A PRELIMINARY STUDY OF MICROSPOROGENESIS OF COTTON (GOSSYPIUM HIRSUTUM L.) CULTIVAR ARKUGO 4.

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RESUMO

Uma técnica de esmagamento para estudos citogenéticos em algodão (*Gossypium hirsutum* L.) cv. Arkugo 4 e de tornar as lâminas permanentes foi descrita em detalhe.

A confirmação do número haploide 26 e a uniformidade de morfologia dos cromossomos dos algodões foi obtida.

Todas as fases e subfases do processo meiótico foram observadas, excetuando a telófase e a citocinese do processo reducional, e a prófase do processo equacional.

SUMMARY

Using tetraploid cotton a squash technique for chromosome cytogenetical studies and a method for making acetocarmine smears permanent has been described in detail.

Meiosis has been described from Arkugo 4 cotton. The haploid number of 26 was confirmed, which is reported to be the number of all cultivated American cottons. Also a considerable uniformity in the morphology of the chromosomes was observed.

A clearly defined first metaphase configuration was rarely observed. The tendency for one of the bivalents to undergo early disjunction was apparent.

Although a few bivalents in cultivated cottons are joined by interstitial chiasmata, the majority were joined by terminal chiasmata.

At the first anaphase, the majority of bivalent partners disjoin normally and move toward opposite poles as single units. Some lagging chromosomes were observed.

The meiotic process in Arkugo 4 like all cultivated American cottons did not present telophase I, cytokinesis I and prophase II.

KEY-WORDS: Cotton, Cytogenetics, Microsporogenesis.

INTRODUCTION

The microspores are produced by meiotic process called microsporogenesis. This meiotic process is of particular importance because it provides an orderly procedure for the reduction of the chromosome number from the

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paired (diploid) number to the single (haploid) condition. Another significant feature of meiosis is that it provides variability, which is accomplished by reduction division, independent segregation of each pair, and crossovers in packytene. Only cells of sexually reproducing species have the capacity to undergo meiosis, and only special cells in the multicellular individual switch from mitosis to meiosis at specified times in a life cycle.

The chromosomes of cotton (*Gossypium hirsutum* L.) have been described and figured by several investigators (4, 6, 7, 8, 13, 14, 15, and 17). DENHAN (7) reported that the cotton plant with its large number of minute chromosomes and its complex cytoplasmic organization makes the cytogenetical analysis very complex. The structures in question are barely within the limits of effective visibility and the possibility of subjective error is large.

The chromosome numbers have been counted in some cotton species and it has been shown that cultivated cottons fall cytologically into two groups, (a) New World cottons with n = 26, and (b) Asiatic cottons with n = 13 (2, 7). The fact that Asiatic cottons have only 13 chromosomes indicates that 13 was the haploid and 26 the diploid chromosome number of the ancestors of our present day cottons (4, 11). The double chromosome number found in the American cottons suggests a duplication of the chromosomes of an ancestral type (11). BEASLEY (3) provided confirming evidence on the origin of the New World type by synthesizing amphidiploids from A x D hybrids* that were similar in morphology and cytogenetically compatible with the natural amphidiploids.

GALIGNER (9) reported that acetocarmine is extremely valuable as a combined fixative and stain for small organisms, smears, or crushed preparations of fresh tissues. It acts very quickly and gives a sharp chromatin stain. He concluded that preparations stained with acetocarmine can be examined to best advantage by the use of artificial light passed through a green or blue filter.

A method for making acetocarmine smears permanent was described by HARVEY (10) which is simply placing the slide in a petri dish filled with 45% acetic acid. When the cover glass has fallen away from the slide, put it in absolute alcohol per 5 minutes and mount in Euparal.

In this karyotypic study of cotton a number of different techniques were tried in order to obtain satisfactory results. Thus this paper is concerned with methods of studying the chromosomes, their number, behavior during the microspore formation and with the technical aspects of producing the squash preparation, which were developed as basic knowledge of cytogenetic studies in cotton.

MATERIALS AND METHODS

Cotton plants (Cultivar Arkugo 4) were grown in greenhouse condition (temperature 30° C and 60% relative humidity) at the University of Arkansas farm to form the material for this study.

Flower buds at various stages of development (Fig. 1) were collected at different times and fixed in carnoy's solution (Ethanol: Chloroform: Acetic acid – 3: 1.5:1.5) for 48h (Fig. 2).

