

The Vegetative Divisions in *Vicia Faba*.

BY

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With Plates LXII and LXIII.

THE nuclear divisions in *Vicia Faba* have long been a common object of laboratory demonstration, and preparations made early in 1910 for this purpose proved so suggestive that a detailed study was undertaken, first of the somatic and later of the meiotic stages.

The discussion of meiosis is reserved for a later paper, and we propose at present to deal only with the vegetative divisions. These were studied chiefly in the root apex, but also in the young petals, stamens, and ovary, and finally in the pollen-grain.

Plants of Carter's 'Monarch' were grown in the spring and summer of 1910 partly in the greenhouse of this Department and partly in a garden at Penge under the care of one of us.

The tissues were fixed in various media, of which Flemming's strong fluid diluted with an equal quantity of water proved the most successful. Sections were cut from 4μ to 5μ in thickness, and were stained sometimes with Flemming's triple stain or with Heidenhain's iron haematoxylin, but most frequently with the combination of Brynal, which, when successfully used, far surpassed either of the others for this purpose.¹

MITOSIS IN THE SPOROPHYTE.

It is convenient to begin the account of the mitoses in the Broad Bean at the stage when the chromosomes are reaching the poles of the spindle, up the outer fibres of which they travel so that they form a hollow truncated cone. The individual chromosomes at this stage are

¹ I am indebted to the Government Grant Committee of the Royal Society for various lenses and certain other apparatus used in this work. H. C. I. F.

straight or hooked rods (Pl. LXII, Fig. 1). Some of them are sharply contracted near the middle, and all of them show a slight irregularity of outline.

As they reach the narrow end of the spindle they shorten and thicken (Fig. 2), become more closely massed, and come into contact with their neighbours on either side.

The daughter nuclei thus newly formed become rounder and more definite in outline, and a faint differentiation of the cytoplasm suggests that the nuclear membrane has been formed. At the same time the close aggregation of the chromosomes loosens, but each remains attached to its neighbours by threads of stainable material. While this is taking place slits are observed here and there in the middle line of each chromosome and seem by their position to be due to the pull of the lateral attachments (Fig. 3).

With the increase of the young nuclei in size both the slits and the cross attachments become more marked (Figs. 4, 5); after a time the nucleus reaches an approximately spherical shape, and the chromosome complex is spread over the surface of the sphere just inside the now clearly defined nuclear membrane (Fig. 6). Now also the nucleolus becomes visible, appearing first as a drop or drops of stainable material (Fig. 7), and usually in relation to one or more of the chromosomes.

The chromosomes themselves become much less regular in outline as their stainable substance is aggregated here and there in irregular masses, leaving a relatively thin thread between (Figs. 7, 9).

At this stage the centre of the nucleus in median section appears empty—except perhaps for the presence of one or two nucleoli—and is surrounded by a ring of chromatin threads. But above or below the middle line a network may be focused, and in it the split and laterally attached chromosomes are clearly recognized (Fig. 8). Their ends are by this time no longer to be found, and have presumably become joined either to those of their lateral neighbours or to those meeting them across the poles of the nucleus; and the longitudinal slits have been pulled out to form diamond-shaped areas (Fig. 9). As time goes on, the limits of the individual chromosome become less obvious, but the extent to which they may be recognized depends partly also on the angle from which the nucleus is viewed. Thus the so-called resting stage is reached, and the nuclei, which may differ one from another considerably in size (cf. Figs. 9, 10), either pass into the 'permanent state', or after a varying period once more prepare for division.

The first indication that division is about to begin is found in the breaking down of certain threads of the reticulum which, in favourable cases, may be recognized as the cross attachments between the chromosomes of the preceding telophase.

At the same time, perhaps we may say in consequence of this change, other meshes approximate so that a double thread is formed (Fig. 11). It shows an appearance similar to that of the split chromosomes when passing into the resting stage (cf. Figs. 5, 11), and may safely be identified with them. The spireme thus produced is from its formation a double structure.

As development proceeds there becomes evident a definite relation between the nuclear thread and the nucleolus; the spireme is arranged in coils and loops radiating somewhat irregularly from the nucleolus in a way that recalls the 'second contraction' of meiosis (Figs. 12, 13), and may perhaps be compared to the polarization observed by Farmer and Shove (3) at a similar stage in *Tradescantia*. The nucleolus becomes vacuolate and decreases in size, doubtless giving up material to the chromatin thread, and as it disappears the spireme straightens (Fig. 14) and becomes coiled with more or less regularity either around or up and down the nuclear area (Fig. 15). The remains of the cross attachments have by this time disappeared, and the stainable substance has come to be pretty uniformly distributed along the thread, which is double throughout its length.

