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Spiral Structure of Chromosomes in *Lilium*

By

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With Plates XVII & XVIII

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In *Lilium* species the chromosomes are large and suitable for the study of chromosome structure. Observing the pollen mother cells in *Lilium longiflorum* by the smear method one of the writers (I) found that in the heterotype anaphase the spiral structure was clearly visible, and that the chromatic spirals were drawn out in various degrees especially often near the point of spindle fiber attachment. The present investigation was undertaken originally with the object of tracing in finer detail this behaviour of chromatic spirals in the anaphase, but was extended to later stages up to the homotype anaphase to follow the successive morphological changes of chromonemata taking place in these stages.

Pollen mother cells of *Lilium longiflorum*, *L. speciosum* and *L. tigrinum* were used as material, and observations were made exclusively with the acetocarmine smear method.

Observation

Heterotype metaphase. In *Lilium longiflorum* and *L. speciosum* the configuration of the mating chromosomes in the heterotype metaphase is considerably different in different pairs of chromosomes according to the degree in which they twist about each other. In *L. tigrinum* which is triploid in nature (TAKENAKA and NAGAMATSU, 1930; SATÔ, 1932) the configurations of these chromosomes differ from those found in the two species just mentioned in that they are considerably more complicated. So far as the structure of chromosomes is concerned, however, the descriptions given below can apply to all these three species of *Lilium* studied except for the cases otherwise indicated.

In the diakinesis the chromosomes are usually obscure of their internal structure and often present a mesh-like appearance (cf. KAUFMANN, 1931), but in the metaphase they become more condensed in form and clearer in structure. In this stage they are found to be composed of two elements: a less stainable core and a chromatic spiral which winds around the former rather than lie embedded in it as a matrix (Figs. 1, 2, 3 and 4). The turns of the spiral run so closely that in many cases the spiral appears as a pile of discontinuous bars, but in favourable cases the bars can be traced by moving the micro-screw from one to the other continuously as on a continuous coiled thread. In each diad of the metaphasic chromosomes, the spiral usually appears to be single, but in certain favourable cases its double nature is recognizable. In Fig. 1 the doubleness of the spiral is shown in the upper member of the bivalent. It becomes manifest when the chromatids in each diad separate from each other in the late metaphase.

In Figs. 2 and 4, it is shown that the spiral mentioned above is not the ordinary simple spiral, but is one formed of a spiral of small gyres or the double-coiled spiral, as has been clearly demonstrated by KUWADA and NAKAMURA (1933, 1934 a) by loosening and unravelling the spiral by means of ammonia vapour. This structure can also be demonstrated by crushing the chromosomes by the press given on the cover-glass with a needle. An example is shown in Fig. 6. From this figure it is seen that the spirals of small gyres or of the lower order can maintain their shape without undergoing any considerable change, and this seems to suggest that these spirals have their own matrix (KUWADA and NAKAMURA, 1934 a, b). The fact that the core part of the spiral of the lower order usually takes the stain more deeply than the chromosome core around which the spiral of the higher order winds seems to support this view.

The time at which the sister chromatids separate from each other differs in different diads. Even in one and the same cell some remain unseparated, while others have separated to variable extents (Fig. 5). In the middle of Fig. 5 chromosome is shown at the critical moment of complete separation of the constituent diads, with the four chromatids, each of a spiral, in the process of being forced to assume the configuration ◊. As is clearly seen from this figure the double nature of the spiral in the diads is brought to light in this stage though it is apt to be concealed in earlier stages when the spirals are much closer together.

