CILIA FORMATION IN CHINESE HAMSTER FIBROBLASTS IN VITRO AS A RESPONSE TO COLCEMID TREATMENT

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INTRODUCTION

Colchicine and its derivative, Colcemid (*N*-deacetyl-*N*-methyl colchicine), have been used widely as mitotic inhibitors. Stubblefield and Klevecz (12) employed Colcemid to obtain synchronized cell populations of Chinese hamster fibroblasts, since the mitotic inhibition was readily reversible in these cells. While studying the effects of Colcemid on the fine structure of mitotic cells (2), we found that there was also an effect on some interphase cells, which resulted in the rapid differentiation of centrioles into basal bodies and the subsequent formation of cilia.

MATERIALS AND METHODS

Chinese hamster fibroblasts (males strain Don C and female strain Dede) were grown at 37°C in McCoy's 5a medium as monolayers, subcultured at 2 or 3 day intervals by trypsinization. Cultures 24 hr old were treated for varying periods with Colcemid (0.06 μg/ml) and in some cases returned to conditioned medium lacking Colcemid for further incubation. Cells were harvested with a brief trypsin treatment (0.2% in 0.9% sodium chloride). Cell suspensions were centrifuged, and the pellets were fixed at room temperature for 1 hr with 3.0% glutaraldehyde in Millonig's phosphate buffer (4) at pH 7.4. The cells were postfixed for 30 min in 1% osmium tetroxide in the same buffer, dehydrated in ethanol, embedded in Epon 812, and sectioned for electron microscopy using a Porter-Blum MT II ultramicrotome. The sections were stained in 2% uranyl acetate followed by lead hydroxide, and were examined with an Hitachi HU-IIA electron microscope.

RESULTS

The centrioles of normal Chinese hamster cells are similar in structure to those described in studies of other cells (3, 8). Typical longitudinal and transverse sections are shown in Figs. 1 and 2. Basal body formation has been rarely observed in extensive surveys of untreated cells (1). In addition, no ciliogenesis has been noted in cells arrested *in mitosis* by Colcemid treatment for 2 hr. Fig. 3 shows a centriole in a metaphase cell after 2 hr of Colcemid treatment, followed by 5 min of incubation in medium from which the drug was omitted. Spindle elements were numerous and the centriole appeared to be normal in structure. Mitotic cells usually divided within 30 min after the Colcemid was removed (12). The central vesicle in the centriole in Fig. 3, also observed by Schuster (9) in the centrioles and basal bodies of a human brain tumor and by Reese (6) in the basal bodies of olfactory cilia of the frog, is a common constituent of normal centrioles in the Chinese hamster fibroblast.

Some *interphase* cells treated with Colcemid for 2 hr contained centrioles in early stages of differentiation into basal bodies. Figs. 4 to 7 show these stages which are very similar to the early centriole modifications observed by Sorokin (10) in a study of ciliogenesis in organ cultures of chick and rat embryonic tissues, and by Renaud and Swift (7) in a study of basal body development in the water mold, *Allomyces arbusculus*. Initially, one of the

parent centrioles became associated with a vesicle which flattened across the end of the centriole opposite the daughter centriole (Figs. 4 and 5). Such vesicles with smooth surface were similar to those associated with the neighboring Golgi complex. At this stage the centriole began to expand into the vesicle to form the ciliary bud (Fig. 6), and the vesicle invaginated around the bud to begin the formation of the ciliary sheath.

In Fig. 7, a case is shown where centrioles became associated with the cell membrane in a manner characteristic of basal bodies. This cell was treated for 17 hr with Colcemid, and, although the centrioles may have replicated, cilium formation did not occur. In fact, no development of cilia beyond the stage shown in Fig. 6 was observed in cells treated continuously with Colcemid for periods up to 17 hr. Further development of a cilium required the removal of Colcemid from the culture medium.