At about this stage the segmentation of the spireme into chromosomes occurs (Figs. 16, 17); this takes place gradually, the spireme breaking across first at one point and then at another, and for some time the separated chromosomes may adhere one to another by fine threads (Fig. 18). Throughout the subsequent stages certain chromosomes are again and again observed, as in the telophase, to be made up of two, or in one or two cases more, distinct segments (Figs. 1, 19, 23), and when separation of the daughter chromosomes has taken place it is possible to recognize this in each member of a pair.

While the development of the chromosomes is proceeding, spindle formation has taken place. A thickening of the cytoplasm around the nucleus occurs at an early stage (Fig. 16), and later, as the nuclear membrane breaks down, delicate fibres are visible (Fig. 18), running from end to end or from corner to corner of the cell.

It would appear that the chromosomes take up their position on the spindle very soon after it is formed (Fig. 18) and remain for some time lying against it; we observed, at any rate, numerous stages such as that shown in Fig. 19, while Fig. 18 represents a stage comparatively rare.

Throughout the development of the spireme the longitudinal fission of the thread is very clear, but after segmentation it becomes inconspicuous, and may sometimes seem to be completely obliterated (Pl. LXIII, Fig. 27). Even at this stage, however, it can be identified in favourable preparations (Pl. LXII, Fig. 18), and by the time that the chromosomes have passed on to the spindle it is once more evident.

The double chromosome is attached to the spindle by one end, and at first lies more or less parallel to or across the spindle fibres (Fig. 19).

Gradually it becomes erected till it stands out at right angles to the fibres (Pl. LXII, Figs. 19, 20), and the ends of the two daughter chromosomes, which at first lay close together, begin to slide away from one another along the spindle (Fig. 20) till the double rod becomes transformed into a wide V or U, at the apex of which the ends remote from the spindle lie pressed together or just crossing one another (Figs. 21, 22). The ends upon the spindle travel further and further apart till the daughter chromosomes are no longer in contact and lie flat or almost flat along the fibres (Fig. 23). They have the form of straight or somewhat sinuous rods; frequently the end towards the pole of the spindle is bent over, forming a hook; whether this takes place depends on the position of the chromosome during the early stages of its attachment to the spindle. Indications that such hooks will be produced may be recognized in Figs. 20 and 21.

The daughter chromosomes have now reached the stage represented in Fig. 1, and they show no recognizable indication of the longitudinal split which is about to occur; indeed, we may perhaps suppose that, for the short period between the metaphase and the telophase, the chromosome is a single structure.

MITOSIS IN THE GAMETOPHYTE.

Various authors have called attention to the double nature of the chromatin elements in somatic cells, and it has been interpreted by some of them (Overton (12), Sykes (14), Takara (15)) as due to the lateral approximation of structures derived respectively from the male and female gametes, while to other investigators (Hof (7), Digby (1)) it has appeared rather that the duplication is brought about by an early longitudinal fission, which in the telophase of one division already prepares for the next.

In the Broad Bean a study of these stages in the cells of the sporophyte seemed to us to point pretty clearly to the latter interpretation; in order to test this conclusion we proceeded to a study of the vegetative divisions of the gametophyte, and examined the nuclei of the pollen-grains in various stages of development.

Here the chromosome number is seven instead of fourteen as in the diploid cells, and there is no question of the association of paternal and maternal structures since only a single set of chromosomes is present. But in the pollen-grain, exactly as in the cells of the root or flower, a double reticulum was observed (Pl. LXII, Fig. 24; Pl. LXIII, Figs. 31, 32), and here also the first evidence of duplication appears in the late telophase (Figs. 30, 31).

The formation of the first 'resting' gametophyte nucleus is preceded by the homotype division. It is proposed to discuss the meiotic stages in a forthcoming paper, and it suffices here to say that in the homotype telophase, as in all others studied, longitudinal fission can be seen.

After the conclusion of the homotype division, while the pollen-grains are still grouped in tetrads, the nuclei are small and very dense, with a fine reticulum in which the details of the arrangement of the thread are not readily made out.