Anaphase. When the chromosomes are pulled from the poles, they are elongated to a considerable extent, and the spirals contained in them are drawn out correspondingly, the drawing out being usually most manifest in the proximal region. In Fig. 15 the gyres of the spiral are considerably smaller in the part where the chromosomes are extensively elongated than where they are not. When the chromosomes are released from tension and contract again on breaking down of the mutual attachment at the distal ends, the spirals come back to their original form in which the gyres are uniformly large. On the midway from the equatorial plane to the pole, the spirals are drawn out once more. This behaviour is widely accepted as characteristic in this stage (cf. SAX, 1930; TAYLOR, 1931; and others). In this stage the two sister chromatids of a diad are clearly separated except for the point at which the spindle fiber is inserted. Most of the chromosomes take, then, the form of single V's and the remaining few that of double V's, the arms of the V's being drawn out to different extents and in different manners. In the telomitic chromosome shown in Fig. 7 the proximal part of each chromatid is drawn out, and exhibits a coiling of gyres smaller than those in the remaining part. In the latter gyres small coils of the lower order are visible. In the similarly drawn out chromosome *a* in Fig. 11, the coils of small gyres are scarcely perceptible in the drawn out parts, but two rows of granules arranged side by side are visible. In the part intact two rows of chromatic rings (optical cross-section of the turns of the spiral of the lower order) are found, an aspect which reminds us of the structure figured by SANDS (1923) in the metaphasic chromosomes of *Tradescantia virginica* (cf. his Figs. 1 and 5, Pl. XXIX).

The spiral is drawn out not only in the region near the proximal end but also in others, and not infrequently in two regions. In the left-hand side arm of the chromosome *b* in Fig. 11 the spiral is drawn out in the middle region, the proximal region being less affected and the distal region remaining intact. In Fig. 8 the spiral in the left-hand side arm of the chromosome is drawn out in two regions, the proximal and the distal near the end, while in the right-hand side arm it is drawn out only in one region extending from the proximal end to the middle. A similar figure of the spirals drawn out is also shown in Fig. 10; in this case too the spirals of the sister chromosomes are not drawn out in the same region.[1] Though in most cases the chromosomes in the heterotype anaphase show the double-coiled structure (Figs. 7, 9 and 11), not infrequently the single-coiled structure is shown in *L. speciosum* and *L. longiflorum*. These chromosomes are thinner and larger than those showing the double-coiled structure, and, except that the single-coiled spirals run often sinuously (Figs. 12 and 13), no coiling of the higher order is recognizable. They present a close resemblance in shape to the chromosomes in the homotype anaphase. The cells containing the chromosomes of both kinds, single-coiled and double-coiled, are found in the same anther. The chromosomes of single-coiled structure may be regarded as representing the extreme case where the spiral of the higher order is drawn out.

Telophase. When the chromosomes reach the poles they are arranged radially with a clear space at the centre (Fig. 16); and when they are released from the tension that has been exerted at both poles, they become shorter. This shortening of the chromosomes in the telophase has been ascertained by LENOIRE (1932) in the living pollen mother cells in *Lilium candidum* mounted with anther slime. When shortened, the chromosomes regain the normal double-coiled appearance in which the turns of the spiral are such close contact with one another that they appear to be a pile of discontinuous bars. In some cases, the chromosomes remain in the drawn-out state. Meanwhile as the chromosomes come together with one another, the new nuclear membrane surrounding the chromosome group is noticeable. The nucleus is first irregular in outline with protuberances formed of the protruding parts of chromosomes (cf. TELEŻYŃSKI, 1931), but later it becomes smooth in outline assuming a semi-spherical shape. When it increases in volume, the semi-spherical shape is transformed into the ovoidal. Then the chromosomes tend to become loose and the spiral of the higher order becomes less manifest through unravelling. In this stage the spirals of the lower order are not clearly visible, though appearing as solid threads (Fig. 17); but shortly after this stage has passed, when the nucleus has grown in volume and the chromosomes are looser, fine coils which

1) In these figures (Figs. 8 and 10) the spirals of the lower order are concealed, both chromonema and matrix being uniformly stained, and only the spirals of the higher order are manifest, while in Fig. 7 both spirals are visible. It seems likely that in his Fig. 1, BELLING (1928) has drawn only the somewhat elongated spirals of the higher order.

represent the spiral of the lower order become clearly visible. These coils of small gyres are seen in Fig. 18 in places, especially in the lower part of the nucleus. In this figure a trace of the spiral of the higher order is still visible, but this aspect becomes gradually less evident.