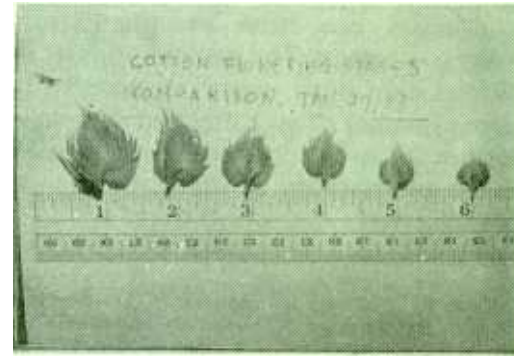

Figura 1 – Comparison of flowering stages in cotton.

*A O a genome form a taxon of the Asiatic diploid group; D = a genome from a taxon of the American diploid group.

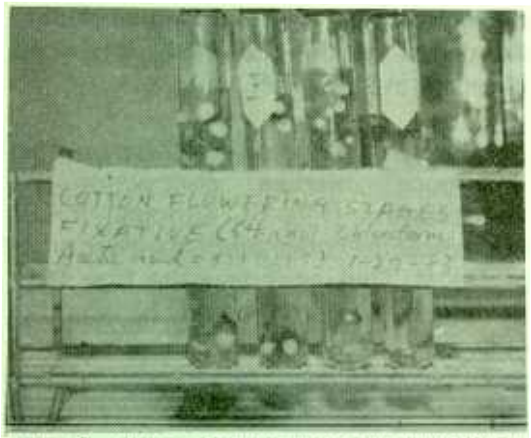

Figura 2 — Cotton flowering stages fixative (Ethanol: Chloroform: Acetic acid = (6:3:3).

SKOVSTED (15) suggests that cotton material is best collected between 8-11 A. M. in full sunlight. In collections made on cloudy days the fixation is inferior, and there seems to be a tendency for the chromosomes to clamp and the cytoplasm to shrink.

The writer agrees with DENHAM (7) that meiosis in cotton is not an especially suitable subject for cytological study, but considers that he has somewhat over-stated the difficulties. These difficulties have been in large part overcome by certain modifications in technique and material. Thus, it was necessary to employ a better fixative, instead just Ethanol: Acetic acid (3:1). The calyx and the top of the corolla were removed (Fig. 3) in order to improve the fixation.

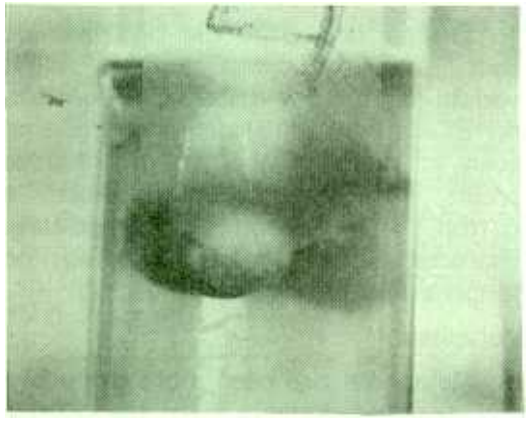

Figura 3 — Flower buds after removal of calyx and top of the corolla.

After fixation the buds were transferred to 70% ethanol and stored at 5 C until examination.

All the stages figured were stained by the acetocarmine solution (9) which was composed of:

Acetic acid (glacial)	45 ml
Distilled water	55 ml
carmine	1 g

These ingredients were boiled gently in a reflux condenser for 5 min. It was then shook well and filtered when cool. The acetorcein was also used as a stain but the results were not satisfactory. After obtaining anthers from the fixed flower buds, the meiotic cells were excised and placed into a droplet of the acetocarmine. All visible debris, anther walls, etc., were removed from the slide before placing the cover slip. Care was taken not to squash the microsporocytes. After cover slip was placed, the slide was warmed on a hot plate (60 C) for about 5 minutes to improve the stainability.

To make slides permanent two techniques were tried: (a) removal of the cover slip by razor blade after the material was frozen over dry ice, then dried over a hot plate (60 C) and mounted in Euparal, and, (b) removal of the cover slip by floating the slide in 45% acetic acid, dehydrated in absolute alcohol, then dried over a hot plate (60 C) and mounted in Euparal. Meiotic stages were studied under a phase contrast microscope (BH-Olympus) in which a camera was set up to photogragh them.

