THE YOLK NUCLEUS OF THE WATER SPIDER, *LYCOSA BIRMANICA* THOR.

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(Communicated by Prof. D. R. Bhattacharya, Ph.D., D.Sc., F.N.I.)

(*Received June 1; read July 23, 1945.*)

INTRODUCTION.

Since its discovery by Von Wittich in 1845, the yolk nucleus has attracted the attention of cytologists all the world over, but there is still a great deal of controversy about its origin, structure and function. Balbiani (1869) at first thought that it was a loose epithelial cell which escaped into the oocyte and functioned like a spermatozoon in parthenogenesis. A few years later, however, he (1893) corrected himself and regarded it as a representative of the centrosome. This view was supported by other workers like Julin (1893) and Munson (1912), but criticised by Gardiner (1927) on the ground that the study of Munson was based on bad preparations. The nature of the lamellae in the yolk nucleus is also controversial. According to some they are specially modified mitochondria (Van der Stricht, 1904; Dyal and Nath, 1933; Jacquiert, 1936) while Koch (1928) believes that they are cytoplasmic in nature.

The present investigation tries to throw light on the origin and function of the yolk nucleus and the homology of its lamellae on the basis of a detailed study of the water spider, *Lycosa birmanica* Thor.

I am extremely grateful to Dr. D. R. Bhattacharya and Dr. Ram Saran Das for their constant criticism and encouragement in the work, as well as to Dr. M. D. L. Srivastava, Dr. L. P. Mathur, and the authorities of the Maharaja's College, Jaipur, for taking a keen interest in the progress of my work and for much assistance. It gives me pleasure to pay my affectionate compliments to Miss Lakshmi Mathur, who volunteered to reduce my coloured plates to black and white to make them suitable for publication.

TECHNIQUE.

The silver techniques of Da Fano, Cajal, and Aoyoma; the osmium technique of Ludford; the chrome-formol technique of Regaud-Tupa; the chrome-osmium technique of Flemming without acetic acid; and the picro-formol method of Bouin were employed for the study of fixed preparations. The mitochondrial preparations were followed by iron-haematoxylin and acid fuchsin. Ripened Janus green B was also used to stain mitochondria in fresh eggs. The silver and osmium slides were not stained. They were simply toned or bleached.

OBSERVATIONS.

An accumulation of the cytoplasmic inclusions at the juxta nuclear position was seen at a very early stage (Plate IV, Fig. 1). Very soon the yolk nucleus appears as a clear vesicle in the juxta nuclear mass of the inclusion particles (Plate IV, Fig. 2 and Text-fig. 6). It is perfectly hyaline in its interior and contains no granule although it has been observed by many workers (Munson, Van der Stricht, Koch, and Narain). The vesicle enlarges but shows no thickening for some time while the inclusion mass is still present in the juxta nuclear position (Plate IV, Figs. 3 and 4). The yolk nucleus continues to grow rapidly and the lamellae formation begins, the outermost being formed first (Plate IV, Fig. 5).

From its initial stages the yolk nucleus occupies a central position in the inclusion mass (Plate IV, Figs. 2, 3, 4 and 5). Very soon it takes over charge of the inclusions and appears to guide all the cytoplasmic activities. In Ludford slides as sketched in Text-figs. 1, 2, 3 and 4 the Golgi bodies are seen in close and definite relationship with the yolk nucleus. In another section as sketched in figure 7 on Plate IV the inclusion mass, the so-called yolk nucleus of

Balbiani, is seen attached to the yolk nucleus. Very few particles are seen near about the nucleus. Dispersal of the Golgi bodies also takes place from the yolk nucleus (Plate IV, Figs. 8, 9, 10 and 11), and during the process the Golgi bodies are sometimes seen forming beautiful patterns. They may be seen forming a common ring round the nucleus and the yolk nucleus (Plate IV, Figs. 8 and 9). The sections mentioned above convincingly show that the yolk nucleus is the chief guiding agent of the dispersal of the Golgi bodies. Fig. 10, Plate IV and Text-figs. 2 and 3 are further evidence of such a relation.