Formation of complete cilia was found in cultures treated with Colcemid for 2 hr and returned to medium without Colcemid. Thirty min after the removal of Colcemid, cilia development had proceeded to the extent depicted in Fig. 8. The ciliary shaft rapidly elongated. The double-membraned sheath surrounding the shaft also enlarged, apparently by the addition of vesicular elements. The central fibers of the cilium are discernible, and the conversion of the centriole into a basal body appears complete, including the formation of a basal foot. The cell shown in Fig. 9 was cultured for 1 hr after Colcemid removal. In this case, two developing cilia were found with the shaft of one clearly protruding from the cell. This figure also demonstrates the fusion of vesicular elements into the ciliary sheath (arrows). The cilia formed as a response to Colcemid treatment have been observed in living cells with phase-contrast light microscopy (Fig. 10). Usually, they showed no apparent motility, but a few have been observed to beat erratically.

An attempt was made to estimate the occurrence of ciliary buds and cilia in cells before and after Colcemid treatment. Quantitation of structures seen in thin sections with electron microscopy is admittedly difficult, but, since no other assay method was available, we used this approach. Several precautions were taken to prevent errors due to inadequate sampling. Serial sections to a field of cell profiles which had been studied were avoided. Sections of cells were considered to be countable profiles when the greatest diameter was about one-third of the average profile diameter (about 8 μ). Each cell profile was carefully studied at moderate magnification for the inclusion of sections through centrioles (or basal bodies). The orientation of each centriole was recorded (cross-

Key to Symbols

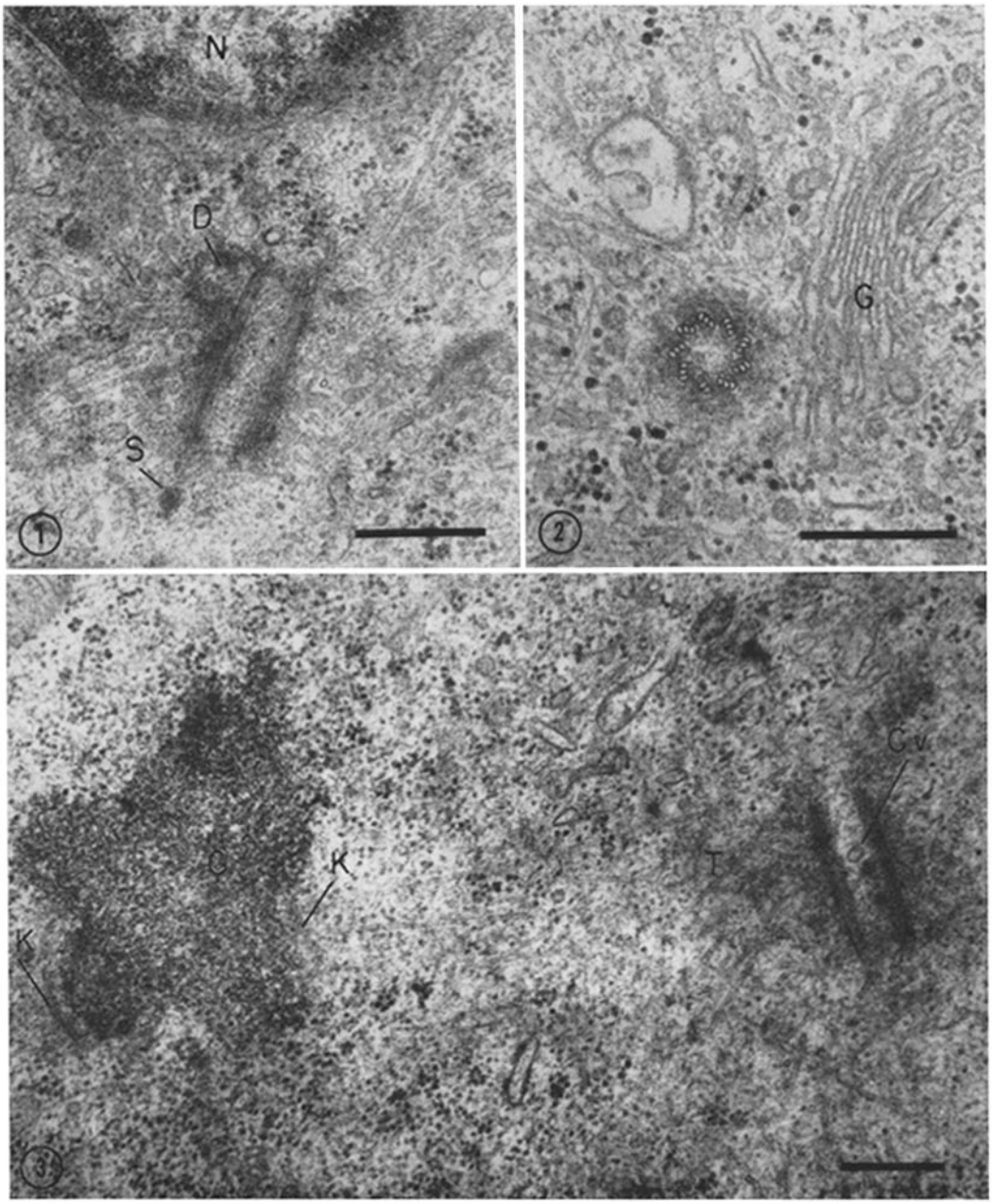
B, ciliary bud	*G*, Golgi apparatus
Bf, basal foot	*K*, kinetochore
C, chromosome	*N*, nucleus of cell
Cs, ciliary sheath	*S*, pericentriolar satellites
Cv, central vesicle	*T*, microtubules of the mitotic spindle
D, daughter centriole	*V*, vesicle

