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THE FINE STRUCTURE OF THE KINETOCHORE OF A MAMMALIAN CELL IN VITRO

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Abstract. The chromosomes of Chinese hamster cells were examined with the electron microscope and the following observations were made concerning the structure and organization of the kinetochore. — The kinetochore consists of a dense core 200—300 Å in diameter surrounded by a less dense zone 200—600 Å wide. The dense core consists of a pair of axial fibrils 50—80 Å in diameter which may be coiled together in a cohelical manner. The less dense zone about the axial elements is composed of numerous microfibrils which loop out at right angles to the axial fibrils. Together the structures comprise a lampbrush-like filament which extends along the surface of each chromatid. Some sections suggested that two such filaments may be present on each chromatid. The fine structure of kinetochores associated with spindle filaments was essentially the same as those free of filaments. The structure and organization of the kinetochore of these mammalian cells was compared to that of lampbrush chromosomes of certain amphibian oöcytes, dipteran polytene chromosome puffs, and the synaptinemal complex seen during meiotic prophase.

Introduction

The kinetochore (centromere) is the site of spindle filament attachment to the chromosome and is believed to be directly involved in chromosome movement during cell division. As yet, however, little is known of its fine structure or relation to either the rest of the chromosome or the other components of the mitotic apparatus. The electron microscope has yielded little information on the structure of the kinetochore. This can be attributed in part to the small size of this component and the difficulty of collecting sufficiently large numbers of mitotic cells for fine structure analysis. The latter is particularly true in the case of mammalian cells. In addition, the older methods of fixation and embedding are generally insufficient for preserving the structure of the kinetochore.

In the course of our electron microscope studies of mitosis in synchronized populations of Chinese hamster fibroblast cells, we observed large numbers of kinetochores which displayed unusually clear structural detail. The present communication, therefore, presents a description of

the structure and organization of the mammalian kinetochore and its relation to the chromosome and other components of the mitotic apparatus.

Materials and Methods

The methods used were similar to those described in a previous study (BRINKLEY, 1965). Chinese hamster cells (strains Dede and Don-C) were maintained as monolayer cultures in McCoy's 5a medium supplemented with 20 per cent fetal calf serum. Colcemid inhibition and reversal were carried out as described by STUBBLEFIELD and KLEVECZ (1965). Cultures in exponential growth were treated with Colcemid at a concentration 0f 0.06 μg/ml. In some cases, cells continuously treated for as long as 17 hours were studied. Usually, however, after 2 hours of Colcemid treatment, mitotic cells were selectively removed from the monolayers by a brief washing in 0.2 per cent trypsin solution. The mitotic cells were centrifuged and the pellet was resuspended in conditioned growth medium without Colcemid. The cells rapidly recovered from the effects of the drug and divided synchronously about 30 minutes later. At various times during recovery from the Colcemid treatment, the cells were centrifuged, and the pellets were fixed in 3 per cent glutaraldehyde buffered at pH 7.4 with phosphate buffer. After one hour in glutaraldehyde, the cells were rinsed in buffer and fixed in 1 per cent osmium tetroxide buffered in a similar manner. Control cells which had not received Colcemid treatment were prepared in the same manner. The pellets were rapidly dehydrated in ethanol and embedded in Epon 812. Thin sections were cut with glass knives on a Porter-Blum ultramicrotome and picked up on Formvar coated, stainless steel grids. Serial sections were picked up on Effa Bar grids (Ernest F. Fullam Inc., Schenectady, N.Y.). The sections were stained in 2 per cent uranyl acetate at pH 5 followed by lead citrate and examined with an Hitachi HU-11A electron microscope.

Results

The kinetochore became evident as a distinct component of the chromosome at prophase before nuclear envelope dissolution (Fig. 1). The spindle filaments, however, were not seen to associate with the kinetochore until after the nuclear envelope disappeared. Fig. 2 shows a group of chromosomes at prometaphase on which numerous profiles of the kinetochore may be seen. In each case, these appear intermediate in density between that of the chromosomes and the cytoplasm. Although they may not be evident at this magnification, the spindle filaments were present.

Longitudinal sections of chromosomes taken though the kinetochore region revealed a filament-like structure extending along the surface of the chromatids (Fig. 3). These were located at the primary constriction and were less dense than the chromosome arms. In this respect, they were similar to structures described as kinetochores in HeLa cells by ROBBINS and GONATAS (1964) and GEORGE, JOURNEY and GOLDSTEIN (1965) and in L strain fibroblasts by KRISHAN and BUCK (1964).