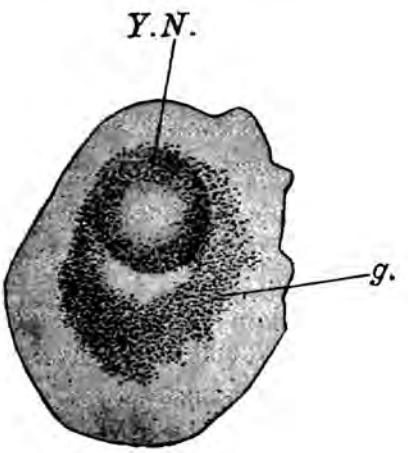


Fig. 1.

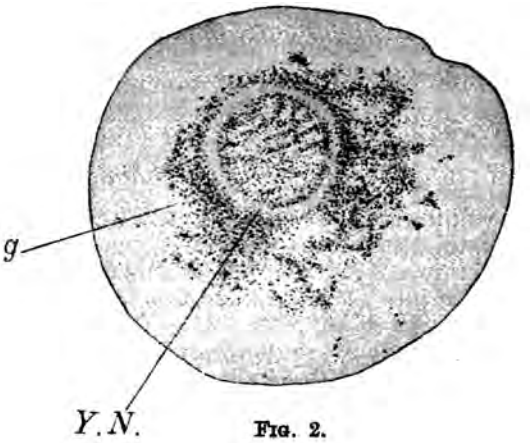


Fig. 2.

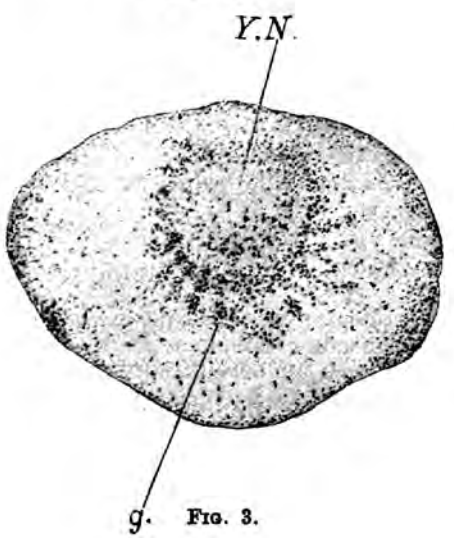


Fig. 3.

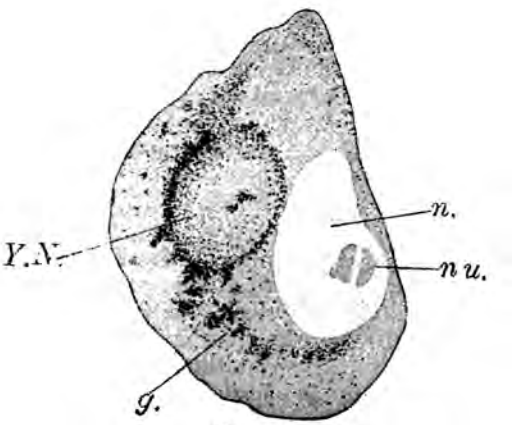


Fig. 4.

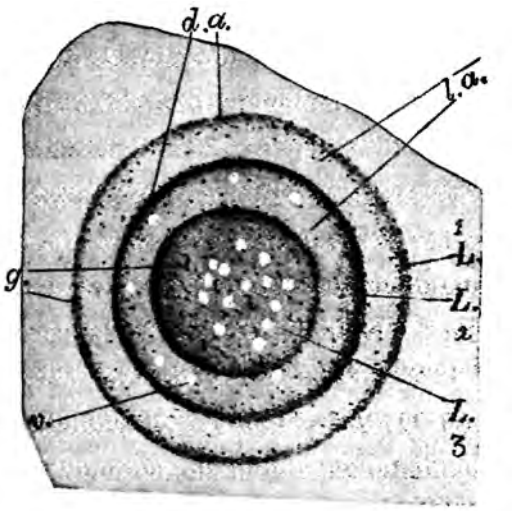
Text-figs. 1, 2, 3 and 4 have been sketched from the material fixed in Ludford and bleached.
Lettering:—*g.*=Golgi bodies; *n.*=Nucleus; *nu.*=Nucleolus; *Y.N.*=Yolk nucleus.

Scale: 0·1 mm.