Interkinesis. The nucleus in the interkinesis appears to be filled with fine spirals with some traces of winding of the spiral of the higher order. With the advance of stage, the spiral of the higher order loses its appearance, even the traces, and only the slender and long spirals which irregularly meander throughout the nucleus are conspicuous (Figs. 19 and 20). In this stage it is noticeable that the two sister spirals are held together at the point of spindle fiber insertion, some assuming the shape of a single V and others a double V or an X (Figs. 19 and 20). The polar, or radial orientation of the chromosomes mentioned above has not been entirely lost even in the mid-interkinesis. In this stage, when the ground substance is almost wholly lost, the spirals of the lower order are most clearly visible. This loss of the ground substance in the mid-interkinesis has been observed by NEBEL (1932 a) and KATO (1934) in *Tradescantia reflexa*. In *Lilium* the spirals (of the lower order) are not intensely drawn out as in the case of *Tradescantia reflexa* (KATO, 1934).

The first step taken up by the nucleus on entering into the homotype prophase commences with the increase of the chromaticity of the ground substance. The spiral threads then gradually grow thicker.

Homotype prophase. In the early prophase the sister nuclei are situated very near the cell membrane. As the stage advances, each nucleus moves to occupy the central position in the cell to which it belongs. In the early prophase the ground substance is as yet weak in chromaticity but becomes gradually stronger. The chromosomes or spireme threads begin to be dislocated from their polar orientation, and come to run rather intricately through a course of a loose helix, which reminds of the spiral of the higher order. As the threads become gradually thicker, the spiral aspect becomes more conspicuous. This spiral is very similar to the spiral of the lower order mentioned before the prophase (Fig. 21). With the gradual thickening of the chromosomes, the coils into a loose helix or the spirals of the higher order are gradually unravelled, but some traces of the winding may be still visible in later stages too (Fig. 22). The chromosomes in the late prophase thus developed are much thicker and shorter than in the earlier stages and are of the single-coiled structure (Fig. 23).

Homotype metaphase. In the metaphase the chromosomes are directed in different directions, but are so arranged as to have their points of spindle fiber attachment on the equatorial plane (Figs. 24 and 25). In *L. longiflorum* and *L. speciosum* the spirals in these chromosomes are considerably slender compared with those in the heterotype metaphase and have a greater number of turns than the latter. But in *L. tigrinum* they are usually almost as thick as the spirals in the heterotype metaphase and show a double-coiled structure (Fig. 24).

In 1932 a case was met in *L. speciosum* in which the chromosomes were much thicker than those in the other cases, a condition that suggests their double-coiled structure. In 1933 this case was met oftener and in 1934 most chromosomes were found to be of this shape. In 1933 and 1934 chromosomes of this type were also observed in *L. longiflorum*, though less often than in *L. speciosum*. In these chromosomes the spirals are thick and short, though slightly thinner than those of chromosomes in the heterotype division, and are coiled regularly. In the chromosome found in the middle of Fig. 26 the spiral is loosened and the spiral of the lower order is clearly visible. In Fig. 27 the spirals are unravelled to a greater extent and show the double-coiled nature as the middle chromosome in Fig. 26. The manner of unravelling is very similar to that observed in the heterotype anaphase in *L. longiflorum* and *L. speciosum* (see, Figs. 8 and 10).

Homotype anaphase. In the anaphase the chromosomes are slender in form. No trace of the spiral of the higher order is visible except for the case in which the chromosomes present more or less sinuous or twisted aspects, but only the spiral of the lower order is conspicuous (Figs. 28 and 29). In the homotype anaphase where the chromosomes are of single-coiled structure no spiral is observed which is drawn out as in the case of the heterotype anaphase. As the division proceeds further, the sinuous chromosomes tend to be straightened, in most cases it being conspicuous near the proximal region of the chromosomes (Fig. 30).