At higher magnifications, the filament-like structures were seen to consist of two components, a dense central element 200—300 Å wide

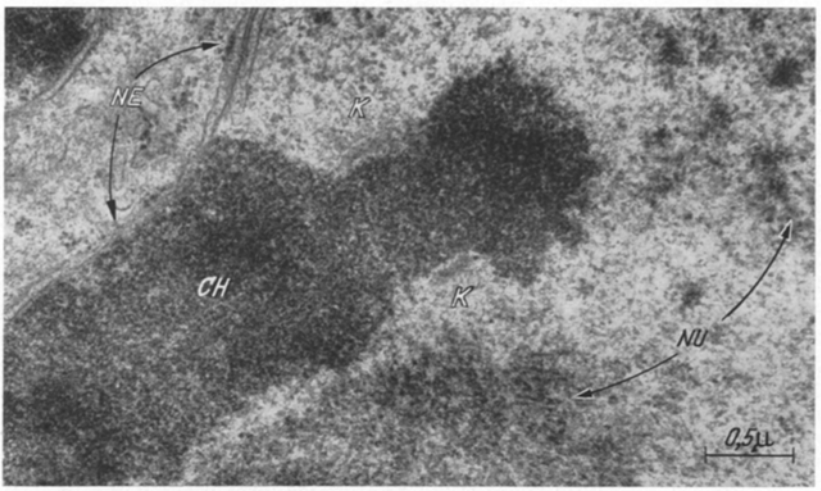


Fig. 1. A prophase chromosome (*CH*) still attached to the nuclear envelope (*NE*). Both sister kinetochores (*K*) are clearly present at the primary constriction. Portions of two nucleoli (*NU*) are also shown. 17 hour Colcemid inhibition. × 23,000

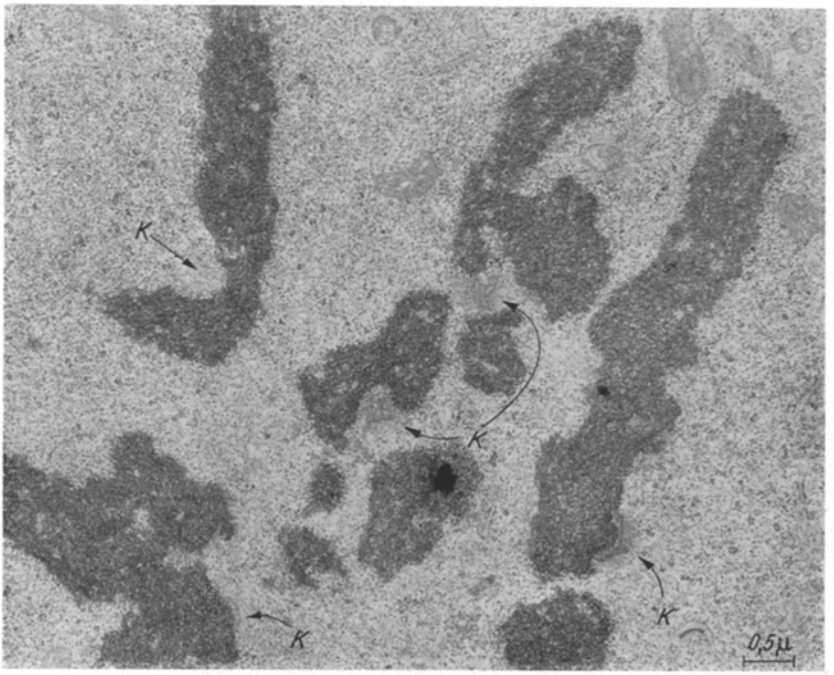


Fig. 2. A group of prometaphase chromosomes showing various profiles of the kinetochores (*K*). No Colcemid treatment. × 13,700

enclosed on each side by a less-dense zone 200—600 Å in diameter. The dense element was bipartite consisting of a pair of axial fibrils 50—80 Å in diameter. These were separated by a distance of 50—80Å and in some sections appeared to be twisted together in a cohelial manner

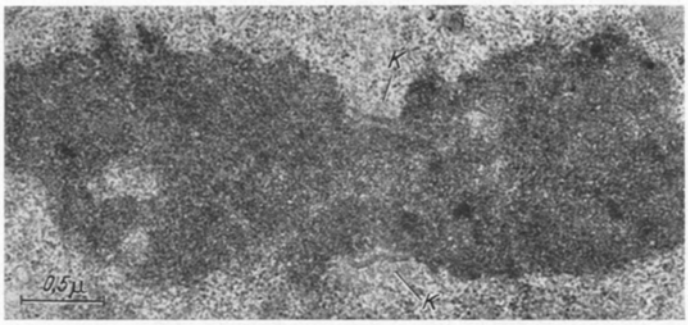


Fig. 3. Metaphase chromosome (*CH*) with a pair of kinetochores (arrows) at the primary constriction. 15 minute recovery after two hour Colcemid inhibtion. × 21,600

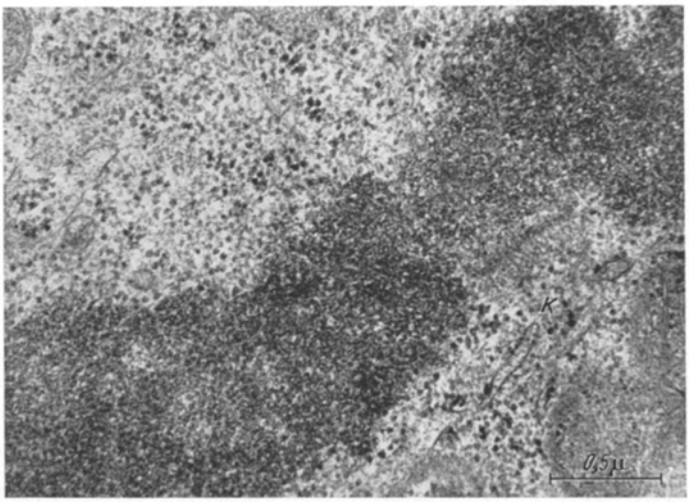


Fig. 4. A kinetochore (*K*) of a metaphase chromosome showing fibrous elements of the less-dense zone. 15 minute recovery after two hour Colcemid inhibition. (CH-chromosome) × 29,600

(Fig. 7). Serial sections through the paired elements provided convincing evidence that they were fibrils rather than tubules or laminated plates as might be envisioned from a cursory examination of a few sections.