RESULTS AND DISCUSSION

Synchrony of Meiotic Division

Fig. 1 presents the various flower bud stages for this meiotic study. In number 6 flower buds the early stages of meiosis were frequently observed. The late stages were observed in number

5 flower buds. Numbers 1, 2, 3, and 4 flowers had already formed pollen (Fig. 1).

The released PMC (pollen mother cells) remained in the immediate vicinity of the anther. Therefore meiotic synchrony could be estimated. In contrast to the tomato (12), a very high degree of sychrony was present within an anther, all PMC being at a similar stage of development (Fig. 4 and 5).

Figura 4 — Cells from an anther at early prophase, which is characterized by sharply stained nucleolus. The synchrony is evident by six cells in the same stage of prophase I.

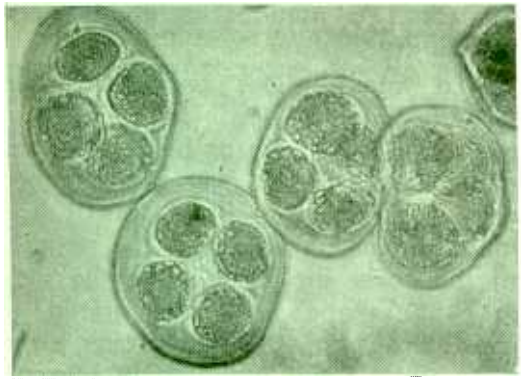

Figura 5 — Tetrad stage in cotton in synchronized condition.

Asynchrony became increasingly evident in comparisons among anthers within a flower bud. Reports on meiotic studies rarely comment on division synchrony. SPARROW et. al. (16) point out that such information may be of interest when studying irradiation effects.

First Meiotic Division

Prophase

Early prophase stages were characterized by the presence of a prominent nucleolus (Fig. 4). A second nucleolus, associated with the first one, was observed in a few PMC.

By late diplonema, the bivalents had undergone marked contraction and condensation (Fig. 6), although it was impossible to count all of the twenty-six bivalents. Diakinesis was the first stage in which bivalents could be counted (Fig. 7).

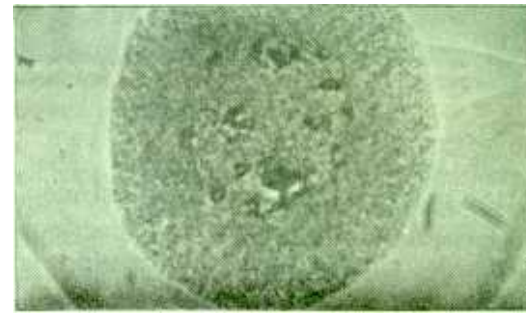

Figura 6 — Diplotene stages from cotton showing the shortening and condensing of the chromosomes.

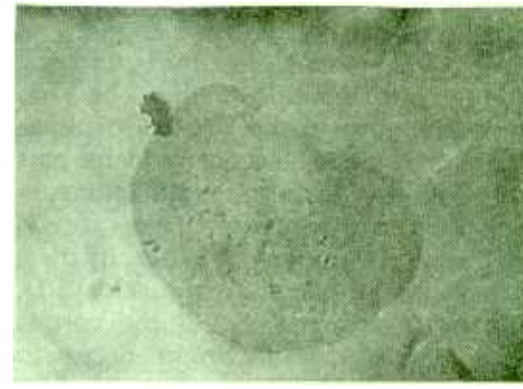

Figura 7 — Diakinesis stage showing the twenty-six bivalents.

During this stage the bivalents are well separated and exhibit slight variations in size and shape. In contrast, WHELAN et. al. (18) demonstrated that even in diplonema it was possible to count the chromosome number in cotton. The prominent nucleolus characteristic of earlier stages was no longer evident in diakinesis (Fig. 7).

Metaphase I

Metaphase has been more throughly and widely studied than the earlier stages, because the contraction of the chromosomes is then completed.

Accumulated data from cytological studies (3, 8, 14) on *Gossypium* species have demonstrated that the tetraploid cotton species have one set of chromosomes similar to the set in Asiatic 13-chromosome species and the other set similar to the set in American 13-chromosome species. Thus, cotton is an allotetraploid and the formation of twenty-six bivalents is evident at the metaphase plate (Fig. 8).

If cotton was an autotetraploid, quadrivalents should be formed instead of bivalents.