As the nucleus increases in size the reticulum loosens, and its double nature is very evident (Pl. LXII, Fig. 24). The nucleus remains in the resting stage for some little time, while the wall of the pollen-grain becomes thickened, and then mitosis begins and hardly differs except in chromosome number from the corresponding process in diploid nuclei. The longitudinal fission is clear in all stages (Pl. LXIII, Figs. 25, 26, &c.), except that here, as in the somatic cells, it may be more or less obliterated when the chromosomes are passing on to the spindle (Fig. 27). The metaphases and anaphases differ from those of the sporophyte in the narrower spindle (Fig. 28); the individual chromosome presents the same appearance and shows the same somewhat irregular outline (Fig. 29). Longitudinal fission of the daughter chromosomes usually appears in the tube nucleus (Fig. 30) a little before it is seen in the generative nucleus (Fig. 31). The reticulum (Pl. LXII, Fig. 24; Pl. LXIII, Fig. 31) is quite like that of the sporophyte, though it gives the effect of being made up of fewer threads. As in the sporophyte the cross attachments break down so that the spireme is double from its formation. The nucleolus, sometimes at any rate, persists longer, and may be seen (Fig. 25) after the spireme has divided into its constituent chromosomes.

DISCUSSION.

The longitudinal fission of the Chromosomes.

In view of the evidence derived both from the diploid and haploid nuclei there seems to us little doubt that in the Bean the separation of the chromosomes into two equivalent portions on the spindle is already foreshadowed by their fission in the preceding telophase.

That such a state of affairs is of common occurrence is suggested by the observations of Grégoire and Wygaerts (6) on *Trilium*, of Grégoire (7) and of Merriman (10) on *Allium*, and of Digby (1), who not only studied the sporophyte, but also observed parallelisms in the nucleus of the young pollen-grain on *Galtonia*.

In *Vicia Faba* the chromosomes do not appear to break up as in *Galtonia* (Digby (1)), but become joined to one another both end to end¹ and laterally. In the region of the lateral attachments the sides of the split chromosomes are pulled apart, and in this way the network of the

¹ Grégoire lays special stress on his observation that in *Allium* the ends of the chromosomes remain independent; it seems to us very improbable that such is the case in *Vicia*, for no free ends can be identified either in the reticulum or in the spireme stage, except such as inevitably occur in nuclei cut by the microtome knife.

resting stages is produced. Later the cross attachments break down so that the spireme consists of the split daughter chromosomes of the last division arranged presumably in the same order in which their ends became united in the telophase.

The double arrangement persists till the two halves separate on the new spindle, and it is only at one stage even temporarily obscured, namely, after the segmentation of the spireme when the chromosomes are passing on to the spindle. This is in part no doubt due to a fresh supply of stainable substance which has been taken up from the nucleolus, but we have also found it useful in this connexion to recall the behaviour of two parallel pieces of elastic band: if these are held just touching one another and then pulled out by their ends, the sides separate to come together once more when the strain is relaxed. But if the now slack pieces are bent or twisted their independence is again obvious. So, it appears to us, the taut spireme shows a duplication which may disappear when it segments, but which is once more obvious when the separate pieces come to lie variously curved upon the spindle. It must further be borne in mind that the chromosome is split only in one plane, and that from certain points of view the fission is therefore invisible. Moreover, in favourable preparations the duplication can even now be recognized, and it seems to us very doubtful whether complete closure of the split ever occurs.

The recognition of the longitudinal fission in the chromosome is thus carried back from the prophase to the preceding telophase, but the mechanism by which it is accomplished is still to seek. Grégoire (4, 5) describes the fission in the telophase as due to the development of alveoli within the chromosome, though he does not connect this process with the subsequent duplication of the spireme; and a corresponding explanation has been put forward by Stomps (13), who described a process of vacuolization in *Spinacia*, which divides the chromosomes of the telophase into series of parallel lamellae. We have seen no indication of more than one line of fission in the Bean, and we are inclined to suggest that the pull of the lateral attachments may play an important part in bringing this fission about.

One is driven throughout the study of mitosis in the Bean to visualize the chromosomes as somewhat elastic, viscous bands, easily adhering to one another, easily splitting in their more fluid interior, but retaining their form by reason of the greater density of their surface layer, which, as Livingston (9) points out for the ectoplasm, may be differentiated by mere contact with the external solution. From such a point of view it will follow that the narrow cross attachments, consisting as they must almost entirely of the transformed outer layer, may cohere sufficiently to pull asunder, as the nucleus expands, the unaltered centres of the chromosomes to which they are laterally attached.

