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Cooperation of kinetochores and pole in the establishment of monopolar mitotic apparatus

(mitosis/microtubules/centrosome/sea urchin egg)

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ABSTRACT **Monopolar mitotic apparatus can be produced in sea urchin eggs by a manoeuvre that distributes the four poles of the second mitosis into four separate blastomeres. The pole of the monopolar mitotic apparatus generates a half-spindle that is similar in structural details to the half-spindle of a normal bipolar mitotic apparatus, although the chromosomes are not as well aligned as in a normal metaphase plate. The chromosomes are oriented; one kinetochore faces the pole while its sister kinetochore faces away from the pole. The poleward kinetochore is connected to the pole by bundles of microtubules. No microtubules are seen on the sister kinetochore that faces away from the pole. Therefore, a single pole can direct most of the events in the establishment of a mitotic apparatus. Our interpretation examines the cooperation of kinetochores and poles in the formation of microtubules between them, stressing the half-spindle as the medium of cooperation and leaving open the question whether the kinetochores are origins or terminations of microtubules.**

The logic of mitosis is displayed in the connections of replicated chromosomes to two poles of a mitotic apparatus. The connections are seen in animal cells as bundles of microtubules running from kinetochores to centrosomal regions that define the poles. In the present work, we induce the formation of monopolar mitotic apparatus in sea urchin blastomeres by a method described some time ago (1). The main question is: Given only one pole, will the chromosomes obey Boveri's rules (ref. 2, p. 181), which prohibit sister kinetochores from connecting to the same pole? The properties of a monopolar mitotic apparatus may illuminate other features of mitosis in which two poles are normally invoked: the formation of a spindle with an axis, the equatorial arrangement of the chromosomes, the orientation of kinetochores with respect to the pole, and the ability of kinetochores to initiate microtubules.

MATERIALS AND METHODS

Material. Eggs of the sea urchin *Strongylocentrotus purpuratus* were used. Jelly coats were removed by passing the suspensions of eggs through bolting silk. Immediately after fertilization, the eggs were suspended in Ca-free sea water and passed through a Nitex filter with a mesh size of about 65 μm to strip away the fertilization envelopes. For further handling, the eggs were attached to glass coverslips. Slips were covered with a 0.75% solution of protamine sulfate, then washed thoroughly with Ca-free sea water to remove unabsorbed protamine. Eggs settling onto these surfaces attached firmly, and the coverslips carrying monolayers of cells could be transferred through the steps of the experimental procedure and through fixation and processing for electron microscopy.

Electron Microscopy. The coverslips carrying monolayers of eggs were immersed in the fixative 1% OsO_4/0.4 M sodium acetate buffer, pH 6.1, at room temperature (3). The fixation continued for 90 min at 4°C. Details of the dehydration and embedding steps followed the procedure described in ref. 4. After embedding, polymerization, and detachment of the glass coverslips, the eggs could be observed with the light microscope. Groups of eggs that had given rise to two monopolar figures and one bipolar figure were selected. Sections were cut with a diamond knife on a Reichert OMU3 ultramicrotome, poststained with lead citrate and uranyl acetate, and observed in the Philips 400 electron microscope.

Polarized-Light Microscopy. Eggs of *S. purpuratus* are not suitable for birefringence studies *in vivo* because of light scattering by yolk particles. In the present work, eggs that retained their fertilization membranes were pelletted and taken up in the following medium: 0.3 M potassium gluconate/0.33 M glycine/10 mM NaCl/3 mM $MgSO_4$/2 mM potassium ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetate/0.5% Triton X-100. The pH was 6.8. The birefringence of the mitotic apparatus was preserved while the cytoplasm was cleared by the action of the detergent.

Isolated Mitotic Apparatus. The above medium yields isolated mitotic apparatus suitable for observation by phase-contrast microscopy and polarized-light microscopy when the fertilization membrane has been removed.

Monopolar Mitotic Apparatus. The procedure is taken from the work of Mazia *et al.* (1). The interpretation is diagrammed in Fig. 1 and is discussed more fully in refs. 1 and 5.