The black bar in the lower left of each figure is 0.5 μ long (except Fig. 10).

FIGURE 1 Centriole in interphase Chinese hamster male (Don C) cell. Longitudinal section showing daughter centriole (*D*) and an abundance of interphase microtubules near the centrioles. No Colcemid treatment. × 37,000.

FIGURE 2 Transverse section of interphase Don C cell showing characteristic triplet arrangement of the tubules in the centriole wall. A prominent Golgi zone (*G*) is nearby. No Colcemid treatment. × 44,000.

FIGURE 3 Don C cell in metaphase demonstrating the normal relationship between the centriole and chromosomes. The cell was treated for 2 hr with Colcemid, briefly trypsinized, and suspended in medium lacking Colcemid for 5 min at 37°C. Rapid regeneration of the mitotic spindle is evident. × 29,000.


N
D
S
1
G
2
K
K
3

section, oblique section, or longitudinal section). The per cent of profiles containing centrioles was the same (6%) in both the control and Colcemid-treated groups; this value is approximately the ratio of the diameter of a sphere whose volume is equal to that of the centriole complex to the cell diameter (0.5 to 9.5 μ) and confirms our ability to find centriole sections in the profiles. However, the percentages of the various centriole orientations observed indicated a significant loss of oblique sections (5), suggesting that this particular orientation was more difficult to find or was occasionally misclassified as longitudinal.

Each centriole section was carefully examined for evidence of ciliary bud formation (as in Figs. 4 to 7) or for the presence of a ciliary shaft containing microtubules (as in Figs. 8 and 9). In the case of oblique or cross-sections the presence or absence of a ciliary sheath was used to distinguish between a centriole and a cilium. A centriole was considered to be functioning as a basal body when either a ciliary bud or a cilium was found attached. In Table I the data comparing control and Colcemid-treated cells are shown in the first two lines. Whereas only 4% of the control centrioles showed evidence of ciliogenesis, about 25% of the centrioles appeared to be functioning as basal bodies following Colcemid treatment. Since many of the centriole orientations prevented a clear diagnosis of ciliogenesis (i.e., cross- and oblique sections), the true rate of ciliogenesis is probably much higher. Of 14 longitudinal centriole sections in the Colcemid group, 6 showed ciliogenesis, whereas only 7 of 29 oblique sections and only 1 of 12 cross-sections were so diagnosed. Therefore, the true rate of ciliogenesis is about 40% following Colcemid treatment as opposed to about 5% in the control cells. Even though the total numbers of cases of ciliogenesis in the two groups of Table I are small, the difference observed between the control and Colcemid-treated groups is significant as determined by the chi square test ($P < 0.01$).

In Table I the data for the Colcemid treated cells are further divided into three groups, to show the development of the shaft of the cilium after reversal of the Colcemid inhibition. The data support our over-all experience that maturing cilia are found only after removing the cells from the Colcemid medium and growing them for a short period of time in conditioned medium. However, only about one-half of the ciliary buds mature following Colcemid reversal.