The less-dense zone around the paired axial elements has been described by some investigators as consisting of a granular matrix (George, Journey and Goldstein, 1965). Our micrographs, however, clearly indicated that this zone was composed of fine fibrils also 50—80 Å

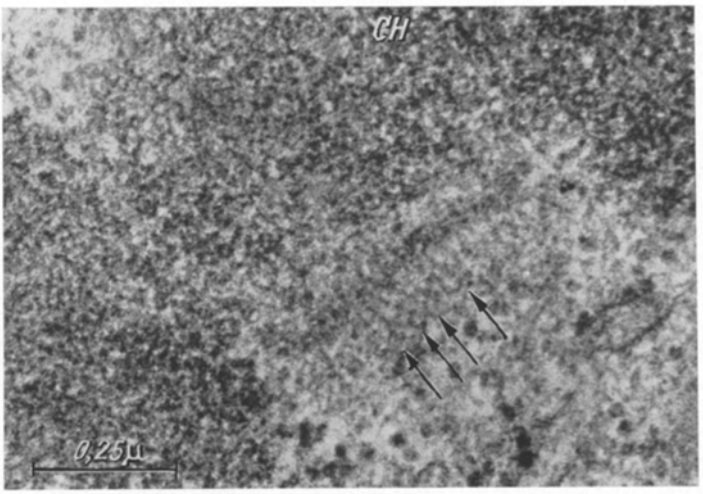


Fig. 5. Higher magnification of the kinetochore region in Fig. 4. Arrows indicate points where fibrils extend out from the dense osmiophilic elements. Note that some of the fibrils appear to form loops. × 80,000

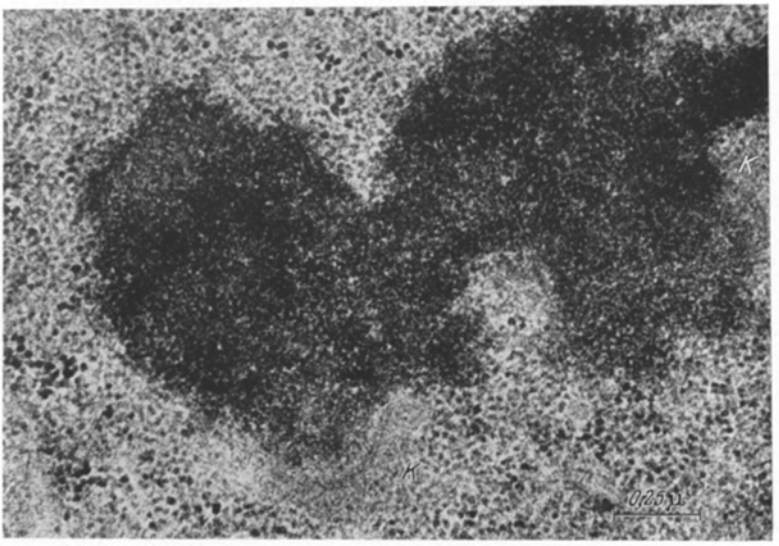


Fig. 6. Section through two chromosomes (*CH*). The paired axial elements of the kinetochore are clearly visible in the upper chromosome. 30 minute recovery after two hour Colcemid inhibition. × 58,000

in diameter (Figs. 5 and 8). In many instances, these appeared to exist in a "lampbrush-like" arrangement in respect to the axial elements (Figs. 4, 5 and 9).

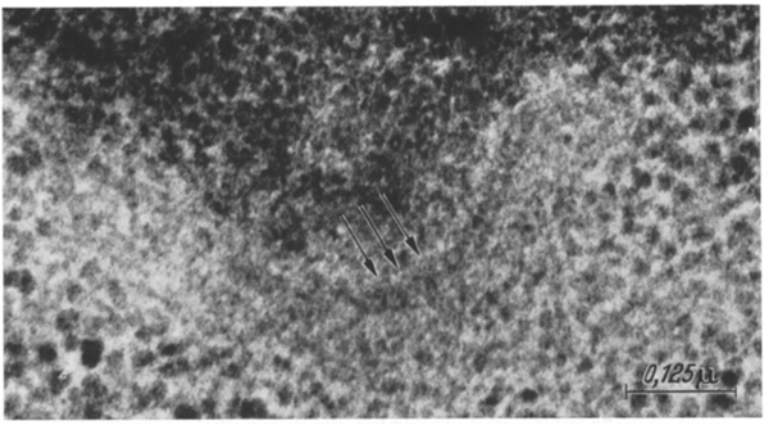


Fig. 7. Higher magnification of the kinetochore in Fig. 6 showing details of the two elements. Points indicated by the arrows suggest a cohelical arrangement of the two elements. × 124,000