Similar observations were made in the mitochondrial slides and the same yolk nucleus was found to be the chief guiding agent of the mitochondrial activities. In quite an advanced egg the mitochondrial concentration was found to be attached to the yolk nucleus leaving the nucleus free from any relation with the mitochondria (Text-fig. 7). Later on the mitochondrial concentration was seen covering the whole yolk nucleus area (Text-fig. 8). In Text-figs. 9 and 10 can be seen how dispersal of mitochondria begins. In certain cases quite an interesting pattern of the mitochondrial arrangement was seen. The mitochondria were arranged in three concentric rings round the yolk nucleus (Text-fig. 11).

Even when the yolk formation is very rapid and the whole egg gets choked with yolk bodies, the yolk nucleus looks as healthy as before, showing no sign of decay (Plate V, Fig. 14). The mitochondrial particles also can be seen proceeding from it to the outer cytoplasm. The nucleus is conspicuous by its large size and lies near the periphery of the egg.

The yolk nucleus was fixed and examined in almost all the fixatives. It was beautifully fixed in all of them. In silver fixatives the lamellae were not found to be affected by silver. The argentophil bodies were lying quite freely on the lamellae, in between them and also on the central core (Text-fig. 5). In osmium fixatives the lamellae did not take up the characteristic blackness. The osmiophil bodies were quite distinct and were lying scattered in intimate relationship with the yolk nucleus. The chrome-osmium and chrome-formol fixed slides, stained with acid fuchsin, showed that the lamellae of the yolk nucleus and the mitochondria gave different staining reactions. Mitochondria always take up the brilliant red colour of the hot fuchsin. But the lamellae did not take up the characteristic stain. They were clearly showing staining affinity towards general cytoplasm while the mitochondria retained the characteristic brilliance. In Bouin's fluid the eggs were kept for more than 96 hrs. Even then the yolk nucleus was found to be as healthy as in other fixatives (Plate V, Fig. 15). These reactions seem to be sufficiently convincing to infer that the lamellae of the yolk nucleus in *Lycosa birmanica* are cytoplasmic in nature.



TEXT-FIG. 5. Yolk nucleus as seen under high magnification in Da Fano's silver nitrate method. Lettering:—*d.a.*=Denser archoplasm; *g.*=Golgi bodies; *l.a.*=Lighter archoplasm; L_1=1st Lamella; L_2=2nd Lamella; L_3=3rd Lamella; *v.*=Vacuole.

Scale: 0·05 mm.

Only one yolk nucleus formed the centre of gravity for both the cytoplasmic inclusions, the Golgi bodies and mitochondria. This body was sometimes applied to the nucleus while at others it remained away from it. In some cases the yolk nucleus was seen near the periphery of the egg. It was found to be present in the oldest egg. Even the egg which had undergone atresia showed this structure (Plate V, Fig. 16). A few young ones were fixed in F.W.A. but they never showed any trace of the yolk nucleus in any part of their body.

In another species of *Lycosa* (*Lycosa sp.*) the yolk nucleus which was apparently a homologue of the structure seen in the egg of *L. birmanica*, had a slightly different structure. At its maximum it never measured more than 0·03 mm. in cross-section while in the previous case it was 0·04 mm. Another important difference was in the lamellar pattern. In the spider *Lycosa birmanica* there could be seen three distinct lamellae, the innermost forming a solid core, but in *Lycosa sp.* only two lamellae were seen. The third one could not be made out in any of the fixatives. The central part of the yolk nucleus was found hollow (Plate V, Fig. 17).

Discussion.

While discussing the problem of yolk nucleus three points are to be considered: its structural peculiarities, its origin, and its function and ultimate fate. As for the first point, the yolk nucleus is composed of a clear central vesicle encircled by lamellae. The central vesicle may also be eaten up by the central core. Certain workers have observed a granule seated in the central vesicle (Munson, 1912; Van der Stricht, 1904; Koch, 1928; Narain, 1940). In the present investigation nothing can be said about the granule. The old workers can be doubted for their imperfect technique. But there is hardly anything which can be said against the observations of Narain.