A clearly defined first metaphase configuration was rarely observed because there was sequential disjunction on separation of the bivalents. The nearest approach to this stage is illustrated in Fig. 8, but the tendency for one of the bivalents to undergo early disjunction was apparent. The smallest bivalents usually are the first to undergo disjunction (18), which was also observed in this study (a, b, Fig. 8). Although no attempt has been made to determine the number of chiasmata in this cultivar, WEBBER (17) indicated that in cotton the number apparently ranges from 1 to 3 per chromosome, with 2 preponderating.

With the cotton plant with its large numbers of minute chromoscomes, the division into chromatids is not always clear.

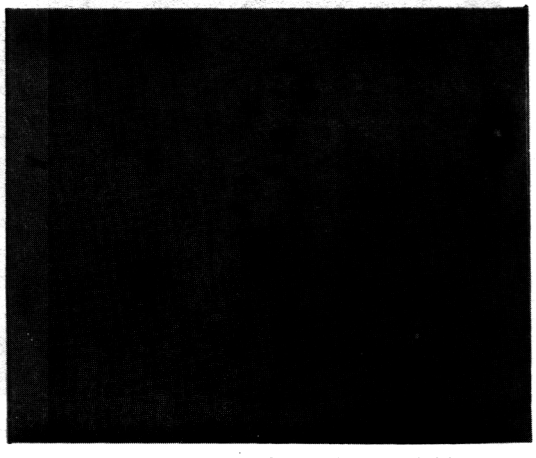

Figura 8 – Metaphase I from allotetraploid cotton with 52 chromosomes.

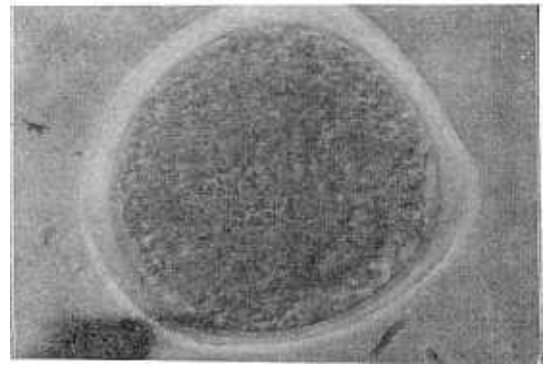

Figura 9 – **Anaphase of the first division with its lagging chromosomes.**

Anaphase-I

During the first anaphase the majority of bivalent partners disjoin normally and move toward opposit poles as single units (Fig. 9 and 10). In Fig. 9 some lagging chromosomes are observed. Such lagging chromosomes exhibit terminal chiasmata. WEBBER (17) has observed as many as 5 lagging bivalents in the Asiatic forms and 8 in the cultivated American forms. Approximately 13 percent of the PMC of the cultivated American forms and 11 percent of the Asiatic forms exhibit lagging bivalents during the first anaphase. At the end of anaphase I, half the number of chromosomes are present at each pole. Thus the reduction process of meiosis is completed. (Fig. 10).

Telophase I

Telophase I is abbreviated in many species and nuclei may go directly to prophase or even metaphase of the second meiotic division. In other cases there may be an extended interphase before the reorganized nuclei divide in meiosis II. An accompanying cytokinesis may or may not occur at the end of meiosis I (1).

In all material studied telophase I and prophase II were not observed. The second division follows immediately without resting stage (interphase). The chromosomes retain their extreme first division contraction (Fig. 11).

In cotton cytokinesis is delayed until both divisions are finished, at which time 4 separate cells are formed to enclose the 4 nuclear products of meiosis. The steps that lead to cytokinesis are shown in Figs. 12, 13, 14, and 15 which demonstrate that the four homologous sets of chromosomes are dived in a common cytoplasm.

Second Meiotic Division

There was nothing particularly unusual in meiosis II with Arkugo 4 cotton. The second meiotic division appeared relatively normal and showed the general character of a somatic mitosis. The nuclei underwent conventional prometaphase, instead of prophase (Fig. 11). The second metaphase plates are well separated and contain sharply distinct chromosomes (Fig. 16).

The main result of meiosis II is separation of the dyads (anaphase II) into individual chromatids, at which time these become fullfledged chromosomes in their own nuclei (Fig. 12). Once telophase II (Fig. 13) is completed, the cell walls arise simultaneously between four nuclei and the tetrads are produced (Fig. 17).