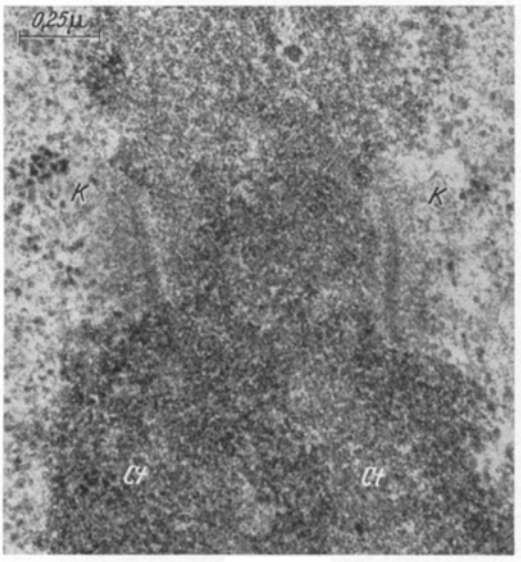


Fig. 8

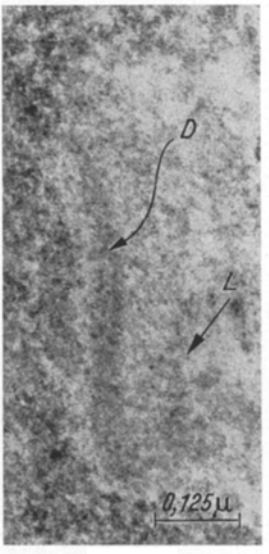


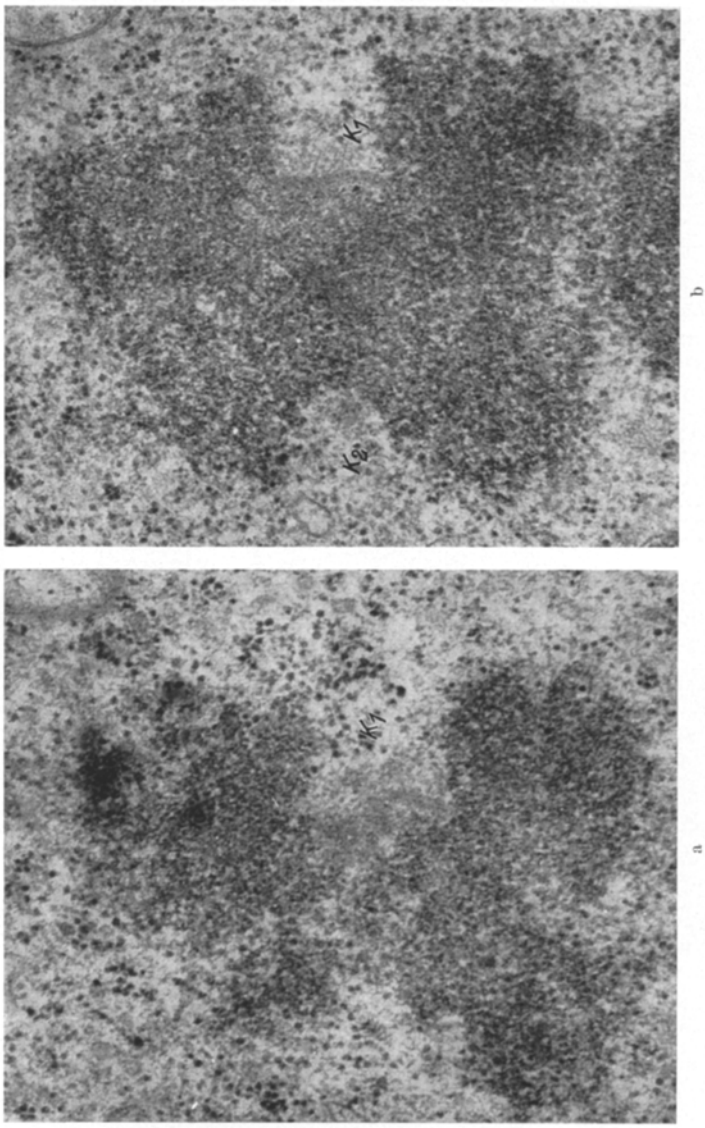
Fig. 9

Fig. 8. Symetrical kinetochore (*K*) associated with each chromatid (*Ct*) of a metacentric chromosome. Continuous Colcemid treatment for 17 hours. × 40,000

Fig. 9. Higher magnification of one of the kinetochores (*K*) in Fig. 8. A fibrous "loop" is shown in the less-dense zone (*L*). (*D*) dense axial elements. × 90,000