As for the chemistry of the lamellar coverings, some workers have considered them to be mitochondrial in nature (Van der Stricht, 1904; Dyal and Nath, 1933; Jacquiert, 1936). Koch (1928) has raised an objection that the lamellae are very well preserved by the acid containing fluids, which, as is known, usually destroy the mitochondrial elements. Jacquiert supports the mitochondrial hypothesis saying that the mitochondria when they are specially modified and hardened are not taken away by the acidic fluids. Dyal and Nath (1933) also favour Jacquiert and adopt the same line of argument. But the lamellae in their morphological features are quite different from the mitochondria met with in the eggs of *Lycosa*. The sketches made by Koch (1928) make it distinctly clear that the lamellae are cytoplasmic in nature, while the sketches made by Jacquiert do not express his viewpoint so clearly. His main argument remains that the lamellae are preserved in mitochondrial fixatives and because they are specially modified and hardened mitochondria they are not taken away by the acid fixatives either. True. But fixatives can by no means be considered the specific and only criterion for identifying particular inclusion particles, and the hypothesis suggested only on this fact cannot be regarded as based on a sound foundation. The Golgi bodies are also known to be fixed by mitochondrial fixatives and *vice versa*. Moreover yolk nucleus is fixed and preserved in all the fixatives without exception. Hence the argument that the lamellae are mitochondrial because they are preserved in the fixatives meant to preserve mitochondria only does not carry much weight. Moreover ovaries fixed in strong Bouin for an exceptionally long period of about 96 hrs. showed all the lamellae intact. They were not at all affected. Considering the strength of the acid and the long period of fixation it does not carry conviction that mitochondria, howsoever specially modified they may be, would remain so unaffected (Plate V, Fig. 15). Jacquiert (1936) has sketched certain filamentary mitochondria and on the basis of their presence he has suggested a relation between the mitochondria and the yolk nucleus lamellae. But not a single filament of mitochondrial nature was ever seen in the eggs of *Lycosa*. Mitochondria make their appearance first in the young oocytes. The yolk nucleus appears later on as a clear vesicle in the mitochondrial patch, and shows no morphological relation with the surrounding mitochondria (Text-fig. 6). At this stage the lamellae have not been formed and the yolk nucleus is apparently a plasma structure. Koch (1928) in a series of diagrams has also shown it to be of the same nature. Before saying anything definite of a structure it should always be put to microchemical tests of staining. Neither Nath (1933) nor Jacquiert (1936) has made use of the acid fuchsin stain and the vital dye Janus green B which are specific for identifying mitochondria. In F.W.A. and Regaud-Tupa slides stained with acid fuchsin the lamellae did not take the brilliant red colour characteristic of mitochondria: rather they had the shade of the stain as was the case in the general cytoplasm. When the fresh eggs were treated with the vital dye, Janus green B, the mitochondria were stained green but the lamellae of the yolk nucleus did not take any characteristic stain (Plate V, Figs. 12 and 13).

As regards the origin of the yolk nucleus, although several workers have recorded its formation as due to nuclear activity (Hirschler, Saguchi, and Heberer as quoted by Srivastava, 1938), no evidence of the kind was ever observed in the present case. Here it appears to arise as a clear vesicle in the ground cytoplasm near the nucleus (Plate IV, Fig. 2 and Text-fig. 6).

Coming to the function of this peculiar structure, it was found to be the principal guiding agent of all the cytoplasmic activities. Fig. 7 on Plate IV and Text-fig. 7 clearly show that the inclusion particles have been detached from the nucleus and are guided by the yolk nucleus. In figure 14, Plate IV, the yolk nucleus is lying in the field of activity while nucleus is seen on one side apparently not having much to do with the inclusion particles. Yolk formation also begins from the area of this structure. In a section of the egg of *Lycosa sp.* the yolk bodies can be seen inside the yolk nucleus, too (Plate V, Fig. 17). Other workers have also shown that it serves as a centre of yolk formation (Munson, 1912; Koch, 1928; Jacquiert, 1936).

