, but is surrounded by deeply stained granules.

Fig. 11. The vacuole, with granular contents, is making its way into the young bud. The nucleolus still retains its position on the opposite side of the cell.

Fig. 12. The nucleolus has made its way to the opening between the bud and the parent-cell.

Fig. 13. Ditto. The vacuole in this case is surrounded by deeply stained granules. Seventy-two hours in Pasteur's solution.

Fig. 14. The nucleolus puts out a projection into the neck of the budding cell.

Fig. 15. Ditto, but a slightly later stage. The vacuolar contents are very abundant.

Fig. 16. Slightly later stage than Fig. 15, just previous to the complete division of the nucleolus. The vacuole is small and irregular in shape.

Fig. 17. The same stage as Fig. 16, but the vacuole has nearly disappeared, and in its place a deeply stained mass of granules nearly equally divided between the two cells.

Fig. 18. Complete separation of the newly formed nucleoli to opposite ends of their respective cells. A granular thread is shown drawn out between the two from the granular network.

Figs. 19–24. After seventy-two hours in Pasteur's solution.

Fig. 19. Shows the nucleolus beginning to divide by constriction in the parent-cell.

Fig. 20. Later stage than Fig. 19, the division is completed even before the vacuole begins to divide.

Fig. 21. Division of nucleolus in the parent-cell. A small vacuole only is present but there are a number of deeply stained granules, which are separated into two equal groups with a granular thread drawn out between them.

Fig. 22. Shows the glycogen-vacuole beginning to form. The chromatin-vacuole is still present with one deeply stained granule, and near the nucleolus numerous deeply stained granules are to be seen.

Fig. 23. Later stage—the large vacuole is the glycogen-vacuole.

Fig. 24. The nucleolus, chromatin-vacuole, and granules restricted to the wall of the cell by the glycogen-vacuole.

Fig. 25. *S. Cerevisiae*, Hansen I, shows nucleolus and granular network and large glycogen-vacuole.

Fig. 26. *S. Cerevisiae*, Hansen I, shows a nucleolus lying on the wall of the cell surrounded by a lightly stained vacuolar protoplasm, containing a few deeply stained granules. The remainder of the thin layer of protoplasm lining the cell is granular.

Fig. 27. Cell showing the gradual formation of the glycogen-vacuole, and the contraction of the chromatin-vacuole. Note the close attachment of the latter to the nucleolus. The protoplasm contains numerous deeply stained granules.

Fig. 28. Young cell with numerous small vacuoles, each enclosing a deeply stained granule, and some granules with a vacuole apparently just forming around each. The whole of the cell contents stain deeply.

Fig. 29. Young cell with three vacuoles, each containing a deeply stained granule. The cell contents stain deeply. The nucleolus is only visible after very careful staining.

Fig. 30. Ditto, with four vacuoles.

Fig. 31. Young cell with one vacuole, containing a deeply stained granule and delicate radiating threads. Near it a nucleolus only visible with difficulty.

Fig. 32. Young cell with nucleolus in the midst of a peripheral ring of granules. The whole cell is pervaded by a deeply stainable substance.

Figs. 33–40. Compressed Yeast examined in the living condition. The black granules represent the highly refractive granules described by Hieronymus.

Fig. 33. Half an hour after being placed in 5% sugar-solution. The nucleolus is visible, and is indicated by a slight depression in the vacuole. Few refractive granules present.

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Fig. 34. The nucleolus is surrounded by the refractive granules, which are now more numerous. Two moving granules in the vacuole. Two hours in sugar-solution.

Fig. 35. The bright granules more numerous. Two vacuoles present and a nucleolus. Two hours in sugar-solution.

Fig. 36. Shows three pairs of granules and small groups on each side of the nucleolus. Two hours in sugar-solution.

Fig. 37. Numerous vacuoles appear, as a preliminary to spore-formation. Three hours in sugar-solution.

Fig. 38. Later stage—the vacuoles are more numerous, the bright granules surround the vacuoles. Three hours in sugar-solution.