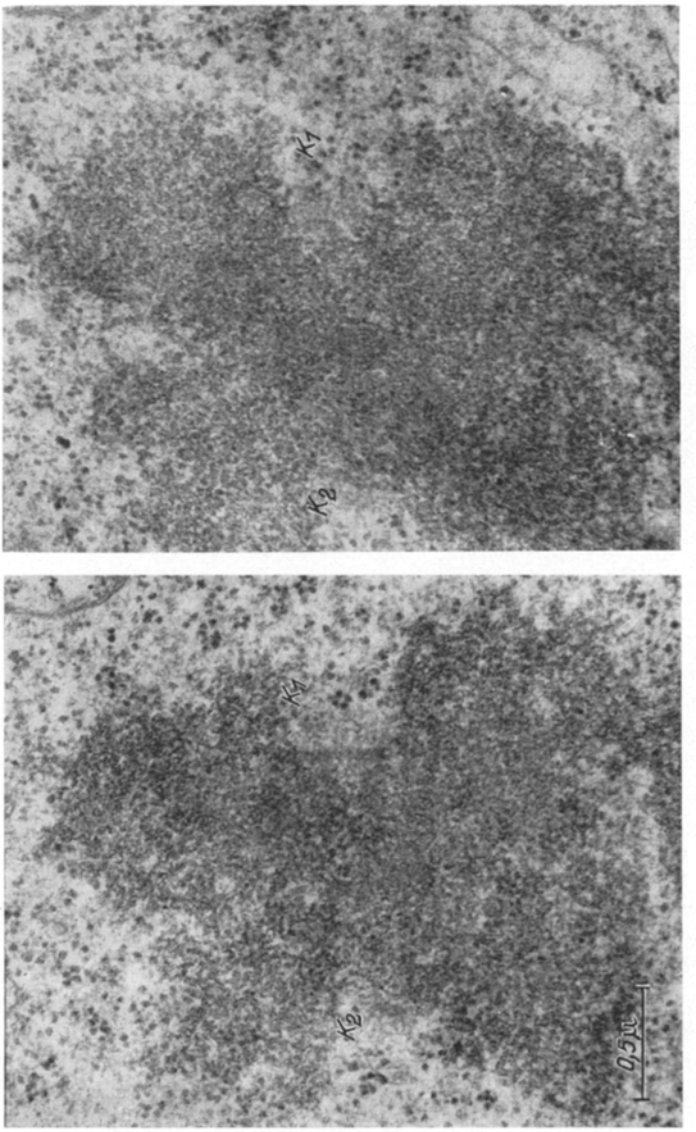
Thus far, we have established that the kinetochore is a structure distinguishable from the remainder of the chromosome which consists of axial elements embedded in a less dense fibrillar zone. Our next objective was to determine the three dimensional organization of these components

with respect to themselves and the remainder of the chromosome. This was attempted by careful examination of kinetochore profiles from a large number of randomly sectioned chromosomes and of serial sec-



tions taken through the kinetochore region. Figs. 3, 6, 8 and 15 represent typical profiles of randomly sectioned chromosomes. In Fig. 6 the kinetochore has a crescent shape partially enclosing the chromatid. In Figs. 1,

3 and 8 it appears to be parallel to the chromatid for a short distance. Fig. 15 shows two profiles of the kinetochore, one linear and the other more rounded in appearance. We interpreted the latter to represent a



d

c

Fig. 10a—d. Four sections of the same small metacentric chromosome. In 10a a portion of only one kinetochore (K_1) is seen. The arrangement of both sister kinetochore filaments (K_1 and K_2) is shown in the remaining three micrographs. 17-hour Colcemid inhibition. × 30,000

transverse section of the kinetochore filament. Taken together, the two profiles shown in Fig. 15 suggest that the elements of the less dense zone completely surround the axial elements resulting in a rounded

filament which extends 0.1 to 0.2 μ along each chromatid. Further support for this interpretation was obtained from serial sections cut parallel to the long axis of the chromosome. Fig. 10a—d show a series of four sections cut through a small metacentric chromosome. From these it can be seen that each sister kinetochore (K_1 and K_2) occupies a relatively

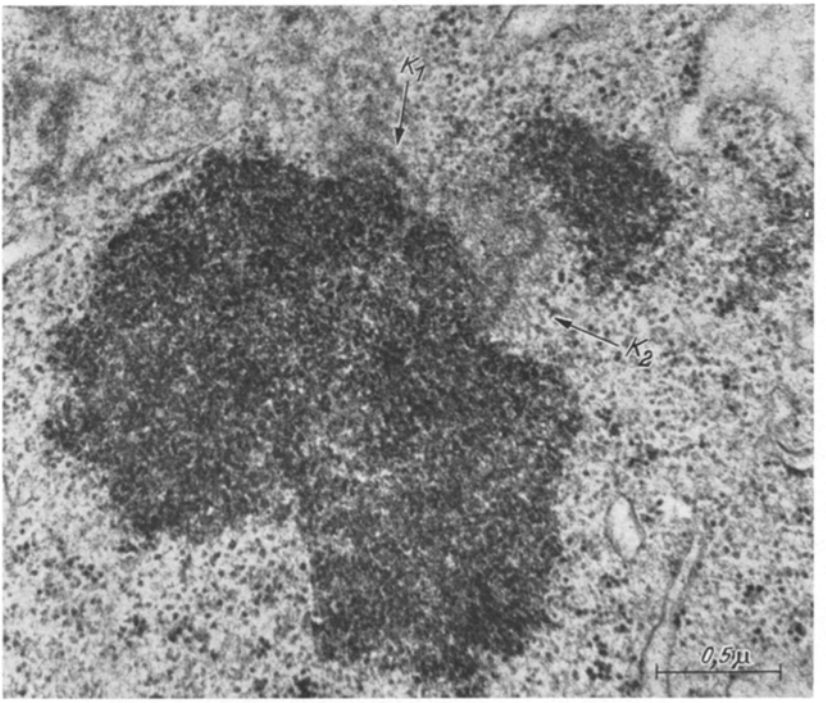


Fig. 11. In this micrograph two kinetochore filaments appear to be on a single chromatid (K_2). Only one filament is seen on the other chromatid (K_1). × 33,600