Taking this fact as a criterion certain others have described equivalent structures in the eggs of other animals as yolk nuclei, attaching little importance to the morphology and lamellar

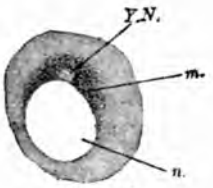
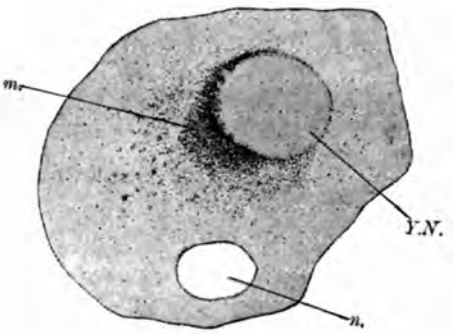


FIG. 6.



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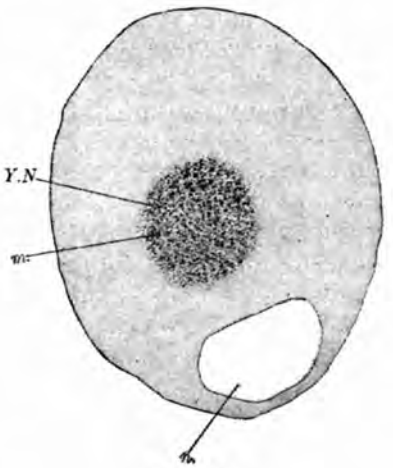

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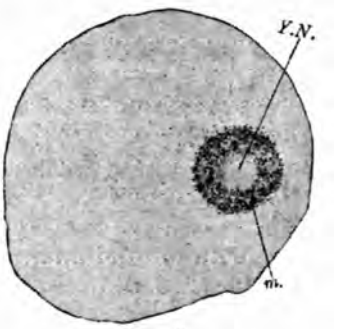

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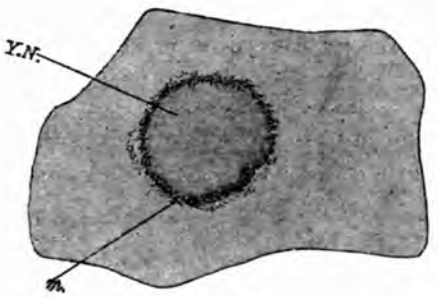

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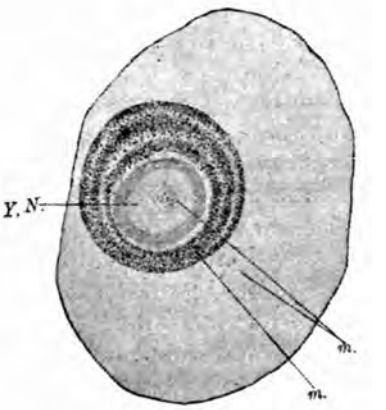

FIG. 11.

TEXT-FIG. 6 is from Regaud-Tupa followed by acid fuchsin. The rest of the TEXT-FIGS. 7, 8, 9, 10 and 11 are from F.W.A. preparations followed by iron-haematoxylin.

Lettering :—*m.*=Mitochondria ; *n.*=Nucleus ; *Y.N.*=Yolk nucleus.

Scale :—Same as for Text-figs. 1, 2, 3 and 4.

pattern of their structures (Das, 1931; Srivastava, 1938; Bhattacharya, 1925; Narain, 1940). L. P. Mathur (1940) has also described a yolk nucleus in the eggs of *Eryx*. The structure has no lamellar covering but he has preferred to give it the same name simply because yolk formation in the eggs of *Eryx* starts from that area.

Summary.

The yolk nucleus is a homologue of Wittich's 'Dotterkern'. It appears in the inclusion mass as a clear vesicle, having no central granule. There are three lamellae arranged concentrically. Since they could be preserved in the acidic fixative (Bouin), which destroys other inclusions, the lamellae are not mitochondrial in nature. Apparently they take origin in cytoplasm. The yolk nucleus from quite an early stage seems to be the principal guiding agent of all the cytoplasmic activities. It apparently takes over charge of all the inclusion particles. The yolk nucleus was found to be present even in the oldest egg. Its ultimate fate could not be observed. Young spiders (as suggested by Koch) were also fixed but no trace of this structure was found. The yolk nucleus remains applied to the nucleus in young oocytes but later on it moves away from it.

Explanation of Plates.

All the figures on Plates IV and V have been drawn on the scale given on Plate IV, excepting figures 15 and 17. Their scale is given separately on Plate V. Figures 12 and 13 are drawn from fresh oocytes approximately on the same scale as given on Plate IV, but strict accuracy in scale has not been observed.