Fertilized eggs are placed in sea water containing 0.1 M 2-mercaptoethanol at a time just before the first mitosis, in early prometaphase. They remain blocked in the first mitosis, giving the two duplex centrosomes time to develop into four mature poles. When the eggs are returned to sea water about the time when the controls have made their second division, they form a tetrapolar mitotic apparatus and divide directly into four blastomeres. Each of the four blastomeres receives half the normal complement of centrosomes and forms a monopolar mitotic apparatus at the next mitosis. Frequently, a furrow fails at the time of the fourway division, thus producing three cells, one of which produces a bipolar mitotic apparatus while the other two produce monopolar figures. Such cases permit internal comparison of monopolar and bipolar spindles (Fig. 2).

RESULTS

One Pole Forms a Half-Spindle. The present description deals with the monopolar mitotic apparatus at metaphase. The

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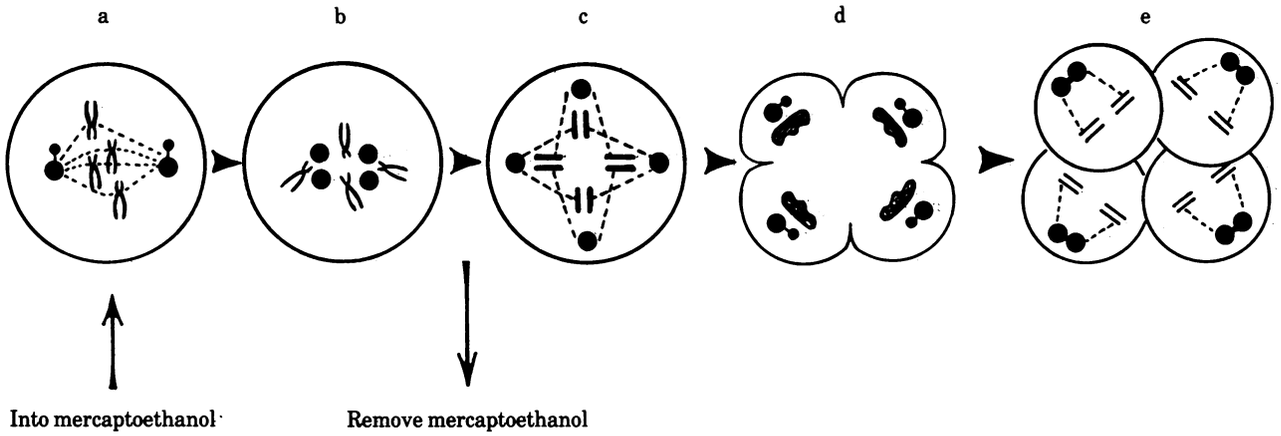

FIG. 1. Experimental design for making monopolar mitotic apparatus (1). (*a*) Fertilized eggs are put into 0.1 M 2-mercaptoethanol in sea water at the time of prometaphase or earlier. (*b*) Chromosomes continue condensation. Mitotic apparatus is arrested. Duplex centrosomes continue maturation to make four potential poles. (*c*) Upon removal of mercaptoethanol, a tetrapolar mitotic apparatus forms. (*d*) Tetrapolar mitosis proceeds; egg divides into four blastomeres. (*e*) At next mitosis, monopolar mitotic apparatus is formed.

main features as observed by light microscopy (phase contrast and polarized light) are: (*i*) The monopolar mitotic apparatus consists of an aster and a half-spindle. (*ii*) The half-spindle corresponds to half of a normal spindle in the orientation of birefringent fibers running from the region of the pole to a virtual equator. (*iii*) The chromosomes are congregated on the margin of the half-spindle but are not aligned in as flat a plane as they are in a normal metaphase plate. (*iv*) Some fibers run past the latitude of the chromosomes; these could be the equivalent of the fibers that connect the poles in a bipolar mitotic apparatus.

In a survey view of the monopolar mitotic apparatus with the electron microscope (Fig. 3) the half-spindle stands out strikingly as a massive population of microtubules running from the region of the pole to the latitude of the chromosomes, with some microtubules running past the chromosomes. Observed at higher magnification, the background resembles that of the normal mitotic apparatus: a zone clear of mitochondria and yolk vesicles containing a dense association of membranes and large numbers of ribosomes. In the figure, one sees one pair of sister chromosomes, with one kinetochore pointing toward the pole, the other away.