It remains to be questioned why the cross attachments formed at this stage are stronger, as the above implies, than the attachments formed between sister chromosomes (Pl. LXII, Fig. 20), or indeed any near neighbours in the prophase and early metaphase. It is probably a relevant consideration that the chromosomes, crowded together at the narrow end of the spindle, seem to be in contact under pressure, whereas they lie loosely side by side in the earlier stages.

As far as we can see, though fission is begun in the telophase, it is not complete till after the spireme is formed, for, even in the early spireme stages (Fig. 11), the thread in *Vicia* is made up of alternate double and apparently single portions, and in Figs. 5 and 8 a clear relation seems to exist between the cross attachments and the points of fission. It must here again be borne in mind that the thread in the regions of cross attachment is comparatively tense, and that it is comparatively slack between these points, so that there the sides of a split thread might readily fall together. The possibility thus remains that the cross attachments do not cause the split, but only make evident a fission already accomplished.

Mechanism of Mitosis.

It is difficult again to obtain useful evidence as to the mechanism which brings about the separation of the daughter chromosomes, but the stages by which this is accomplished in *Vicia* seem fairly simple. The chromosomes become attached by one end to the spindle, they lie lax for a time along it, they are swung out at right angles to the spindle axis, and the separation of the two halves of the attached end begins. The halves remain in contact at the free end for a considerable time, so that the daughter chromosomes form first an acute and later an increasingly obtuse angle, till they come to lie almost along the same straight line. By this time they are quite free one of another; they show no decrease in length till they approach the poles, when they are shortened and thickened, forming the dense mass described. The contraction of the chromosomes at this stage is no doubt due to the continued action on their lagging ends of whatever force is responsible for their movement along the spindle.

It is well known that after good fixation the spindle fibres are often less conspicuous than when inferior fixatives are used. This fact is evidence against the recognition of the fibres as definite cell entities: Farmer and Moore (2) have regarded them as protoplasm modified by the forces at work in the cell, and we are led to suggest that an important part may be played by currents of altered cytoplasm in Angiosperms, much as these have been suggested (Fraser and Welsford (4)) to be responsible for the changes taking place in the cytoplasm of the ascus among Fungi.

If it be credible that such an alteration is in the direction of greater osmotic activity, then we should have a mass of osmotically active substance

(the end of the central spindle) lying within each group of chromosomes at the pole. When the nuclear membrane is formed water would pass into this mass from the outer cytoplasm, which is *ex hypothesi* less osmotically active, and the nuclear area would enlarge. If with the nuclear membrane the chromosome complex is carried outwards and therefore distended, the necessary conditions are obtained for the fission of the chromosomes in their more fluid interior under the pull of the lateral attachments.

Abnormal Conditions.

Some study has been made in recent years of the effect of abnormal conditions on nuclei, and *Vicia* has often been employed for this purpose. Němec (11) in an investigation of the effect of chloral hydrate and other reagents lately described a stage similar to that shown in Fig. 19 as representing the separation of the gemini in a heterotype division consequent on the nuclear fusions he records. This stage is a very common one in our material, both in the roots and in other vegetative organs grown under natural conditions, and we are constrained to regard it as a normal phase of karyokinesis. That this is the case is perhaps worth recording in view of Kemp's failure to find meiotic stages even in material subjected to abnormal conditions (8).

We noticed, also, considerable variation in the size of the nuclei even in neighbouring cells (cf. Pl. LXX, Figs. 9 and 10), but nuclei of irregular form, tripolar spindles, and other evidences of abnormality were entirely absent, as was indeed to be expected.

Segmented Chromosomes.

In a considerable number of cases some of the chromosomes on the spindle were seen to be made up of two or occasionally more distinct segments. It is perhaps possible to imagine that the segment rather than the chromosome or chromomere represents a discrete (hereditary) unit, and it might be suggested that the arrangement of these units in the chromosomes is indifferent and may vary. Such a speculation may throw light upon the fact that the number of independent Mendelian characters is in certain organisms greater than the haploid number of chromosomes, and moreover, if it were of at all general application, it would account for the often described variation in the chromosome number. Thus, if the average number of segments in certain chromosomes be two, their occasional independence or the union of three or four together would increase or diminish the apparent number of chromosomes. Again, the more or less permanent association of two segments would produce an appropriate physical basis for the Mendelian phenomenon of coupling.

SUMMARY.

1. There are fourteen chromosomes in the sporophyte and seven in the gametophyte of *Vicia Faba*.

2. On reaching the pole of the spindle the chromosomes become massed together and come into contact one with another.

When this aggregation loosens, the chromosomes remain laterally attached to their neighbours and show longitudinal fission.