Plate IV.

All the figures are from the material fixed in Ludford's modification of Mann-Kopsch. Unstained.

Plate V.

Fig. 12. Fresh oocyte of *Lycosa sp.* treated with ripened Janus green B.
Fig. 13. Fresh egg of *L. birmanica* treated with Janus green B.
Fig. 14. F.W.A. with acid fuchsin.
Fig. 15. Bouin with iron-haematoxylin.
Fig. 16. Egg undergoing atresia. Ludford. Unstained.
Fig. 17. Yolk nucleus from the egg of *Lycosa sp.* Bouin with Mann's methyl blue-eosin.

Explanation of Lettering on the Plates.

A. = Albuminous yolk; *d.a.* = Denser archoplasm; *G.* = Swollen Golgi bodies; *g.* = Golgi bodies; *l.a.* = Lighter archoplasm; L_1 = 1st Lamella; L_2 = 2nd Lamella; L_3 = 3rd Lamella; *M.* = Swollen Mitochondria; *m.* = Mitochondria; *n.* = Nucleus; *n.u.* = Nucleolus; *v.* = Vacuole; *Y.N.* = Yolk nucleus.

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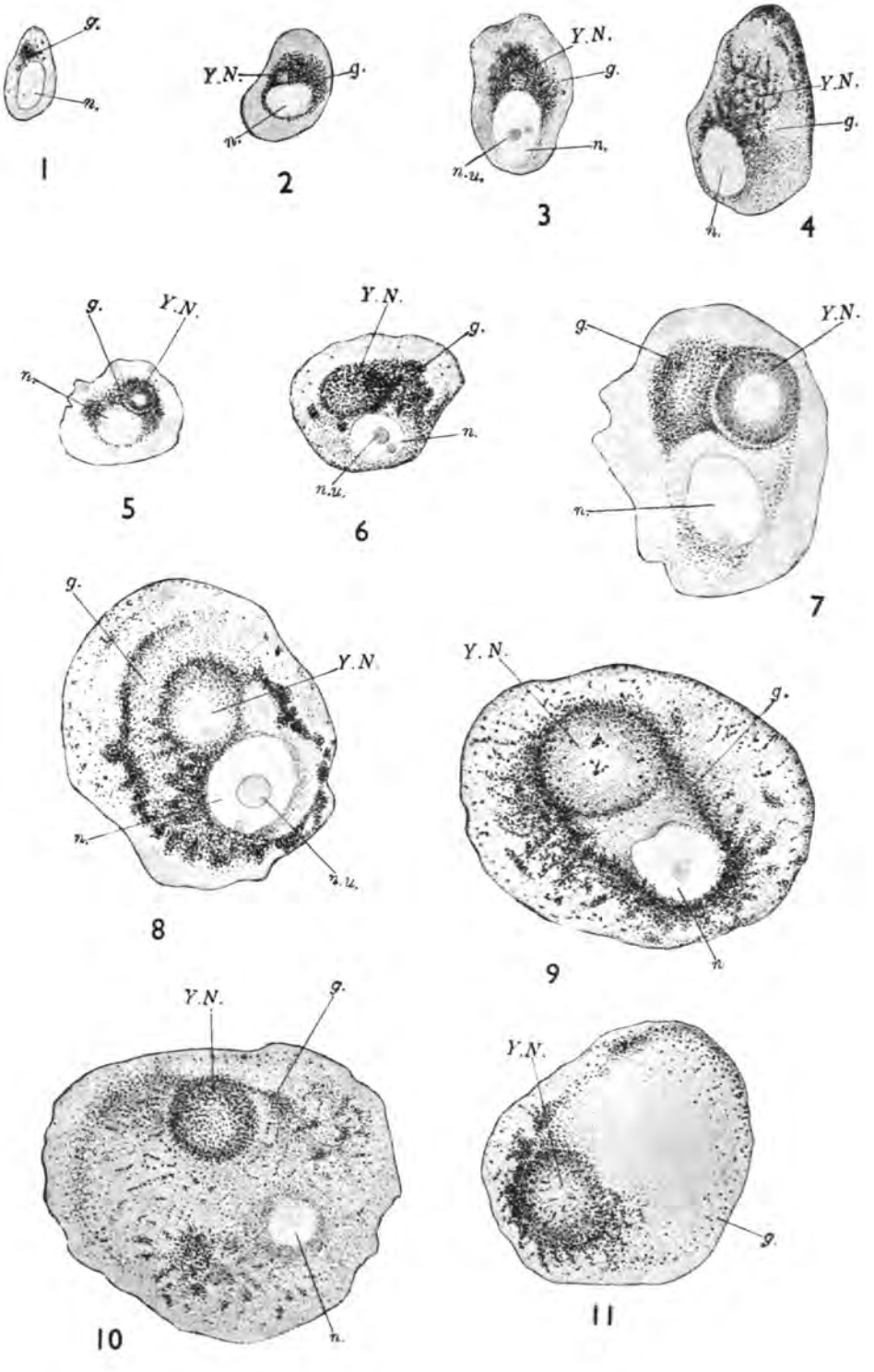
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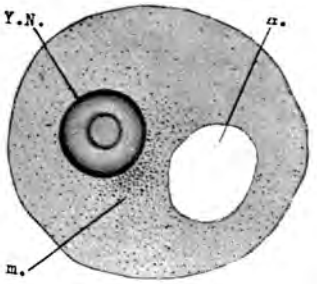