The structure at the pole as shown in Fig. 3 is the one encountered most commonly in sections through the axis of the half-spindle. The single centriole is surrounded by a dense accumulation of osmiophilic material. Peripheral to this mass is a less compact zone from whose surface the spindle microtubules are directed. In rarer cases, the polar structure was observed as two somewhat-separated masses, each containing a centriole.

Microtubules Are Associated Only with Kinetochores Facing a Pole. The kinetochores stand out in sections as dense, sometimes curved, plaques in good contrast to the much less dense chromatin.

The sister kinetochores in the monopolar mitotic apparatus are oriented. One faces into the half-spindle and toward the

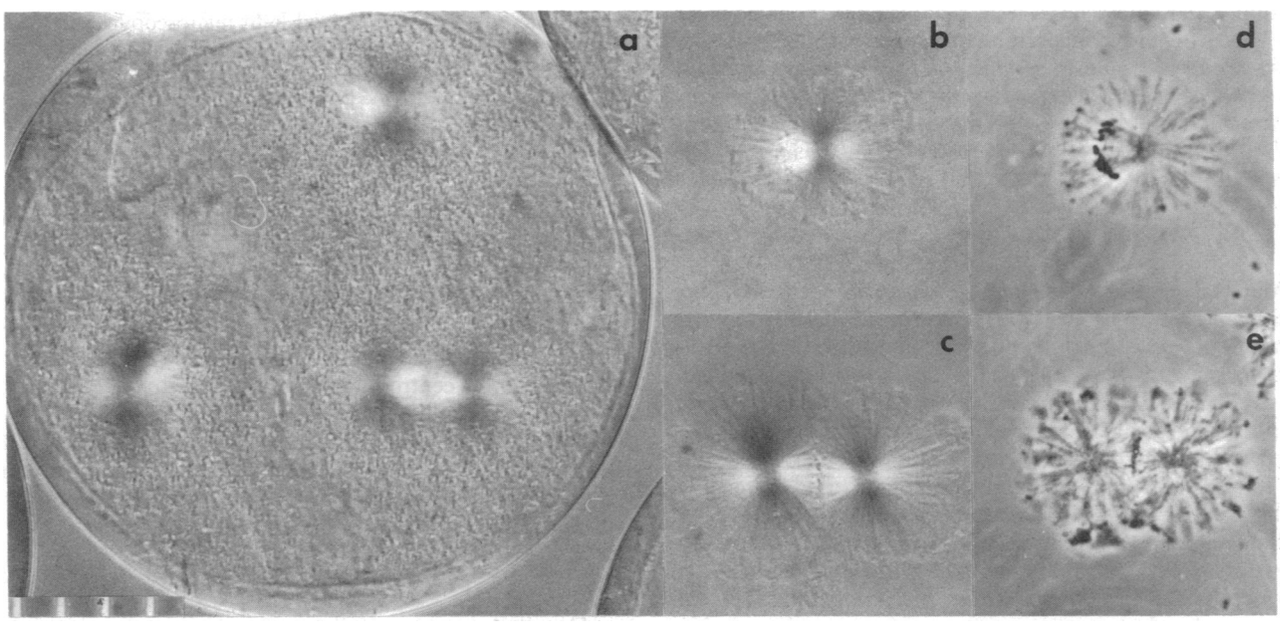

FIG. 2. Monopolar and bipolar mitotic apparatus. In the protocol shown in Fig. 1, some eggs divide into three blastomeres. One blastomere receives two poles and forms a bipolar apparatus at the next mitosis. The other two receive one pole and each forms a monopolar mitotic apparatus. (Scale division = 10 μm.) (*a*) Two monopolar and one bipolar, retained within the fertilization envelope; polarized light. (*b*) Isolated monopolar, polarized light. (*c*) Isolated bipolar, polarized light. (*d*) Isolated monopolar, phase contrast. (*e*) Isolated bipolar, phase contrast.