The fission persists and is pulled out in the regions of the cross attachments to form the diamond-shaped meshes of the reticulum. The ends of the chromosomes also unite, and one or two nucleoli appear. Thus the 'resting stage' is produced.

3. On the initiation of a new division the cross attachments break down, the sides of the diamond-shaped areas approximate, and a double spireme is formed; this ultimately breaks transversely into longitudinally split chromosomes.

4. The line of separation of the daughter chromosomes on the spindle is therefore marked out in the preceding telophase, and persists throughout the intervening stages.

5. The chromosomes are frequently constricted into segments, and it seems probable that the way in which the segments are grouped to form chromosomes may vary. An explanation here suggests itself of the often recorded variation in the chromosome number, and possibly also of the Mendelian phenomenon of coupling.

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EXPLANATION OF PLATES LXII AND LXIII.

Illustrating Dr. Helen Fraser's and Mr. Snell's paper on Vegetative Divisions in *Vicia Faba*.

All figures were drawn with a camera lucida under a 2 mm. apoch. hom. imm. Zeiss N.A. 1.40 comp., oc. 12. Magnification $\times 2,000$ throughout.

PLATE LXII.

- Fig. 1. Root; late anaphase, showing segmented chromosomes.
 Fig. 2. Ovary; telophase, chromosomes contracted.
 Fig. 3. Stamen; loosening of aggregation of chromosomes; cross attachments formed; longitudinal fission begun.
 Fig. 4. Root; later stage of same.
 Fig. 5. Root; same, still later.
 Fig. 6. Root; early reticulum.
 Fig. 7. Root; early reticulum; nucleolus present.
 Fig. 8. Root; reticulum in upper nucleus; in lower (sister) nucleus spireme formation has already begun.
 Figs. 9 and 10. Root; 'resting' stages.
 Fig. 11. Root; breaking down of cross connexions in formation of spireme.
 Fig. 12. Root; early spireme, showing relation to nucleolus.
 Fig. 13. Root; same, rather later.
 Figs. 14 and 15. Root; later stages of spireme; disappearance of nucleolus.
 Fig. 16. Root; beginning of segmentation of spireme.
 Fig. 17. Root; tangential section of nucleus, showing newly formed chromosomes.
 Fig. 18. Root; chromosomes passing on to spindle.
 Fig. 19. Root; chromosomes attached to spindle by one end, and for the most part lying parallel to it.
 Fig. 20. Root; individual chromosomes on spindle, (a) cross attachment between members of a pair of daughter chromosomes; (b) and (c) early stages of separation of daughter chromosomes.
 Fig. 21. Root; metaphase.
 Fig. 22. Root; later stage of same.
 Fig. 23. Root; early anaphase, showing segmented chromosomes.
 Fig. 24. Young pollen-grain in transverse section; 'resting' nucleus, longitudinal fission visible in threads of reticulum.

PLATE LXIII.

Fig. 25. Pollen-grain in oblique section ; longitudinally split chromosomes ; remains of vacuolate nucleolus.

Fig. 26. Pollen-grain in transverse section ; chromosomes after disappearance of nuclear membrane.

Fig. 27. Pollen-grain in longitudinal section ; chromosomes passing on to spindle ; split, not recognizable.

Fig. 28. Pollen-grain in longitudinal section ; metaphase.

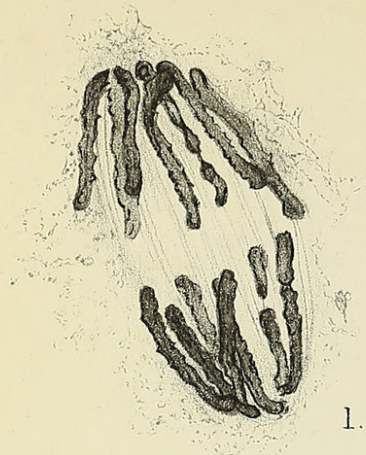
Fig. 29. Pollen-grain in longitudinal section ; anaphase.

Fig. 30. Pollen-grain in longitudinal section ; late telophase ; in the larger (tube) nucleus the chromosomes have already undergone fission.