In these experiments no differences were found in the response of Don C and Dede cells to Colcemid treatment, so the data from both strains have been pooled throughout this report. Since the Don C strain is a cloned line in which all cells contain three chromosomal markers (11), it appears unlikely that the cells which form cilia were partially differentiated before treatment and therefore intrinsically different from the remaining population. We suspect that all cells are capable of cilia production, but the process occurs only during a specific period in their growth cycle.

DISCUSSION

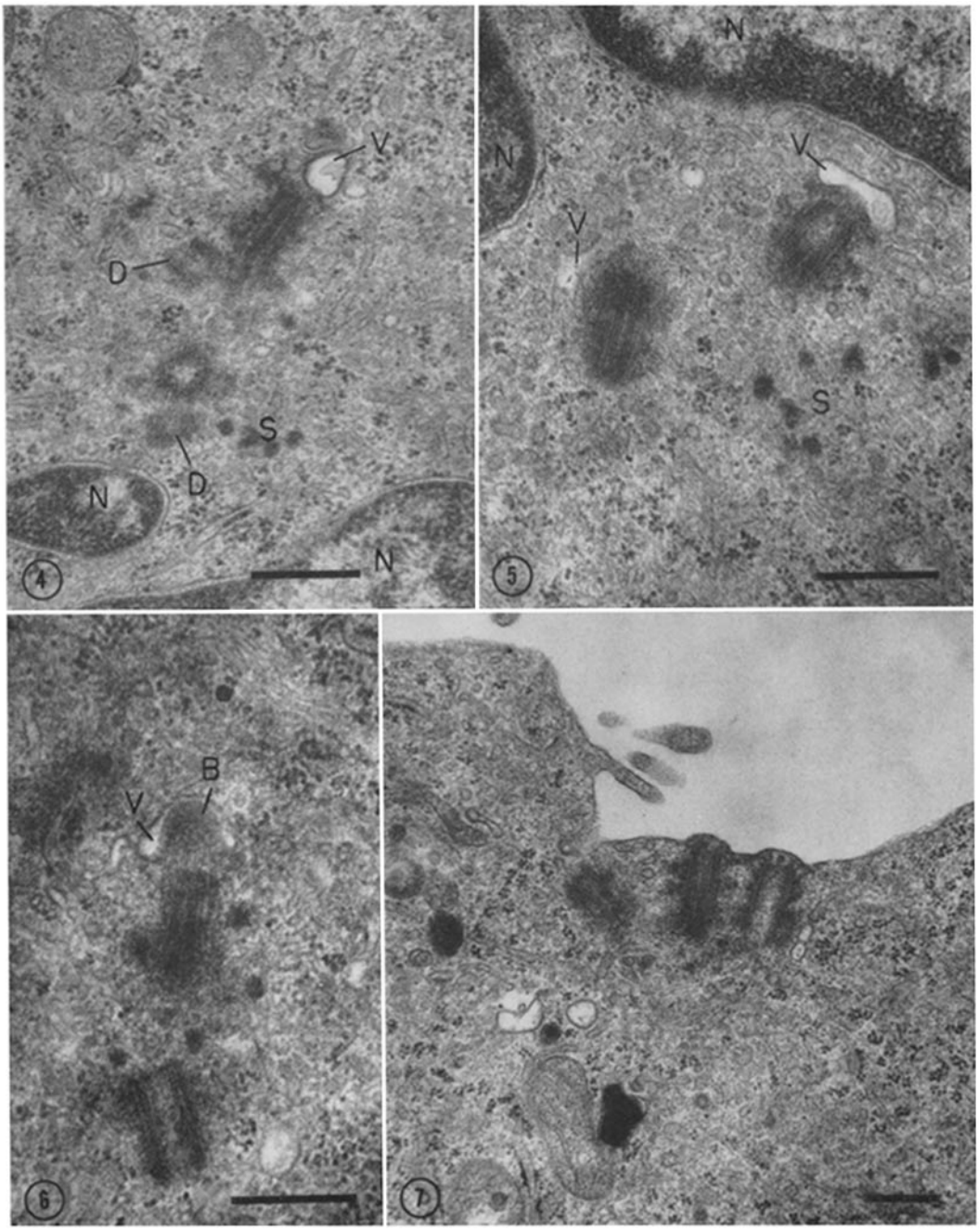
Colcemid and colchicine are well known for their inhibition of mitosis, although the molecular mech-

FIGURE 4 Centriole complex in Don C interphase cell treated with Colcemid. Each parent centriole, one in longitudinal and the other in transverse section, has a daughter centriole (*D*). One parent centriole is closely associated with a vesicle (*V*) at the end opposite to the daughter centriole. Colcemid treatment for 17 hr. × 31,000.