Conclusion

Since the double-coiled structure of chromosomes in the heterotype metaphase was first announced by FUJII (1926) in the pollen mother cells of *Tradescantia reflexa*, this structure has been studied and confirmed by various authors in this and some other plants, namely :—

Tradescantia reflexa	FUJII (1926), ISHII (1931), KUWADA (1932), KUWADA and NAKAMURA (1933, 1934 a), KATO (1934)
Hosta japonica	ISHII (1931)
Sagittaria Aginashi	SHINKE (1934)
Lilium longiflorum	SHINKE (1934)
Lilium tigrinum	SHINKE (1934)
Trillium kamtschaticum	MATSUURA (1934)

In the present investigation *Lilium speciosum* was added to this list.

The peculiar shape of chromosomes in the heterotype metaphase which in *Tradescantia reflexa* distinguishes itself from that of the chromosomes in the somatic division as well as in the homotype division by thickness and shortness was attributed by FUJII (1926) to the double-coiled structure of the chromosomes. While in the homotype division no generalization is possible as to the form which the chromosomes assume (cf. TISCHLER, 1921–22, p. 423), it is widely known that in the heterotype division they are generally of massive forms, markedly thicker and shorter than in the somatic division (cf. SHARP, 1926, p. 263). This constancy in the form of the heterotype chromosomes may perhaps be taken as showing that in the heterotype division the chromosomes are generally of double-coiled structure. In the homotype division, on the other hand, the structure should be indefinite as indicated by the variability of chromosome form, and this has been already demonstrated elsewhere in other plants and here in *Lilium speciosum* as follows :—

	Heterotype metaphase	Homotype metaphase
Tradescantia reflexa	double coiled	single coiled
Sagittaria Aginashi	double "	double "
Hosta japonica	double "	——
Trillium kamtschaticum	double "	——
Lilium longiflorum	double "	some single coiled and others double coiled

Lilium speciosum	double	"	"	"	"
Lilium tigrinum	double	"	"	"	"

The variability of the form of chromosomes in this division is, therefore, regarded largely as due to the condition of coiling whether it is double or single. As shown in the *Lilium* species mentioned above, the structure or the mode of coiling, double or single, can differ even within the same species, and it is also different in different years which mode dominates the other. We may, then, perhaps conclude that whereas in the heterotype division the reason for the constantly occurring double-coiled structure is probably fundamental, it seems to be superficial in the homotype division in which this structure is not of a constant occurrence. In the heterotype anaphase too the instability of the double-coiled state mentioned above is due to the elongation of chromosomes. This modified structure is generally brought back to the original double-coiled structure in the telophase, probably by the action of the matrix substance, and in this no fundamental cause seems to be involved. In a certain sense the occurrence of the double-coiled structure in the homotype division may be compared with the restoration of the drawn-out spirals in the heterotype telophase, though the mechanisms by which the spiral is drawn out and by which it is restored may differ in these two cases.

Summary

1) In *Lilium longiflorum*, *L. speciosum* and *L. tigrinum* the double-coiled (spiral-within-spiral) structure of the heterotype chromosomes was ascertained in the metaphase.

2) In the heterotype anaphase the spirals of the higher order are drawn out. The phenomenon is most manifest in the proximal part of the chromosomes. Although they are drawn out in various parts in various degrees in this stage, this is only transitory and the double-coiled structure is essentially maintained up to the end of the telophase. In some particular cases, however, the spirals are drawn out to so a considerable extent that the chromosomes are of single-coiled structure.

3) The interkinetic nucleus is filled with the spirals of the lower order (chromonemata) which run more or less sinuously with visible traces of the coils of the higher order.

4) In the homotype metaphase two forms of chromosomes are

found in *Lilium longiflorum* and *L. speciosum*. In one form the chromosomes are slender being of single-coiled structure, and in the other they are massive, as in the heterotype metaphase, and of double-coiled structure. In *L. tigrinum* the chromosomes are also found to be of double-coiled structure but no single-coiled chromosome is observed.