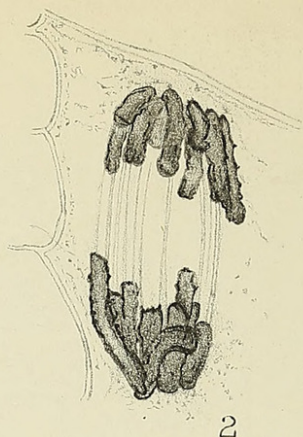
Fig. 31. Pollen-grain in longitudinal section ; tube nucleus showing reticulum ; chromosomes, in which fission has taken place, still recognizable in generative nucleus.

Fig. 32. Pollen-grain in transverse section ; reticulum in 'resting' nucleus.

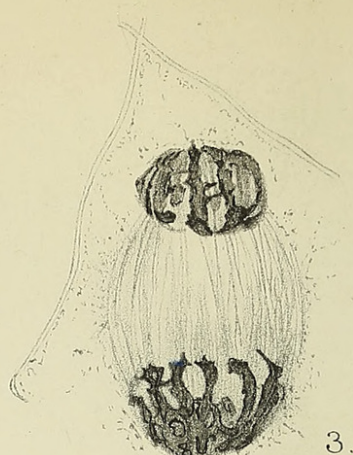
Fig. 33. Pollen-grain in transverse section ; nucleus with spireme beginning to form.