FIGURE 5 Oblique sections through a centriole pair, each associated with a vesicle which has flattened across the end of the centriole. Don C, treated with Colcemid for 17 hr. × 35,000.

FIGURE 6 Longitudinal sections through a centriole pair in female (Dede) Chinese hamster cell. Only one centriole is differentiated, and the typical right-angle orientation between the members of the pair has been modified. The ciliary bud (*B*) has been formed in an invagination of the vesicle (*V*). Colcemid treatment for 2 hr. × 36,000.

FIGURE 7 "Basal body" orientation of centrioles in a Don C cell treated with Colcemid for 17 hr. Cilium formation did not proceed in the presence of the drug. × 21,000.


V
D
S
D
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4
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7

anism of this effect is not yet understood. It appears that these drugs somehow block the formation of spindle tubules. In our material, spindle fibers persist in some Colcemid-treated cells, but they are not so frequently seen as in control cells (2). Upon removal of the drug, numerous spindle fibers reappear within a few minutes (Fig. 3); so it is probable that Colcemid merely blocks the assembly of spindle protein into fibers, rather than the actual synthesis of the protein subunits from amino acids. Therefore, it seems likely that in these cells the primary site of Colcemid action is in the centriole (perhaps also the kinetochore region of chromosomes). The effects of Colcemid on ciliogenesis support this concept.

Colcemid appears to promote an interaction between the centriole and vesicles of the Golgi complex, resulting in the establishment of a ciliary bud. The observation that further development of the cilium is inhibited by Colcemid suggests a homology between the components of the ciliary shaft and the mitotic spindle tubules. Removal of the drug resulted in the rapid regeneration of the mitotic spindle in metaphase cells and in the rapid formation of the ciliary shaft in interphase cells where the centriole–Golgi apparatus interaction had already occurred.

Inasmuch as the results demonstrate the formation of a specialized structure in the brief interval

TABLE I

Quantitation of the Occurrence of Ciliary Buds and Cilia following Colcemid Treatment