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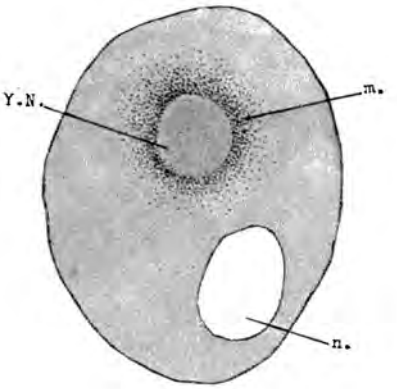

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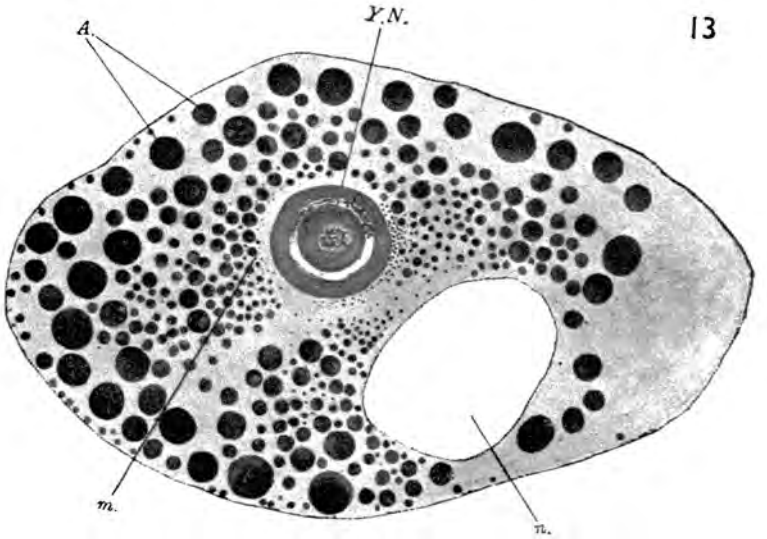

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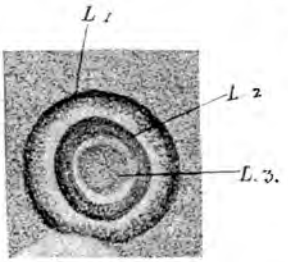

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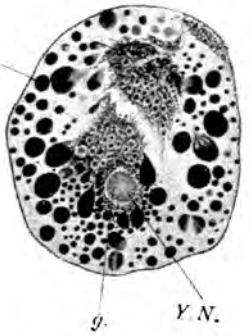

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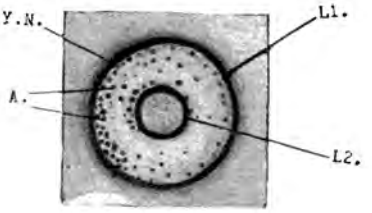

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FIGS. 15 & 17.