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THE CHROMOSOMAL PELLICLE

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INTRODUCTION

THE problem whether the chromosomes have a distinct limiting membrane has elicited differing opinions.[1,2] Micrurgical studies on the salivary chromosomes of *Chironomous*[3] indicated the presence of an elastic membrane which when ruptured made the chromosomes sticky. In some flagellates of termites the new nuclear membrane was found to originate by the fusion of the membranes of the chromatids.[4]

Evidence from electron microscopy regarding the structure of the chromosomes is rather confusing.[2,5,6] The disagreements range from an inability to confirm "the presence of any highly organized structures"[5] (p. 143) in the chromosome to its resolution into a large number of paired fibrils suggestive of a polynemic structure.[2] Electron micrographs showing a wealth of detail do not seem to have rendered possible a distinction of the sheath, the matrix and the chromonemata as separable entities, though such a distinction was said to be possible in favourable living material[4] (p. 12). In sea urchin eggs, the limiting membranes of anaphase chromosomes have been considered to be an assemblage of material for the future nuclear membrane, since they resemble the nuclear membrane in their structure[6] (pp. 309–310, Fig. 75).

Our knowledge of the patterns of association of the nucleic acids and proteins in chromosomes has been collated mostly from staining acetic-alcohol fixed onion roots treated with enzymes and acids[2] (pp. 110-111). The structural details of the chromosomes could be revealed by very simple procedures.[7,8] A distinct membrane delimiting the metaphase chromosomes was observed during an evaluation of the suitability of acetic-alcohol for a study of chromosome structure. Perhaps, the discovery of the chromosomal membrane itself was due to the superiority of the hæmatoxylin squash technique[7,9–11] in its crisp delineation of the chromosomal details.

OBSERVATIONS

Root-tips from bulbs of *Allium cepa* germinated in an incubator at 30° C. were fixed in acetic-alcohol (1 : 3) for 24 and 96 hr. at a room temperature of 26–30° C. Interspaced with washes in repeated changes of distilled water of 10–15 min. duration, the fixed material was hydrolysed in N HCl at 60° C. for 10 min., mordanted in 4% ferric ammonium sulphate for 10 min., stained in a 0·5% solution of hæmatoxylin (B.D.H.) for 5 min., teased into small bits and squashed in a drop of 45% acetic acid. The edges of the coverslip were sealed with paraffin wax.

In material fixed for 24 hr. the structure of the meta- (Photos 1 and 1 A) and anaphase chromosomes were exactly similar to those illustrated earlier from other techniques.[7,8] Attention is invited to the diverging SAT-threads and the vesiculate SAT-grain in Photo 1 A. Three types of chromosome configurations were observed in roots exposed to the fixative for 96 hr. Very few metaphases showed the quadri-partite structure illustrated in Photos 1 and 1 A. There is a gradual digestion and consequent disorganization of the chromonemata on long storage of the roots in the fixative resulting in the chromosomes appearing either as structureless ribbons, or as closed hollow tubes composed of pellicles, with stainable granular matter disposed along their inner borders.

This is illustrated in Photos 2, 2 A, 3 and 3 A of the same cell taken under ordinary illumination. Only one chromosome is in focus in Photo 2 and an enlarged picture of it is presented as Photo 2 A. The chromosomal membrane is clear and distinct in the region indicated by an arrow and the granular remnants of the chromonemata are disposed along the inner border of this membrane. To emphasize the distinctness of this sheath and for comparison its contour is stippled with Indian ink in Photo 2 B.

The ends of the chromosomes directed towards the bottom in Photo 2 are in focus in Micrographs 3 and 3 A. The various stages of disappearance of the stainable matter within the pellicle are seen in the enlargement (Photo 3 A) of the chromosomal arms. Further confirmation of the presence of a chromosomal membrane is offered by Phase Micrograph 4 of the same cell, where the majority of the chromosomal arms are in focus. The pellicle is clear in the chromosome indicated by an arrow