In conclusion the writers wish to express their cordial thanks to Prof. Y. KUWADA for his suggestions and criticisms throughout the course of the investigation.

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Explanation of Plates

All the figures are photomicrographs taken from acetocarmine preparations using ZEISS's apochr. imm. 2 mm. and comp. oc. 12.

Fig. 1. *L. longiflorum*. Heterotype metaphase. Doubleness of the spiral of the higher order is seen in the upper diad, the component spirals being almost separated into two single spirals in contact laterally.

Fig. 2. *L. speciosum*. Chromosomes in metaphase showing double-coiled structure.

Fig. 3. *L. speciosum*. The same.

Fig. 4. *L. tigrinum*. A trivalent chromosome in metaphase showing double-coiled structure.

Fig. 5. *L. longiflorum*. Metaphase chromosomes showing spirals of the constituent chromatids.

Fig. 6. *L. speciosum*. Chromosomes in the same stage the spirals of which are loosened out by the press given on the cover-glass. The spirals of the lower order are manifest in most of these chromosomes.

Fig. 7. *L. speciosum*. A telomitic chromosome in anaphase. The spirals of the higher order are drawn out in the proximal region.

Fig. 8. *L. longiflorum*. The same in late anaphase, in which both arms are drawn out in different regions.

Fig. 9. *L. speciosum*. The same showing double-coiled structure.

Fig. 10. *L. longiflorum*. The same as in Fig. 8 showing different aspects of coiling in both arms.

Fig. 11. *L. longiflorum*. The same. In the chromosome marked *a*, the spiral is drawn out in the proximal region in both arms and in the left-hand side arm of the chromosome *b* it is drawn out in the middle region and less markedly in the proximal region.

Fig. 12. *L. speciosum*. The same with the unusual single-coiled structure.

Fig. 13. *L. longiflorum*. The same in which the chromosomes are extremely drawn out into the single-coiled structure.

Fig. 14. *L. longiflorum*. Telophase. Chromosomes are short and the spirals are regular in coil.

Fig. 15. *L. longiflorum*. The same with two homologous atelomitic chromosomes lagging in the equator. The two arms which are in contact with ends are drawn out.

Fig. 16. *L. speciosum*. The same with the chromosomes radially arranged about a clear space.

Fig. 17. *L. speciosum*. Late telophase showing unravelling of the spiral of the higher order.

Fig. 18. *L. speciosum*. The same where small coils (of the lower order) are manifest. Traces of spirals of the higher order are still visibie in places.

Fig. 19. *L. longiflorum*. Interkinesis. Spirals of the lower order are most manifest, but traces of the spirals of the higher order are also visible.

Fig. 20. *L. speciosum*. The same.

Fig. 21. *L. longiflorum*. Early homotype prophase showing the spirals of small gyres running through a large helix course which resembles that of the spiral of the higher order in the heterotype division.

Fig. 22. *L. longiflorum*. Middle prophase with the spirals of large gyres loosened.

Fig. 23. *L. longiflorum*. Late prophase. Large gyres of the spirals are almost completely unravelled.

Fig. 24. *L. tigrinum*. Homotype metaphase showing chromosomes of the double-coiled structure.

Fig. 25. *L. longiflorum*. Metaphase in side view showing slender chromosomes of single-coiled structure.

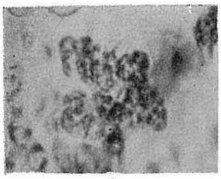
Fig. 26. *L. speciosum*. Metaphase showing chromosomes of double-coiled structure.

Fig. 27. *L. speciosum*. The same in which the spirals of the lower order are loosely coiled into the spirals of the higher order. Each pair of these spirals of the longitudinal halves of chromosomes are widely extended and attached only at the insertion point of spindle fiber.

Fig. 28. *L. speciosum*. Anaphase showing unravelling of the spirals of the higher order in various degrees.

Fig. 29. *L. tigrinum*. An anaphase chromosome or the spiral of the lower order showing no coiling of the higher order.