Then the primary common membrane dissolves and the microspores separare (Fig. 18).

The nuclei of microspores divide and form the generative and vegetative nuclei (Fig. 19).

In some species (soybean, alfalfa, tobacco, tomato etc.), the pollen grain are considered to be mature and ripe for pollination when they reach the binucleate state (13). In some others (cotton, wheat, corn, rice, sorghum etc.) only after the generative nucleus underwent a second mitotic division to produce the two nuclei were the pollen grains able to fertilize the female gamete (5)

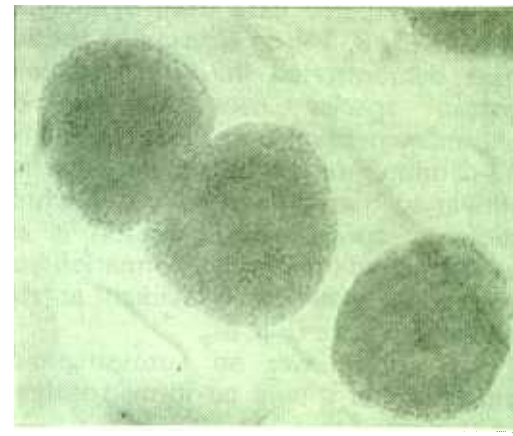

Figura 10 — Anaphase I completed and showing two haploid sets of chromosomes.

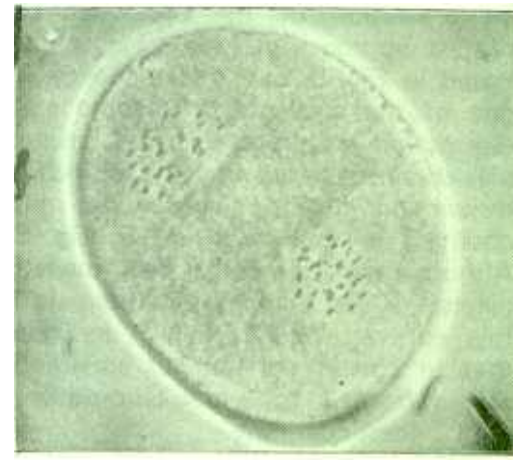

Fig. 11 — Prometaphase II showing the retention of the first division contraction.

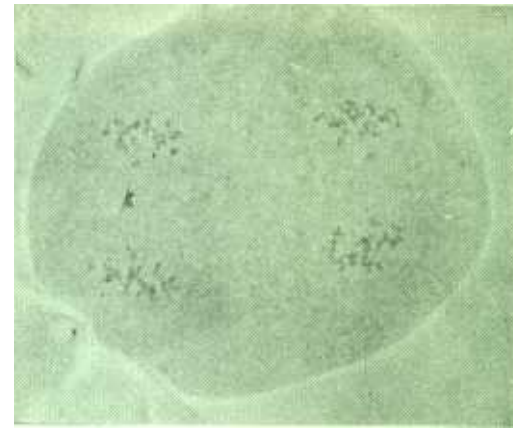

Figura 12 — Anaphase II. The four sets of chromosomes in a common cytoplasm showing that cytokinesis does not occur after the first nuclear division.

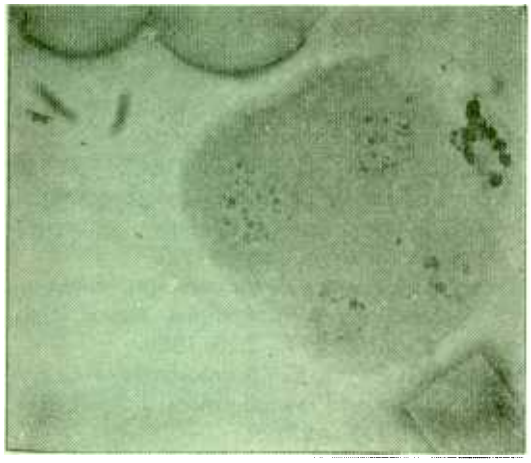

Figura 13 — Telophase II. The discondensation of the chromosomes is apparent before cytokinesis.