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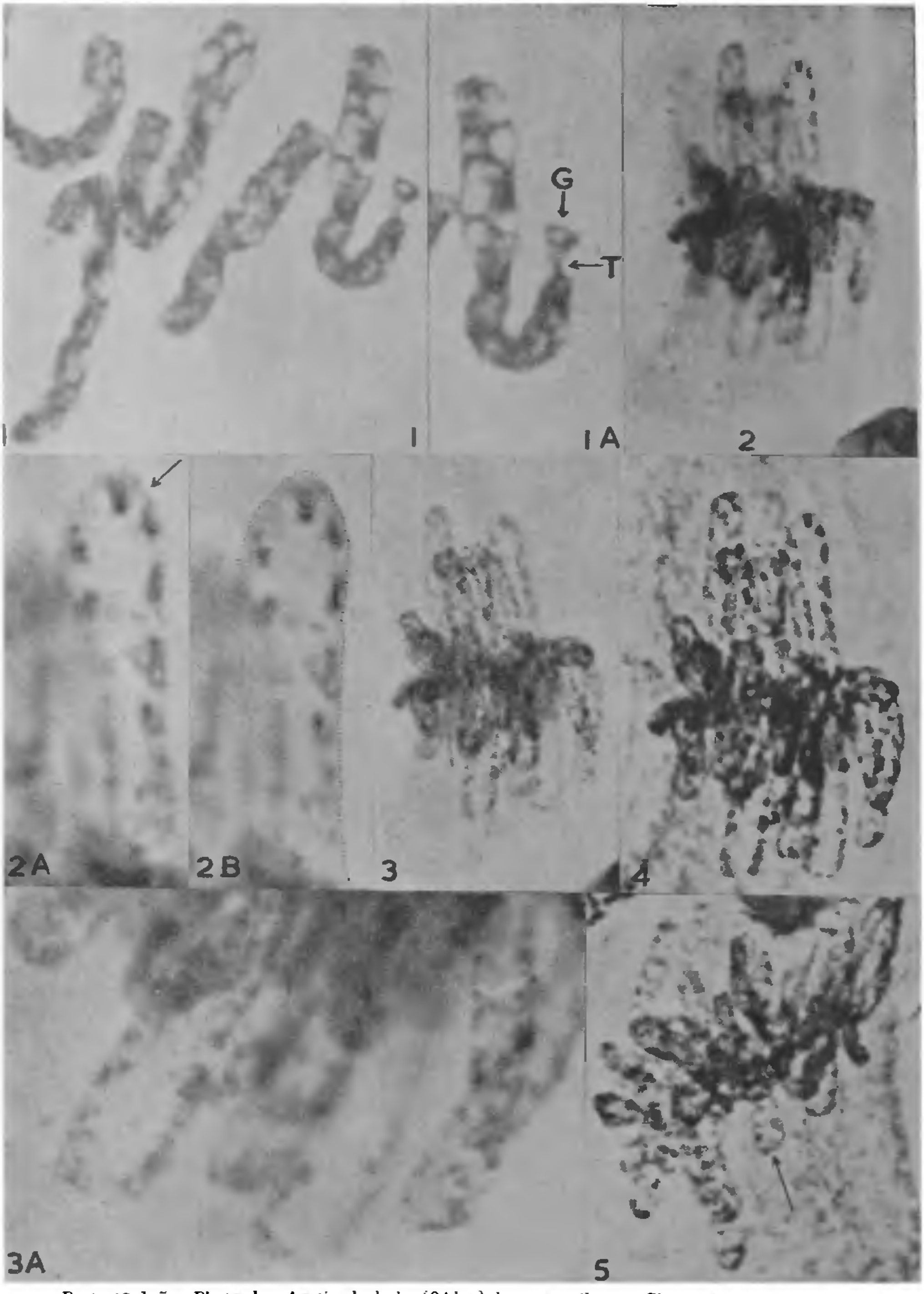
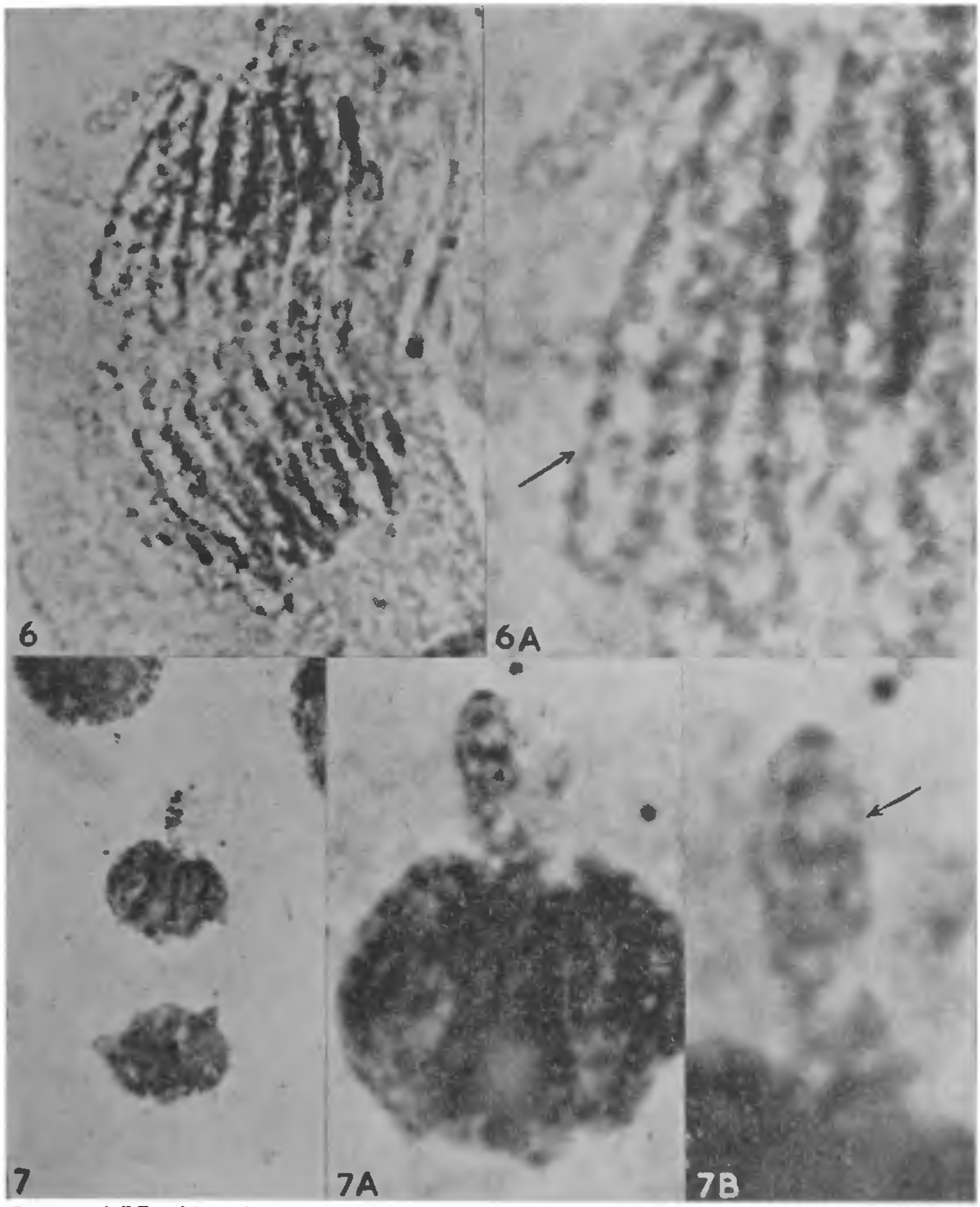