small area on the surface of the chromatids. Also, since it is possible to section through only one kinetochore of a pair (Fig. 10a), they apparently do not completely encircle the chromatids. Furthermore, there was no evidence that one sister kinetochore was structurally continuous with the other. Instead, each appeared to arise from a condensed region of a chromatid, wind around its surface for a short distance and reinsert at a point further along the same chromatid.

Most sections showed only one kinetochore filament associated with each chromatid. However, a few sections indicated that two filaments must be present. In Fig. 11, what appears to be a pair of kinetochore filaments are shown on one chromatid.

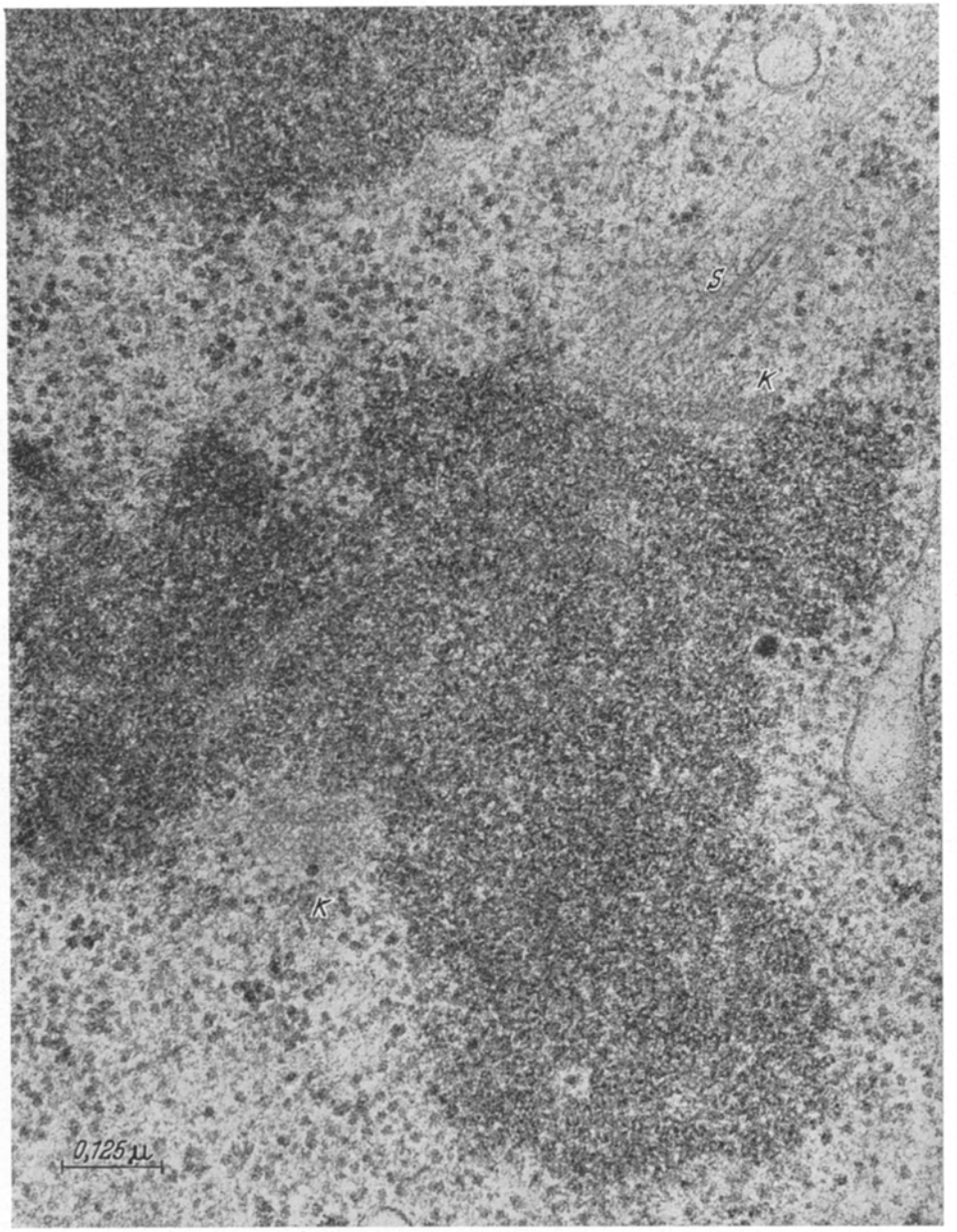


Fig. 12. Several spindle filaments (*s*) are shown extending from the kinetochore (*K*) inward toward the centrioles (not shown). The sister kinetochore appears to be free of spindle filaments. Two hour Colcemid treatment. × 51,000