Fig. 39. The vacuoles disappear. The protoplasm, as shown by means of reagents, exhibits a foam-structure at this stage. Twenty-four hours in sugar-solution.

Fig. 40. Two groups of granules on opposite sides of the nucleolus in a hyaline protoplasm. Twenty-four hours in sugar-solution.

*S. Ludwigii.*

Fig. 41. Cell showing chromatin-containing vacuole and nucleolus.

Fig. 42. Ditto. Two lines of granules run from one end of the cell to the nuclear vacuole. The figure shows the nucleolus as seen above the vacuole, not inside it.

Fig. 43. Young bud just forming. Nucleolus beginning to divide. One row of granules stretching from one end of the cell to the nuclear vacuole.

Fig. 44. Cell showing two vacuoles and a nucleolus between them.

*S. pastorianus.*

Fig. 45. Cell showing two large normal vacuoles, and in the strand of protoplasm across the middle a nuclear vacuole and a nucleolus on one side.

Fig. 46. Shows the nucleolus as seen from above. On focussing down the vacuole could be seen.

Fig. 47. Cell showing nucleolus as seen through the vacuole.

Fig. 48. Cell with nucleolus and three small chromatin-vacuoles.

Fig. 49. Cell showing nucleolus at one end surrounded by granules which are connected to a small vacuole at the other end by a deeply stained row of granules.

Fig. 50. Nucleolus in the middle of the cell in contact with a curving line of granules running from a chromatin-vacuole at one end to the other end of the cell.

Fig. 51. Cell with young bud. The nucleolus is in contact with a vacuole containing a deeply stained granule and network.

Fig. 52. Later stage than Fig. 51; the nucleolus is beginning to divide.

Fig. 53. Case of budding in which the nucleolus is about to divide in the neck joining the two cells. The daughter-cell contains a chromatin-vacuole in close contact with its share of the nucleolus.

*Spore-formation.*

Fig. 54. Cell just at commencement of spore-formation—protoplasm reddish blue, nucleolus light blue.

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Fig. 55. Cell in which the deeply stainable substance is becoming concentrated in a central mass of protoplasm. The nucleolus stains light blue as before, but a little more deeply.

Fig. 56. Later stage. The nucleolus shows a deeply stained granular substance inside or in close contact with it.

Fig. 57. The deeply stained granular mass inside or in contact with the nucleolus has increased in size. The deeply stained central portion of the protoplasm is surrounded by granules.

Fig. 58. Later stage. The deeply stained mass of protoplasm is smaller.

Fig. 59. Still later stage. The deeply stained nucleolus is now the most prominent structure.

Fig. 60. Commencement of division. The nucleolus and its deeply stained mass begin to elongate.

Fig. 61. The deeply stained granular mass at a later stage in the process of elongation.

Fig. 62. The row of deeply stained granules stretching across the cell, surrounded by a faintly stained substance.

Fig. 63. Shows the gradual accumulation of the granular substance at both ends to form the daughter-nucleoli.

Fig. 64. Complete separation has now taken place.

Fig. 65. Later stage in the division; the two daughter-nucleoli are still connected together by a less deeply stained substance.

Fig. 66. Division-stage, as shown in a preparation stained with haematoxylin, Heidenhain's method. One portion of the protoplasm is still shown more deeply stained than the other, with a distinct line of separation between them.

Fig. 67. Same method of preparation. The division is transverse.

Fig. 68. The two groups of deeply stained granules completely separated, each surrounded by a less deeply stained substance with a faintly stained granular thread drawn out between them.

Fig. 69. Division into four.

Fig. 70. Slightly later stage than Fig. 68.

Fig. 71. Shows a cell in which division is taking place into eight nucleoli instead of four.

Fig. 72. Four groups of deeply stained granules, still connected together by less deeply stained substance. The division apparently has taken place at one end of the cell.

Fig. 73. Stage similar to Fig. 71, but the groups of granules are more irregularly distributed at one end of the cell.