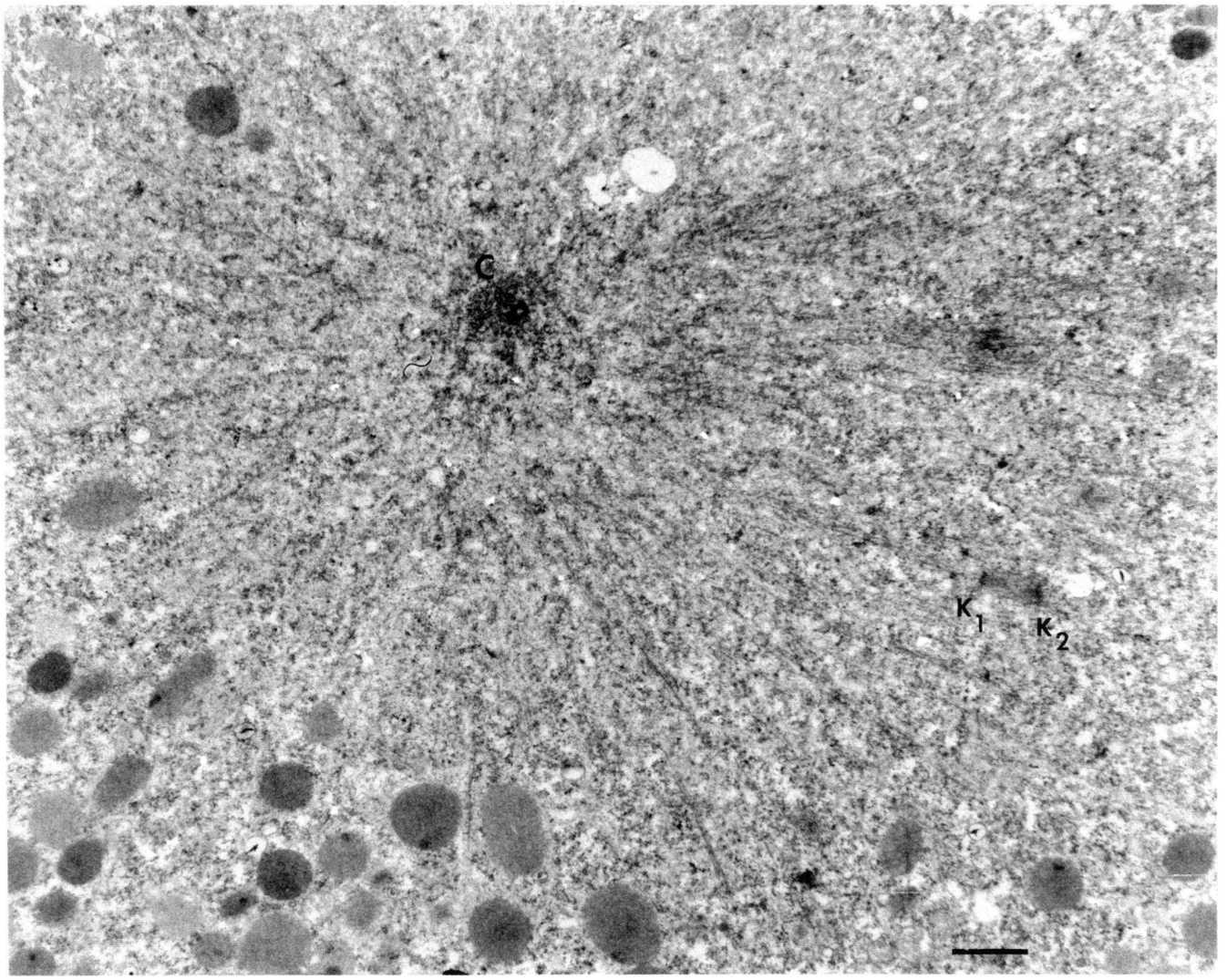

FIG. 3. Survey view of a monopolar mitotic apparatus. Microtubules run from the edge of a centrosphere, at the center of which a centriole (C) is surrounded by osmiophilic material. The half-spindle is seen as a sector in which numerous microtubules run from the pole to the latitude of the chromosomes. In this section, one chromosome is seen with one kinetochore (K_1) oriented toward the pole; its sister (K_2) faces away from the pole. (Bar = 1 μm.)

pole, as is especially evident in cases in which sections include a pole and pair of kinetochores (Fig. 3). The other faces away from the pole. Evidently, the orientation does not require the action of the two poles.