Inhibition of the mitotic process with Colcemid did not result in the complete dissolution of the spindle filaments. Colcemid treatment did, however, inhibit the alignment of chromosomes on a metaphase plate. After two hours in Colcemid, the chromosomes became grouped in a

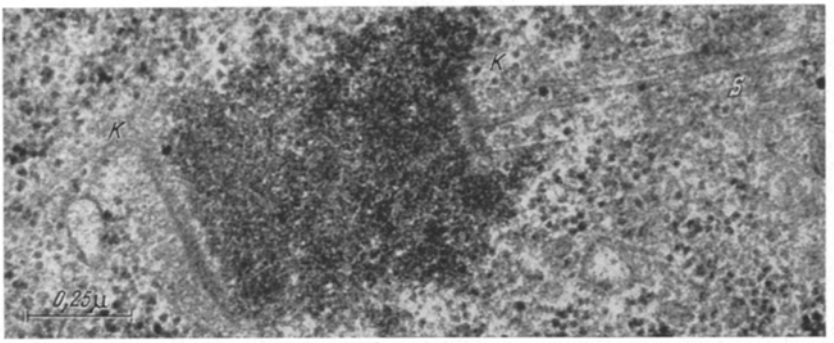


Fig. 13. Sections through a chromosome (*CH*) in the region of the kinetochore (*K*). Spindle filaments (*S*) are seen to make contact with the osmiophilic axial elements. 30 minute recovery after two hour Colcemid inhibition. × 56,000

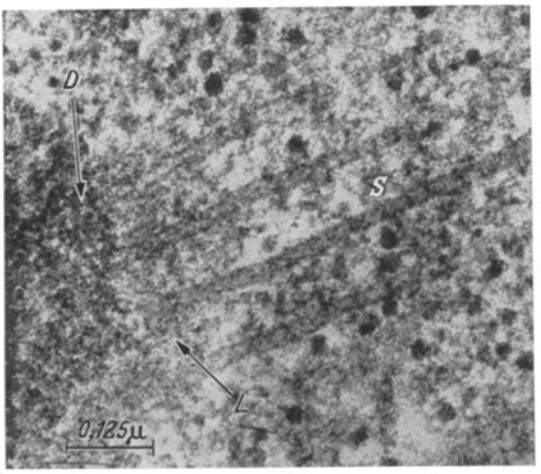


Fig. 14. The details of spindle filament attachment are shown in this micrograph. (*L*) fibrous, lessdense zone. (*D*) dense, osmiophilic elements. Continuous Colcemid treatment for 17 hours. × 94,000

sphere near the center of the cell. The centrioles failed to separate and assumed a position near the center of the chromosomal sphere. Under these conditions, one sister kinetochore of each chromosome was oriented toward the center of the sphere while tho other was directed to the outside. The inner kinetochore was, in most cases, associated with several spindle filaments which extended from this structure toward the centrioles. The outer kinetochore on the other hand, was always free of spindle filament while under the influence of Colcemid. Fig. 12 shows

a chromosome from a cell which has been treated with Colcemid for two hours. The inner kinetochore has several spindle filaments extending toward the centriole (not shown). The outer one, while identical in structure, is free of spindle filaments. Other spindle filament-kinetochore associations are shown in Fig. 13 and 14. In some sections, the spindle filaments were seen to penetrate the less-dense zone and make contact with the dense axial elements (Fig. 14). In others, the filaments

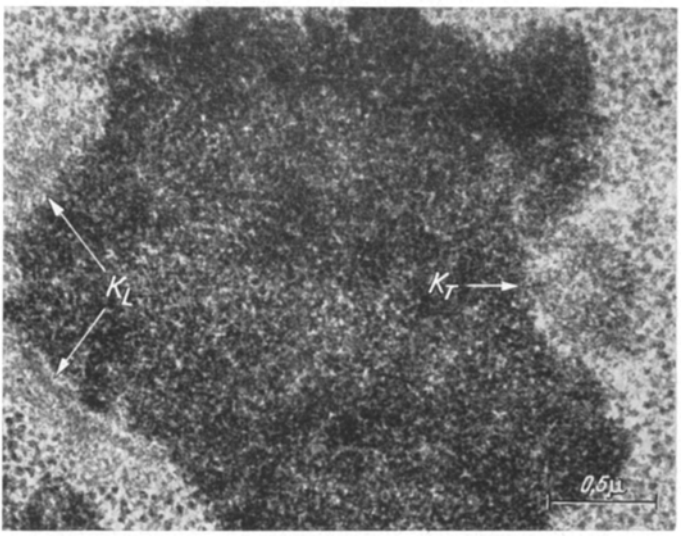


Fig. 15. Section showing two aspects of the kinetochore. The upper arrows (*KL*) indicate two segments of a kinetochore in longitudinal section. *KT* indicates a kinetochore in transverse section. 30-minute recovery after two hour Colcemid inhibition. × 28,000

appeared to be embedded in the fiberous loops of the less-dense zone. A few sections showed what appeared to be spindle filaments passing through the kinetochore into the condensed chromosomal regions. It is difficult however, to determine the actual points of termination of filaments in thin section since such points may merely be where filaments pass out of or into the plane of section. In cases where serial sections were available, the filaments appeared to terminate at the dense axial elements.