Fig. 74. The four products of the previous two divisions are now lying in a slightly more deeply stained portion of the protoplasm at one end of the cell.

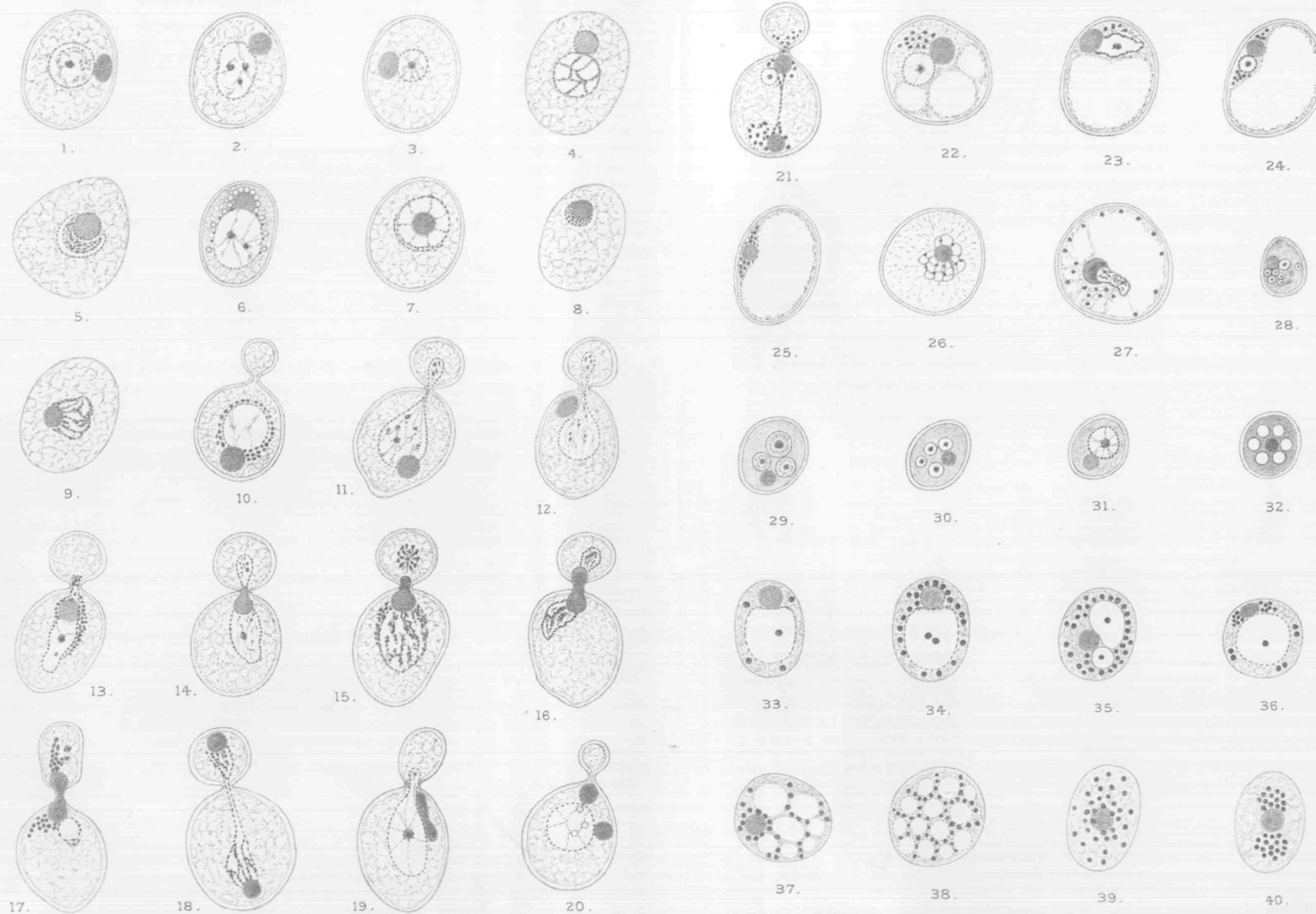
Fig. 75. Later stage than Fig. 74. The spores beginning to separate out.