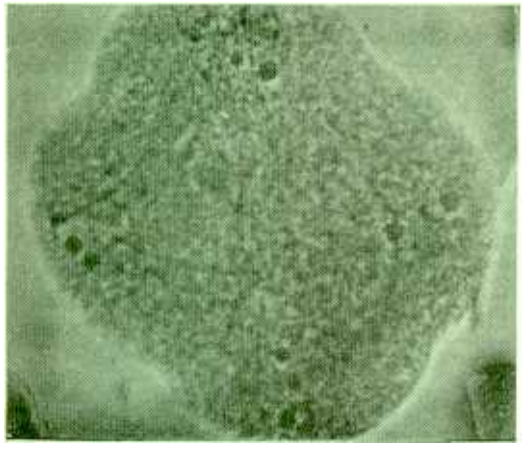

Figura 14 — The beginning of cytokinesis is evident in the bulging of the cell wall.

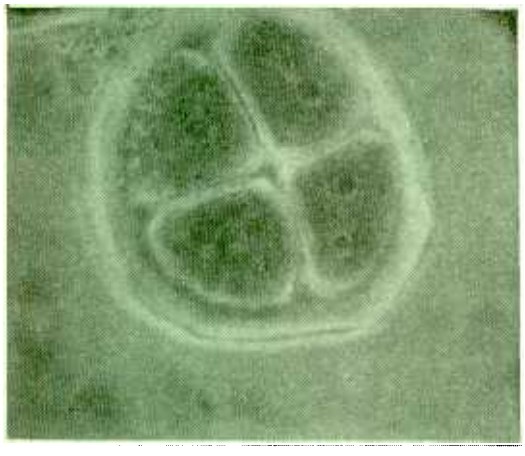

Fig. 15 — Citokinesis. The four meiotic cells after cytoplasm division.

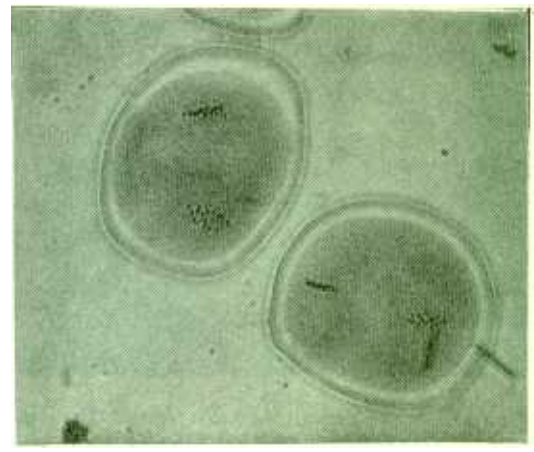

Figura 16 — Metaphase from the second division with the chromosomes in each plate.

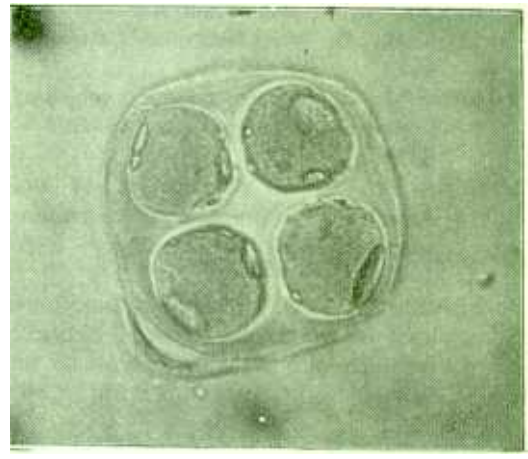

Figura 17 — The four microspores showing that the tetrad stage is completed.

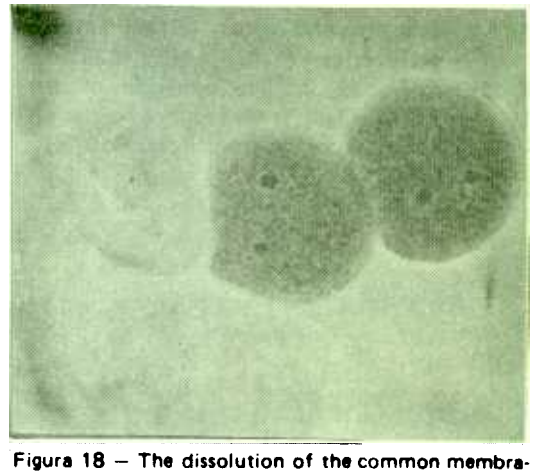

Figura 18 — The dissolution of the common membrane is apparent.

Figura 19 — The binucleate stage is reached. The vegetative and generative nuclei are visible.

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