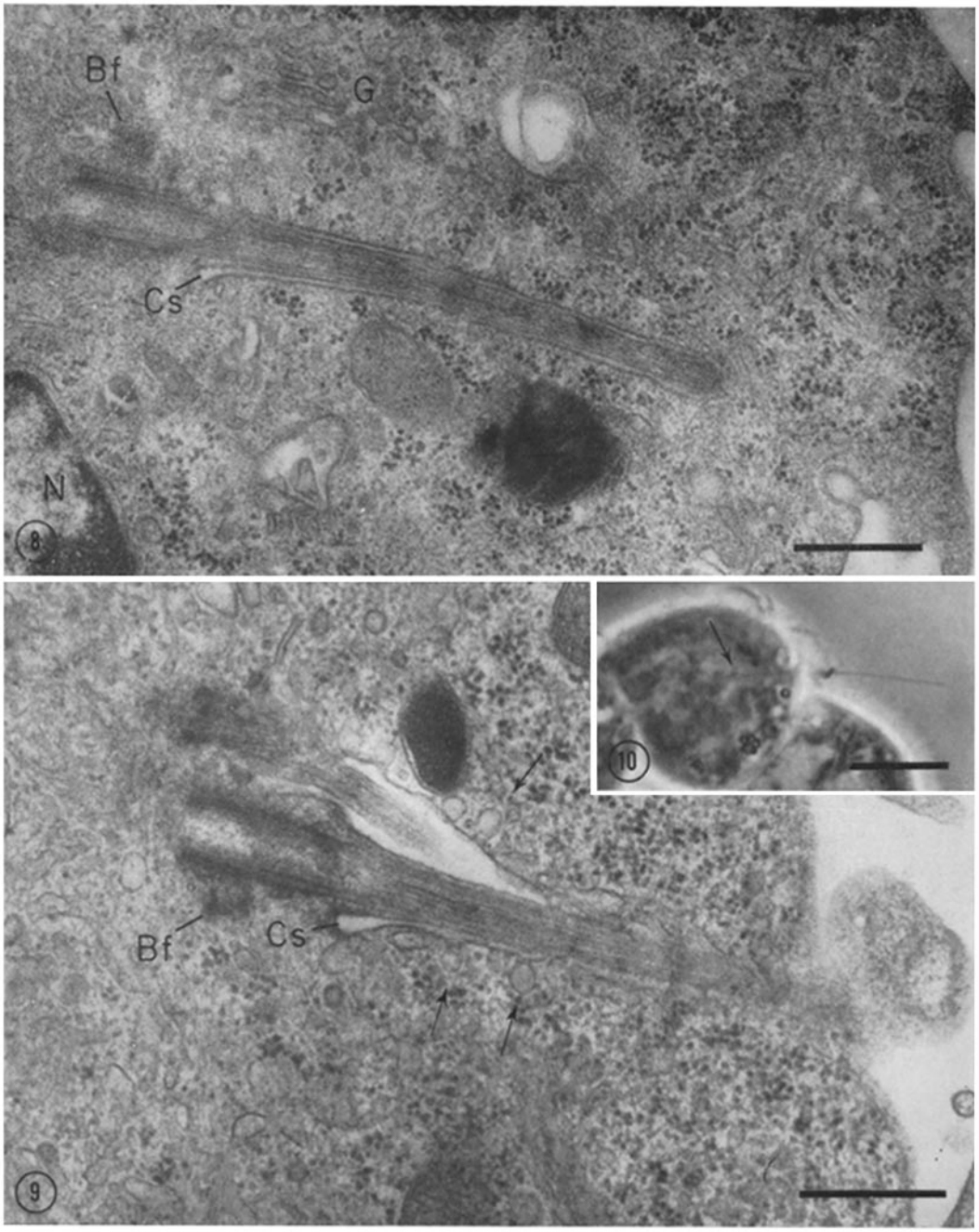
Treatment	Number of profiles examined	Total number of centrioles and basal bodies found	Number of cases with ciliary bud	Number of cases with ciliary shaft	Total number of basal bodies
Control	953	55	2	0	2*
Colcemid treated, all groups combined	940	55	9	5	14*
Colcemid, 2 hr	162	9	3	0	3
Colcemid, 2 hr, 0.5 hr reversal	182	13	2	1	3
Colcemid, 2 hr, 1.0 hr reversal	596	33	4	4	8

* Difference significant, $P < 0.01$. Calculated by the method of Yates to correct for discontinuity.

FIGURE 8 Longitudinal section of basal body and growing cilium in a Debe cell. This cell was treated with Colcemid for 2 hr and then incubated in medium omitting Colcemid for 30 min. The vesicle (*V*) in Figs. 4 to 6 expands to form the ciliary sheath (*Cs*), probably by the addition of vesicles from the nearby Golgi zone (*G*). The central elements of the cilium are visible in longitudinal section, and the basal foot (*Bf*) is seen forming near the basal body. × 37,000.

FIGURE 9 Longitudinal section of basal body and cilium in Don C cell treated with Colcemid for 2 hr and then incubated in medium lacking the drug for 1 additional hr. A second basal body and cilium were cut tangentially. The larger cilium now extends through the cell wall. Small vesicles appear to be fusing into the ciliary sheath (*Cs*) at the regions indicated by arrows. × 42,000.

FIGURE 10 Living Don C cell treated with Colcemid for 2 hr followed by 2 hr of incubation in the absence of Colcemid. The projection of the cilium through the cell wall is evident. The cilium extends several microns into the cell interior to the basal body located at the arrow. Such cilia were observed to beat erratically in some cells. Phase contrast. Magnification bar is 10 μ long. × 1400.


Bf
G
Cs
N
8
10
Bf
Cs
9

of 2 or 3 hr, we feel this must be interpreted as a specific ultrastructural differentiation induced by Colcemid treatment. Since the effect can be produced in vitro in a cloned line of fibroblast cells that can be readily synchronized (12), this material should be excellent for studies of the molecular relationships between the cell genome, the centriole, and Golgi apparatus during the various phases of the cell reproductive cycle. Such experiments are now in progress.

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