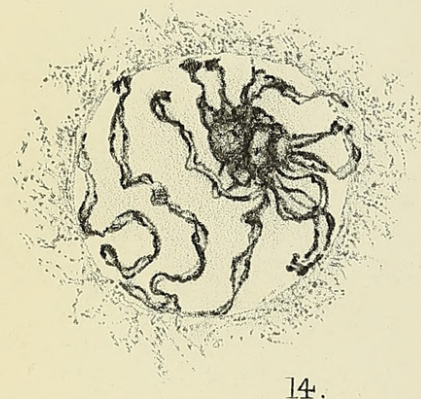
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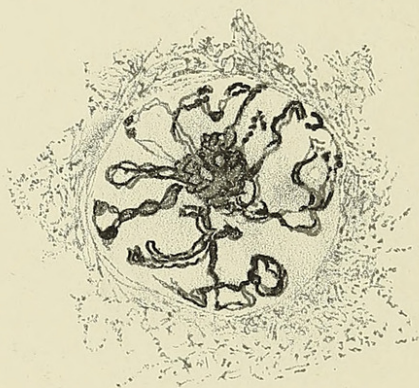
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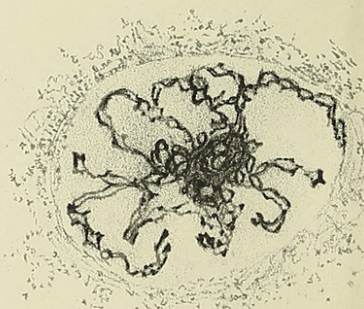
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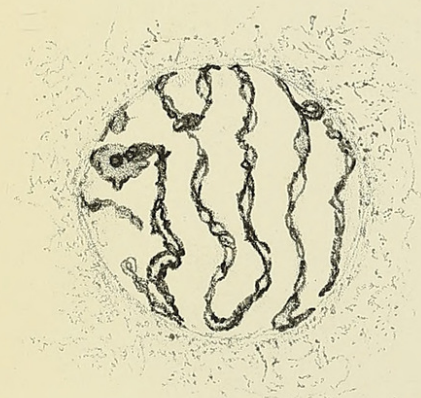
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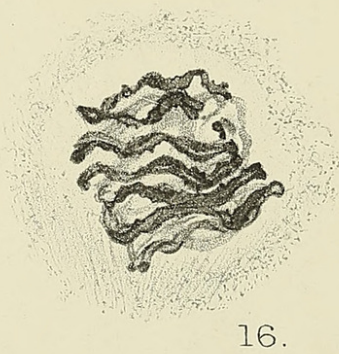
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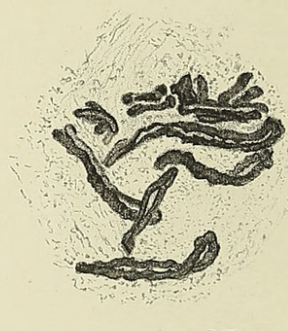
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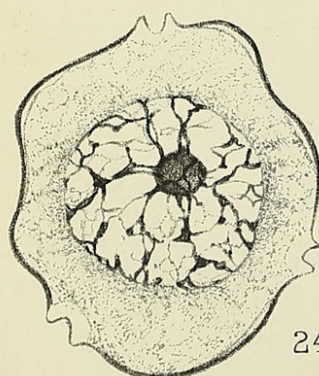
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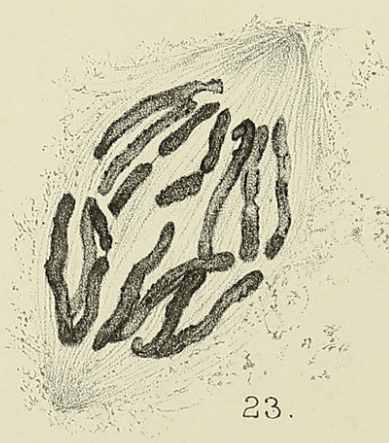
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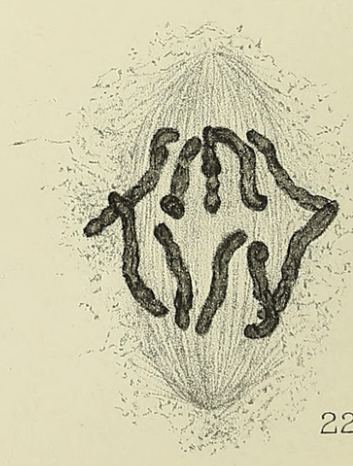
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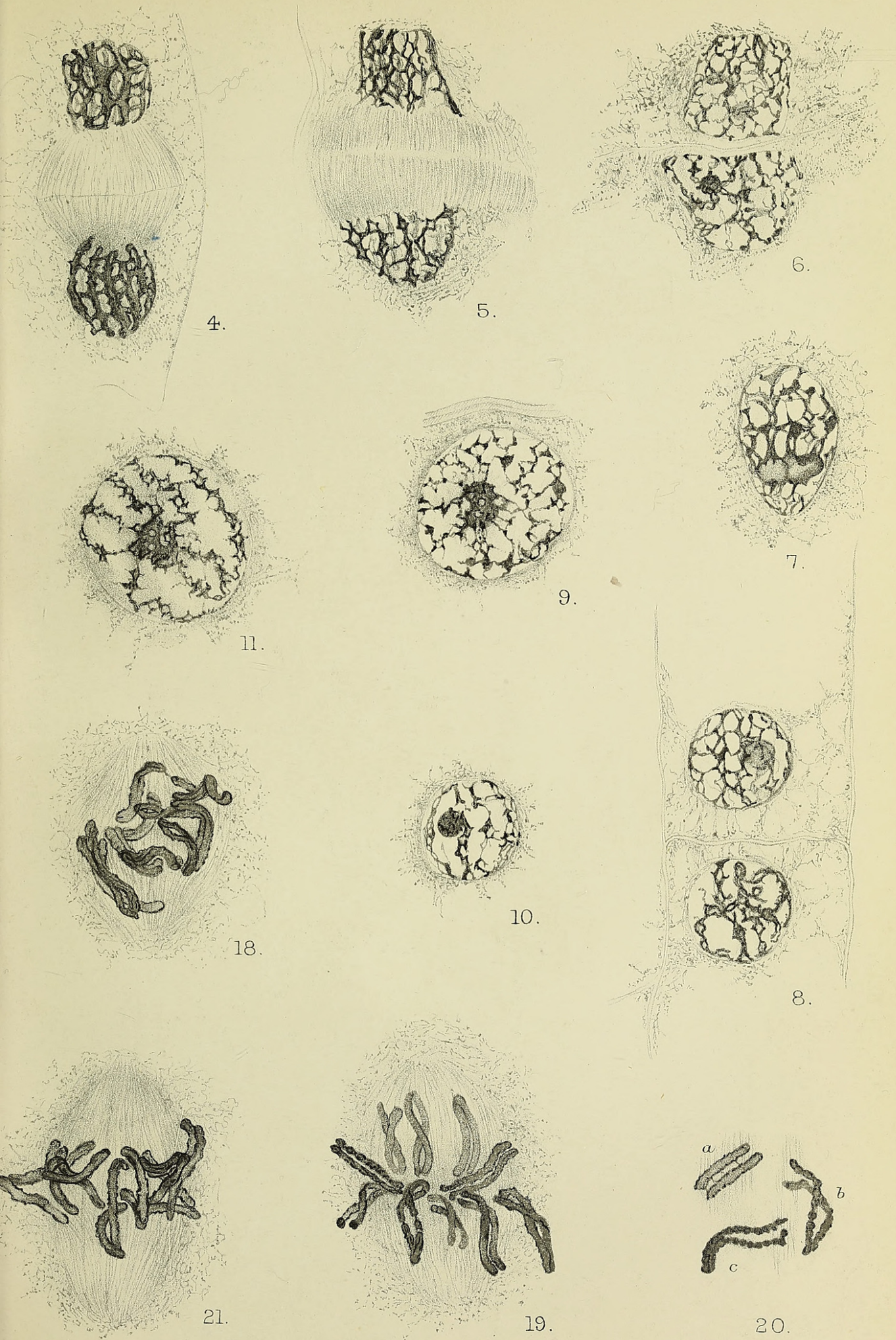