Kinetochores facing the pole are directly connected with bundles of microtubules that run straight toward the pole (Fig. 4 *b*, *c*, and *d*). The bundles resemble the kinetochore-to-pole fibers observed in bipolar mitotic apparatus (Fig. 4*a*). In the monopolar mitotic apparatus no microtubules are observed on the kinetochores that face away from the poles (Fig. 4 *b*, *c*, and *d*). In most cases, the poleward kinetochore is sharply defined and the microtubules appear to be inserted directly into the osmiophilic plaque (Fig. 4 *c* and *d*). The surface of the sister kinetochore facing away from the pole often seems to be associated with an aggregation of material that appears to be finely fibrous. No exceptions to these observations have been encountered; where sister kinetochores can be seen in the sections, those facing the pole are associated with bundles of microtubules, while their sisters facing away from the poles are devoid of microtubules. Bajer (6) reports the same finding in newt cells with monopolar mitotic apparatus.

Our observations of the later phases of the monopolar mitosis are fragmentary. The sister chromosomes do split apart, as is typical of the transition from metaphase to anaphase. The chromosomes connected to the poles do not separate further from their sisters. A single nucleus is reconstituted; there is no cleavage. The next mitosis is bipolar.

DISCUSSION

In a previous study (4) we described the nonpolar mitotic apparatus induced in unfertilized eggs that had not received normal centrosomes by insemination. The nonpolar mitotic apparatus took the form of an aster. The chromosomes were arrayed all around the aster. The kinetochores were not oriented and did not make connections to the microtubules, which were present. In the present work, the blastomeres that made monopolar mitotic apparatus contained true poles, derived through normal fertilization. The contrast between a nonpolar mitotic apparatus and a monopolar mitotic apparatus defines a pole: it can form a half-spindle, organize chromosomes in a quasi-metaphase arrangement, orient kinetochores, and make connections to one of the sister kinetochores.

In the monopolar mitotic apparatus the kinetochore facing the pole connects to that pole by a bundle of microtubules. Its sister, facing away from the pole, is devoid of microtubules.