Fig. 30. *L. speciosum*. Anaphase showing sinuous aspect of chromosomes. Drawing-out of spirals of the higher order is manifest in the proximal region.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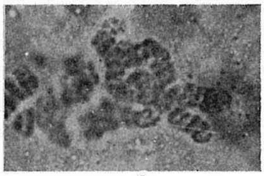
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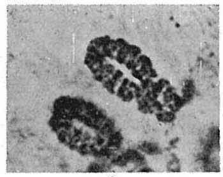
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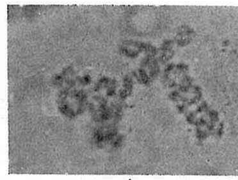
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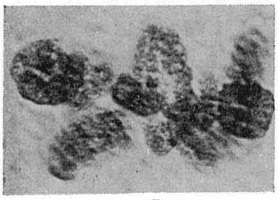
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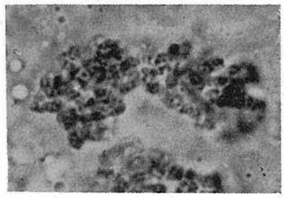
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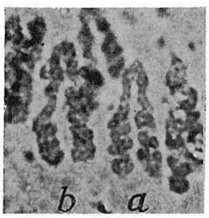

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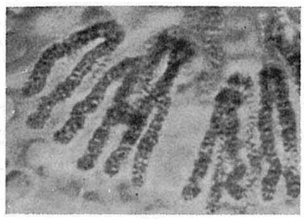
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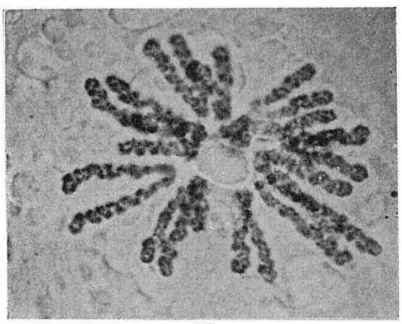
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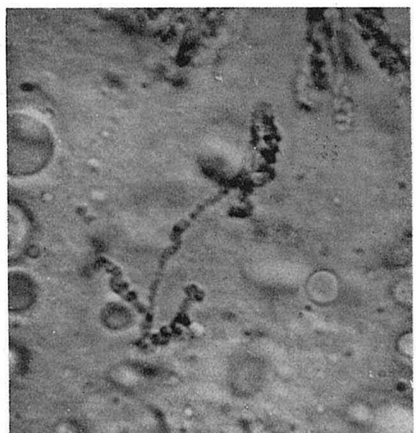
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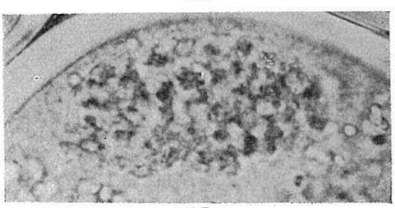