Except for the apparent absence of spindle filaments at some kinetochores, we were unable to detect any differences in the structure of kinetochores as a result of Colcemid inhibition. Identical structures were seen on Colcemid arrested chromosome, chromosomes of control cells and cells completing mitosis after Colcemid reversal.

Discussion

If we define the kinetochore simply as that region of the chromosome where the spindle filaments are attached, then the structures described in the present report must represent a part, if not all of the kinetochore apparatus of mammalian chromosomes. Any interpretation of the kinetochore fine structure, however, should take into consideration the light microscope descriptions provided in earlier studies. Unfortunately, the widely differing descriptions reported in the literature make such a comparison difficult. Schrader (1939) maintained that the kinetochore possessed one granule or "spherule" per chromatid. Later others reported that each spherule was longitudinally double (*cf.* Giménez-Martin, Lopez-Saez and Marcos-Morena, 1965).

Thus even at the light microscope level it is possible to see four subunits associated with the kinetochore in some chromosomes. The kinetochores of Chinese hamster chromosomes measured some .1 to .2 μ in their greatest length. Thus, they are large enough to be visible with the light microscope. Their cross sectional diameter might also be visible by such means. However, it would be difficult if not impossible to resolve any finer structure in these kinetochores with light optics. The fact that the kinetochores appear filamentous in thin section rather than globular or spherical is probably due to differences in methods of preparation and examination. Indeed the varying descriptions of the kinetochore given from both light and electron microscope studies may be a result of widely differing methods of preparation. In fact, our earlier studies of OsO_4-fixed, methacrylate-embedded mammalian cells failed to reveal any structure other than an electron dense, amorphous mass at the kinetochore region. It was only after double fixation in glutaraldehyde and OsO_4 and subsequent embedding in Epon that the structures described in the present study were seen. The latter procedure is known to give superior morphological preservation of most biological structures.

Previous electron microscope studies (Nebel and Coulon, 1962; Harris and Mazia, 1962; Robbins and Gonates, 1964; and Krishan and Buck, 1965) describe the kinetochore as resembling a plate or laminated disc. As mentioned previously, we interpret the images shown in the present study to represent a filament rather than a plate. The term plate implies a flattened structure of uniform thickness. While some sections of the kinetochore may give such an impression, examination of a composite of sections showing a variety of profiles of the kinetochore argue against a plate-like arrangement. Instead, this structure appears to be a coarse filament some 100—200 mμ in diameter composed of a pair of axial fibrils from which similar fibrils loop out on all sides. Serial sections suggest that the filament originates at one point on each chromatid, winds around the surface of the chromatid and reinserts into

a point further along the same chromatid. As mentioned previously, it is possible that two such filaments are present on each chromatid.

The arrangement of microfibrils into a lampbrush-like configuration is evident in a variety of chromosome types. Such organization is visible with the light microscope in the chromosomes of certain amphibian oöcytes (e.g. *Triturus*) during meiotic prophase (GALL, 1963; CALLAN and LLOYD, 1960). Also, electron microscope studies of dipteran salivary gland chromosomes show the polytenic threads in the large RNA puffs to loop out in a lampbrush-like manner (BEERMANN and BAHR, 1954). Perhaps an organization more closely allied to that of the kinetochore is seen in the arrangement of microfibrils of pachytene chromosomes (synaptinemal complex). Electron microscope studies of these chromosomes generally show a pair of axial filaments 100 Å in diameter surrounded by similar microfibrils which are interpreted as being lateral extensions from the axial filaments (MOSES and COLEMAN, 1964; NEBEL and COULON, 1962). MOSES (1964) interpreted the elements of the synaptinemal complex as representing the "basic units" of the chromosome. It is true that in each of the above cases, the chromosome is greatly extended and uncoiled. Also, such chromosomes are still active in RNA synthesis. In fact, MONESI (1965) has generalized that the extent of "loop organization" is a measure of the synthetic activity of the chromosome. Such ordered structures are not visible in mitotic chromosomes because of their complex folding and packing. The kinetochore, however, may be an exception. This component represents a small region of the chromosome which remains active during mitosis. In lieu of our present knowledge however, it is not possible to directly relate the activity of the kinetochore to that usually ascribed to the lampbrush-type organization, viz. RNA synthesis. On the other hand, it is obvious from many studies that the kinetochore is more than just an anchor point for chromosomal spindle filaments. Its behavior during cell division implies a direct role in chromosome movement. Evidence for its function as an organizer of chromosomal spindle filaments is reviewed by SCHRADER (1953) and MAZIA (1961). More recently, the studies of INOUÉ (1964) on living cells gives strong support for the theory that the kinetochore is directly involved in the organization of microtubular subunits into chromosomal spindle filaments. Taken together, these data suggest a dynamic function of the kinetochore during cell division. Thus, while most of the chromosome is inert during mitosis and remains highly condensed, the kinetochore remains active and persists in an extended state.

Additional studies utilizing cytochemical and autoradiographic procedures at the electron microscope level are now in progress and should further elucidate the activity and chemical organization of this component.

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