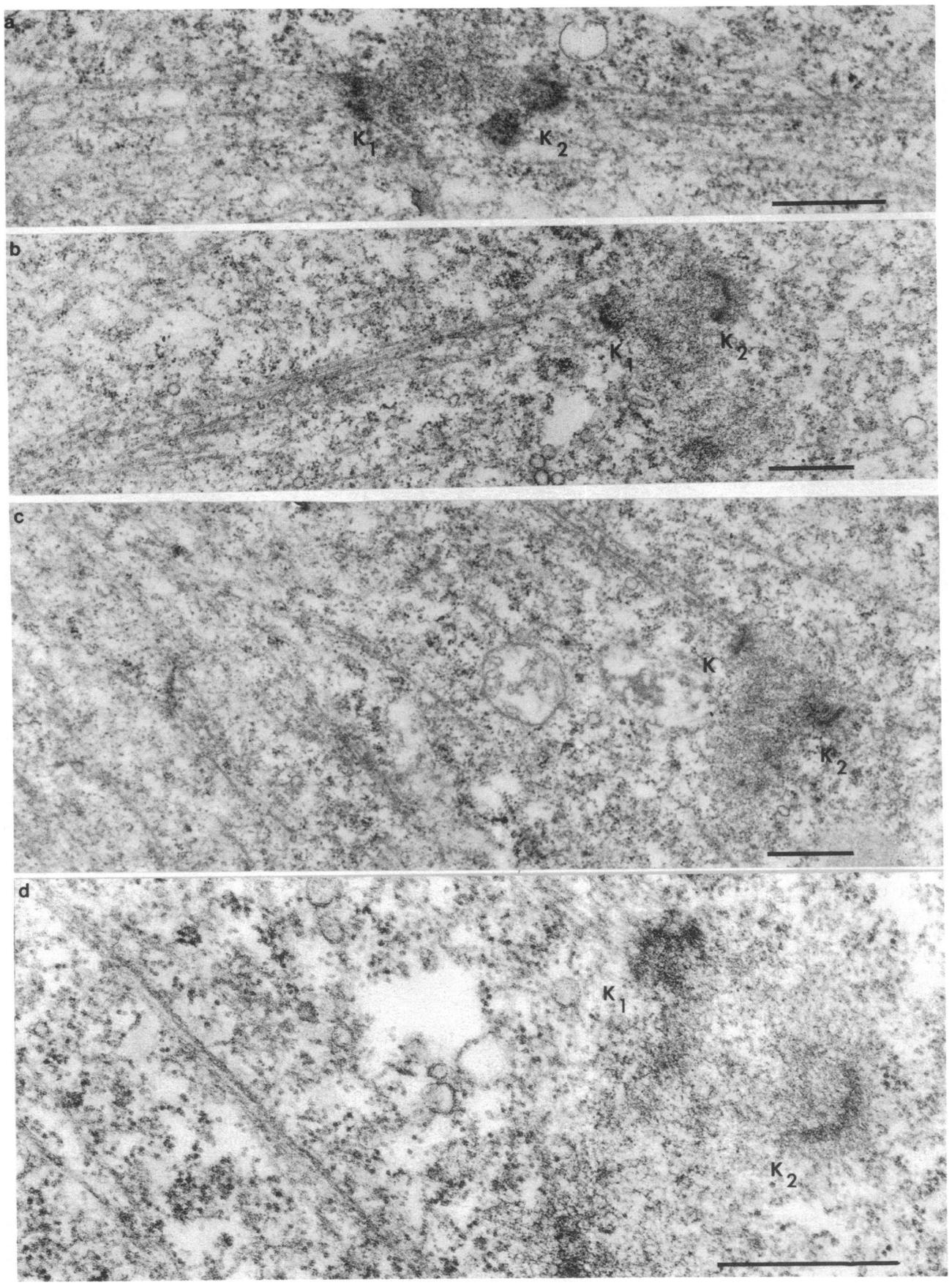

FIG. 4. Connections of sister kinetochores (K_1 and K_2) to microtubules. (*a*) Bipolar mitotic apparatus. (*b*, *c*, and *d*) Monopolar mitotic apparatus. (Bars = 0.5 μm.)

Viewed simply, the observation might seem to contradict the evidence (7, 8) that kinetochores are sites of initiation of microtubules and favor the alternative opinion (9) that kinetochores capture microtubules coming from the poles. However, a broader view accomodates either interpretation.

We can regard the formation of microtubules connecting kinetochores and poles as a cooperation between the two. The medium of cooperation is the half-spindle, organized by the pole. It can be regarded as an environment that supports the initiation of microtubules. At least some microtubules are initiated at the pole and run past the chromosomes. If kinetochores are the origins of chromosome-to-pole microtubules, such microtubules can be generated only by the kinetochore that faces the pole, is engaged to the "equatorial" margin of the half-spindle, and sees the environment within the half-spindle. Its sister, facing away from the pole, does not see the environment of the half-spindle and cannot initiate microtubules. The observations do not explain why it is impossible for both sister kinetochores to engage to the half-spindle and connect to the same pole, but they confirm this all-important law of mitosis even for the case in which only one pole is present.

If it is permissible to compare the half-spindle of the monopolar mitotic apparatus with that seen at anaphase in normal mitosis, we can assign to it at least one component that may be decisive for the initiation of microtubules. The regulatory molecule calmodulin is highly localized in half-spindles at anaphase (10, 11).

Some older interpretations of the formation of a normal mitotic apparatus viewed the positioning, orientation, and engagement of the chromosomes as the interplay of two poles. It is now seen that a single pole can direct these operations in forming a monopolar mitotic apparatus. The bipolar mitotic spindle can be viewed as a conjunction of two half-spindles. In each half-spindle, one of the sister kinetochores sees the "equatorial" margin and engages to the pole. If the margin of the other half-spindle is sufficiently close, the other sister kinetochore would be engaged by the second pole. In this image, the microtubules connecting the poles would be the means of holding the half-spindles together in a bipolar mitotic apparatus.

It is only a hypothesis that the mitotic apparatus at metaphase is made of two half-spindles, but it is a fact that two half-spindles, with an interzonal region between them, are discerned as soon as anaphase begins. The significance of half-spindles for the anaphase events has been discussed by Bajer and Bajer (12). At least one kind of molecule, calmodulin, distinguishes the half-spindles at anaphase from the rest of the mitotic apparatus (10, 11). Either the bipolar spindle is composed of two half-spindles from the start or else there must be a mechanism for cleaving a spindle into two halves at the beginning of anaphase.

Our conclusions are: (*i*) The establishment of microtubular connections between kinetochores and poles depends on the cooperation of the two, mediated by half-spindles. (*ii*) One pole can orient a sister pair of kinetochores so that only one of them can connect to one pole, as required by Boveri's laws.

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