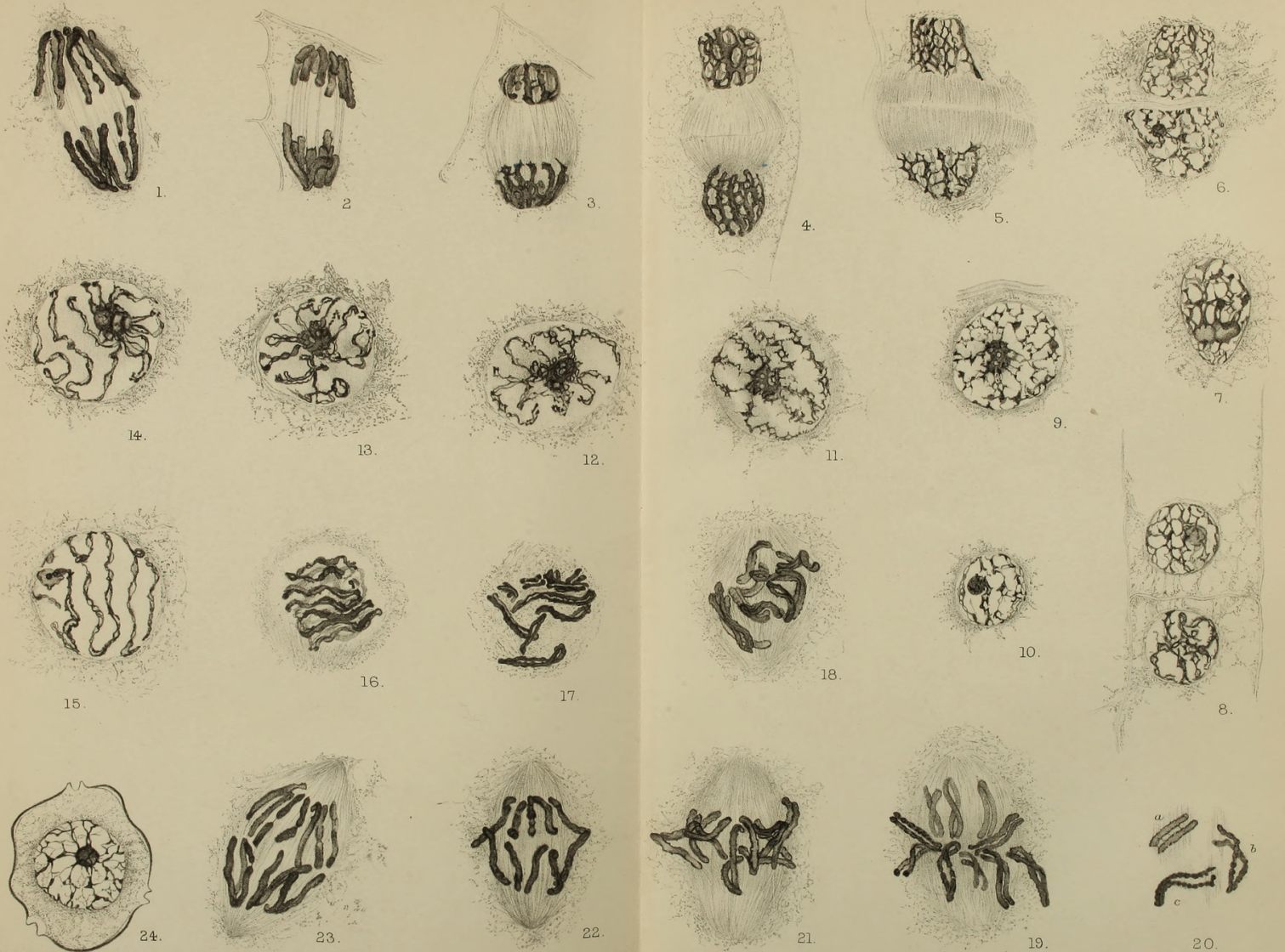
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