Fig. 76. The spores now visible, each with a distinct outline due to the presence of a thin membrane.

Fig. 77. Later stage. The membrane is more distinct.

Fig. 78. Same stage as Fig. 77, but five spores shown, two smaller than the others. From observations made recently I am inclined to think that these two fuse together to form one.





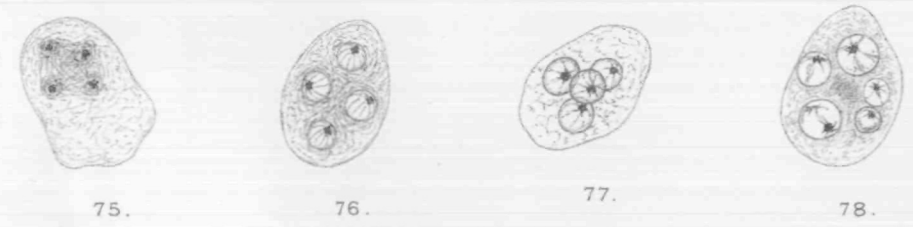
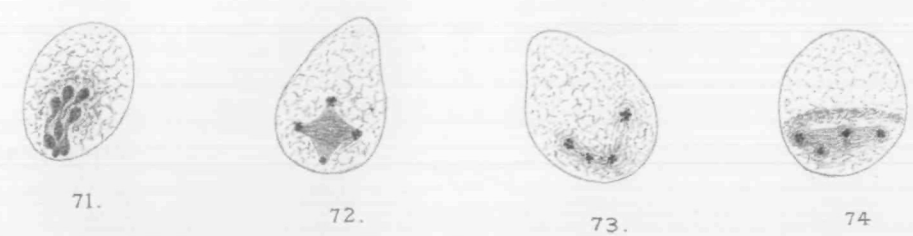
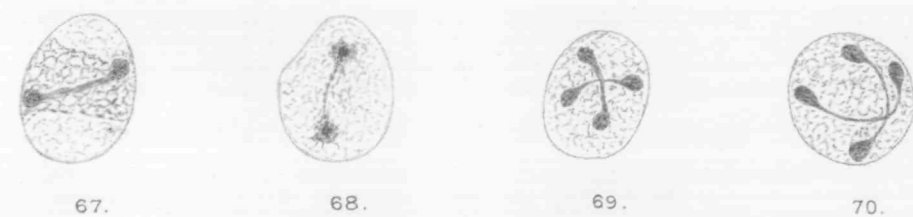
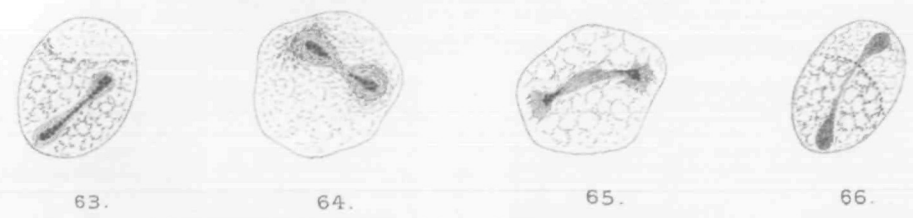
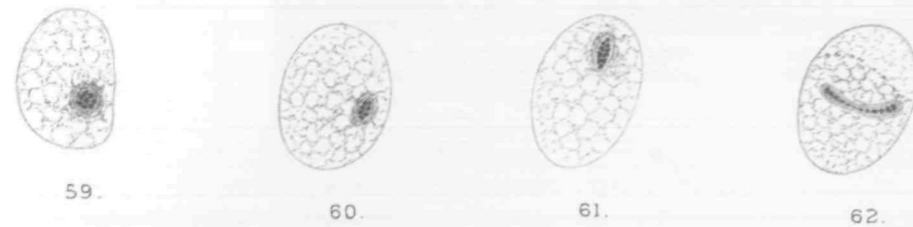
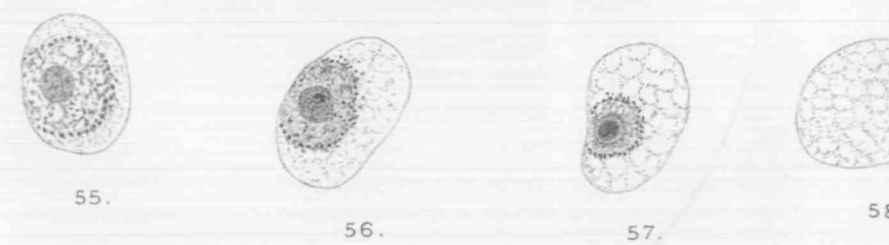
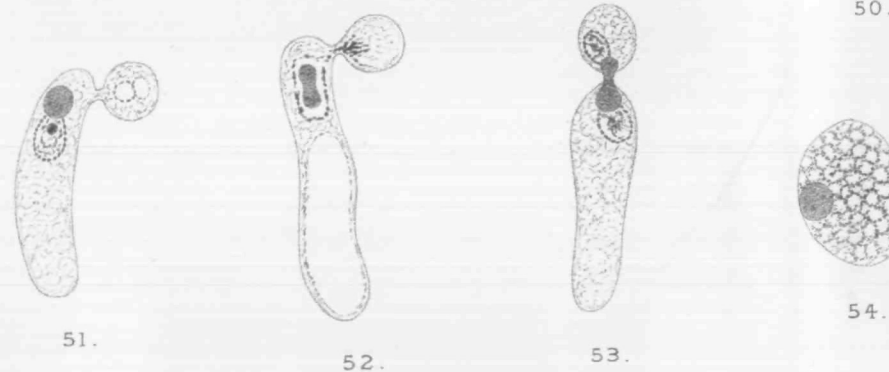
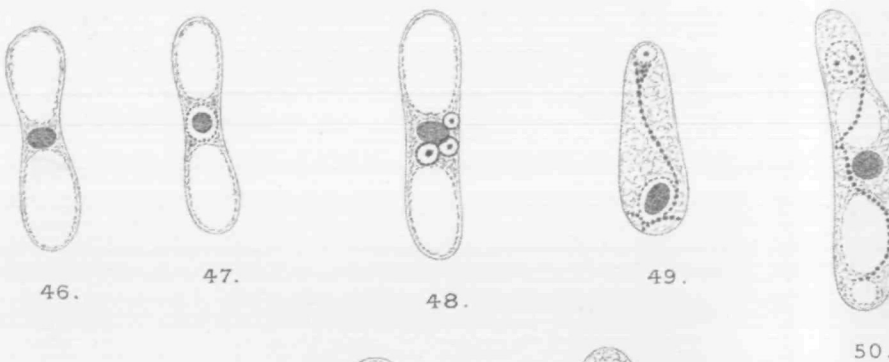
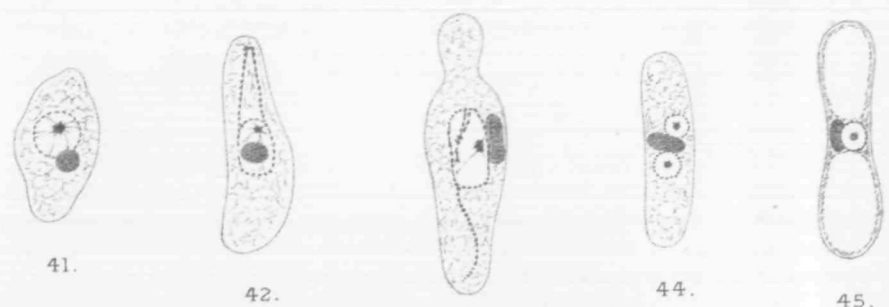
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