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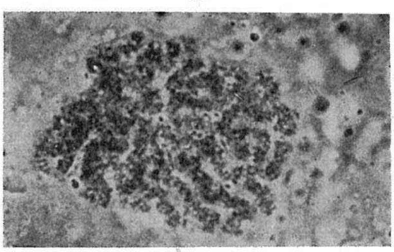
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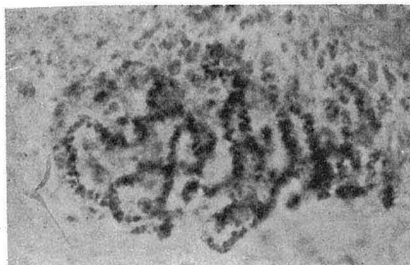
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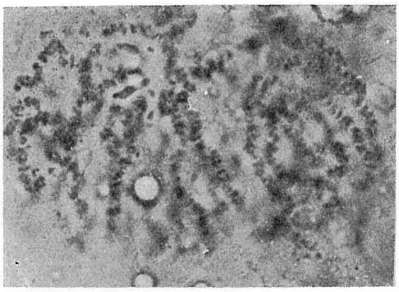
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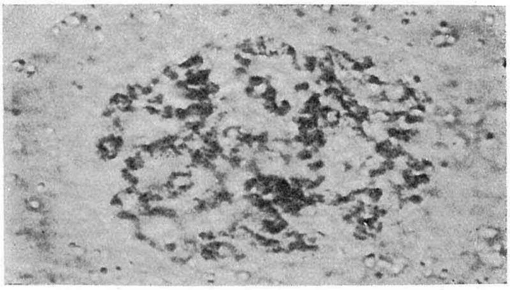
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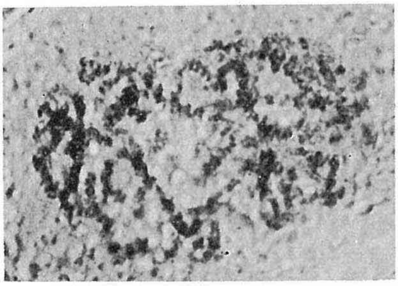
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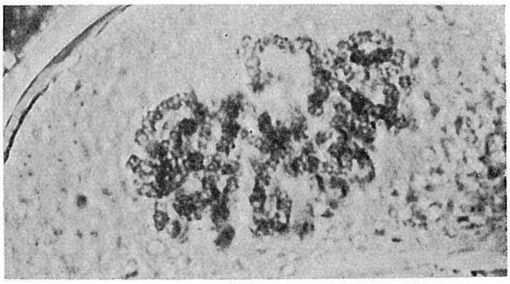
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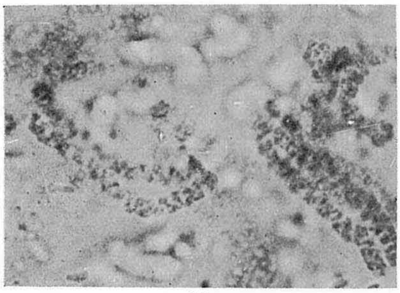
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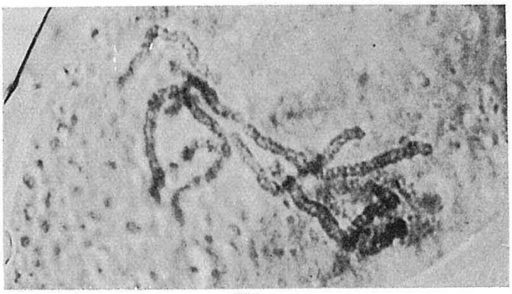
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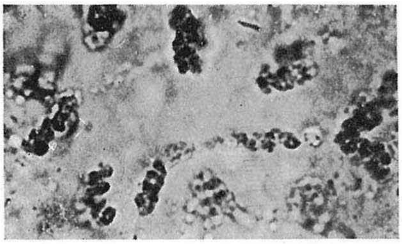
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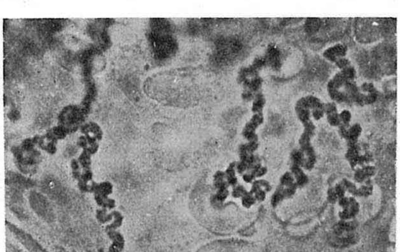
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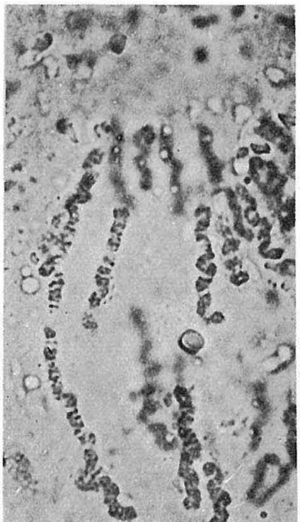
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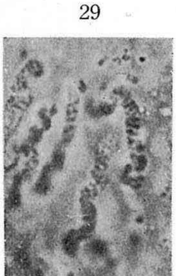
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