PHOTOS 1–5. Photo 1. Acetic alcohol (24 hr.)–hæmatoxylin. Chromosomes quadripartite at metaphase. Ordinary illumination, × *ca.* 3,350. Photo 1 A. SAT–chromosome with diverging SAT–threads (T) and SAT–grain (G), × *ca.* 3,950. Photos 2–4. Acetic-alcohol (96 hr.)–hæmatoxylin A cell in metaphase under ordinary (Photos 1-3 A) and phase contrast (Photo 4), types of illumination showing the chromosomal pellicles. Photos 2 and 3, × *ca.* 1 200. Photos 2A, 2 B and 3 A, × *ca.* 3,500. Photo 4, × *ca.* 1,360. Photo 2 B. The chromosomal pellicle is stippled with Indian ink. Photo 5. Metaphase. The arrow indicates the pellicle of a chromosome, × *ca.* 1,360.

in another metaphase illustrated in Phase Micrograph 5. At anaphase the outlines of the chromosomes alone are visible under phase contrast (Photo 6). The arrow indicates the pellicle of a chromosome (Photo 6 A) lying at the extreme left in the top group.

The presence of a sheath for meta- and anaphase chromosomes suggested that these membranes may be transformed into the nuclear membrane.[4] In squashes where the caduceus coilings of the chromonemata are seen (Photos 1 & 1 A) a loosening of these coils was observed in one or two of the chromosomes which projected out of the packed telophase mass. These were bounded by a limiting membrane. The staining of this membrane could be accentuated by the



PHOTOS 6–7 B. Photo 6. Anaphase. The chromosomes appear as hollow tubes, × *ca.* 1,360. Photo 6 A. Part of Photo 6 enlarged. Arrow indicates the pellicle, × *ca.* 9,350. Photos 7, 7 A and 7 B. Acetic-alcohol-formaldehyde (3 hr.)–hæmatoxylin. The nuclear membrane could be seen bounding the protruding chromosome arm. Photo 7. × *ca.* 1,100. Photo 7 A, × *ca.* 3,500. Photo 7 B, × *ca.* 7,100.

addition of 1 ml. of 40% formaldehyde to every 4 ml. of acetic-alcohol (1 : 3. *cf.*[11]). Therefore. roots fixed in the above mixture for 3 hr. were stained with hæmatoxylin, squashed and made permanent. Photos 7, 7 A and 7 B are of the same cell at different magnifications. The finger-like projecting arm of the chromosome is bounded by a membrane resembling that of the metaphase chromosomes.

Discussion

The free ends of the chromonemata were seen but rarely[7,8] in preparations giving a crisp delineation of the structural details (Photos 1 & 1 A). In an end view, the paired chromonemal threads had the appearance of rings (*cf.*[2], p. 85). Our curiosity was roused by the possibility that such a configuration may be due to the alignment of the free ends of the chromonemata along the contour of a chromosomal pellicle. Contrary to the general belief,[2,6] *the chromosomes of* Allium cepa *do have a distinct limiting membrane* as illustrated in the micrographs.

The structural integrity of the chromosomes has been assigned variously to (*a*) DNA,[12] (b) a combination of DNA with residual protein[13] and (c) to a complex of RNA, DNA, histone and non-histone proteins.[2] It has been stated: "No one component can be singled out as the essential structural material. As the metabolic properties change from phase to phase and from cell type to cell type, structural patterns also change. In this flux and flow, first one chromosomal material and then another may be displaced or accumulated"[2] (p. 116).

The photographs presented would show that chromosomes not enclosed within a nuclear membrane retain their configuration even when their contents are digested and disorganized. It would appear that it is the chromosomal membrane which gives the mitotic chromosomes their morphologial integrity.

Summary

Hæmatoxylin squashes of root tips of *Allium cepa* fixed for 96 hr. in acetic-alcohol (1 : 3) exhibited three types of chromosome configurations. Very few cells in metaphase shc ved the quadri-partite structure seen in material fixed for 24 hr. There is a gradual digestion of the chromonemata on long exposure to the fixative resulting in the chromosomes appearing either as structureless ribbons or as closed hollow tubes of chromosome pellicles. It would appear that it is the chromosomal membrane which gives the mitotic chromosomes their morphological integrity.

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A NEW FUNDAMENTAL PARTICLE—ANTI XI-PLUS $\bar{\Xi}^+$

THE observation of a predicted hyperon, the anti Xi-plus ($\bar{\Xi}^+$) particle, produced in the collision of an antiproton with a proton has been reported simultaneously from two centres of high energy nuclear research.

The one is from the Brookhaven National Laboratory, Upton, New York, where the reaction $\bar{p} + p \rightarrow \bar{\Xi}^- + \bar{\Xi}^+$ has been observed in a 20-inch liquid hydrogen bubble chamber exposed to a separated antiproton beam of 3·3 Bev/c. momentum produced in a tungsten target in the Brookhaven alternating gradient synchrotron. The particular event was found after 34,000 pictures had been scanned for strange particle production. Each picture contained an average of 14 antiproton tracks.

The second report is from CERN, Geneva, Switzerland, where the interactions of fast antiprotons 3·3 Bev/c. with protons have been studied with the CERN proton synchrotron, and observed in the Scalay 81-cm. hydrogen bubble chamber. The event was observed in the course of the methodical scanning of the first 10,000 photographs with an average of 7 antiproton tracks per photograph.

While expected to exist the anti Xi-plus particle has so far not been observed, and these are the first observations reported.—(*Phys. Rev. Letters*. March 16, 1962.)