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Cilia in cell-cultured fibroblasts

II. Incidence in mitotic and post-mitotic BHK 21/C13 fibroblasts

F. L. ARCHER* AND D. N. WHEATLEY†

Department of Pathology, University Medical Buildings, Foresterhill, Aberdeen, Scotland

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INTRODUCTION

Numerous descriptions have appeared in recent years of the occurrence of cilia of the 9 + 0 pattern in a wide variety of cell types, but little is known concerning their development and fate within the cell, or of their functional significance. The investigations of Sorokin (1968) indicate that at least in one mammalian tissue, the fetal rat lung, primary (9 + 0) cilia differ in site of origin and mode of formation from motile (9 + 2) cilia. Since, as described by Sorokin, primary cilia are formed directly from 'reproductive' centrioles, obvious questions arise as to their fate during mitosis and their relationship to the mitotic apparatus. A previous study establishing the frequency of occurrence of primary cilia in cultured fibroblasts of a number of different types (Wheatley, 1969) offers an opportunity of adopting a more experimental approach to these questions. An established line of hamster fibroblasts, the BHK 21/C13 line of Stoker & Macpherson (1964), was chosen for further investigation because the cells have a consistently high incidence of cilia. The present communication reports our observations of the incidence and appearance of primary cilia in these cells during normal mitosis, in Colcemid-arrested mitosis, and in the subsequently formed interphase cells observed throughout 2 days recovery in Colcemid-free medium.

MATERIALS AND METHODS

Cell line and culture conditions

BHK 21/C13 fibroblasts originating from the kidney of a newborn Syrian hamster (Stoker & Macpherson, 1964) were regrown from stock which had been frozen about 40 generations from the original cloning. The cells were grown in Eagle's medium supplemented with 10% tryptose phosphate broth (Difco), 10% calf serum, and crystamycin (200 units/ml), in 4 oz. pharmacy bottles in a gas phase of 5% CO_2 in air. They grew rapidly in monolayer, with a mean doubling time in the exponential phase of about 12–14 h, and were subcultured at 3-day intervals.

* Present address: Department of Pathology, University of Chicago, Chicago, Illinois 60637, U.S.A.
† To whom reprint requests should be directed.

Collection of mitotic populations

BHK cells undergoing mitosis were collected by gentle mechanical agitation of flasks containing asynchronous, randomly growing populations after culture in a Ca^{2+}-free medium for 24 h (Terasima & Tolmach, 1963; Robbins & Marcus, 1964). This method yielded at best only up to 13 % mitotic cells because of the tenacious properties of BHK fibroblasts. For obtaining larger numbers of BHK cells in mitosis it was necessary to arrest cells in metaphase by exposure of log-phase cultures for 10–12 h to vinblastine sulphate (Velban, Eli Lilly & Co., Indianapolis, U.S.A.) 10^{-7}M, or Colcemid (Ciba, Horsham) 0·15 μg/ml, at which time they were collected for examination.

In preliminary experiments it was found that a modification of the method of Stubblefield & Klevecz (1965) was best for yielding large numbers of BHK cells arrested in metaphase and capable of recovery and renewed growth on subculture. Cells were incubated for 2 h in medium containing 0·45 μg/ml of Colcemid, then exposed briefly (30 s) to 0·2 % trypsin in balanced salt solution, followed immediately by gentle agitation and collection. The method consistently yielded 80–95 % metaphase cells. These were inoculated into 2 ml of fresh medium at a concentration of 2×10^5 cells/ml in a series of tightly stoppered test tubes, and incubated at 37 °C. The incidence of mitosis was estimated by counting 1000 cells which had been fixed in acetic-alcohol and stained with acetic–orcein. Growth rates were estimated by collecting and counting the cells in successive duplicate tubes at appropriate intervals; cells from the same tubes were fixed and embedded for electron microscopic examination.

For electron microscopic examination, cells from the culture flasks were centrifuged into pellets and fixed for 2 h in 6·0 % glutaraldehyde buffered at pH 7·2 with sodium cacodylate (0·1 M), followed by post-fixation in 1 % osmium tetroxide

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ABBREVIATIONS

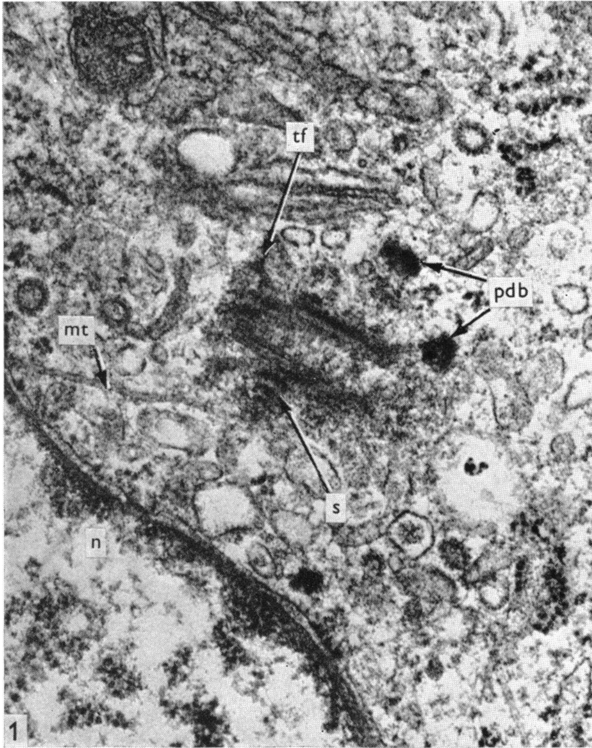
ce	centriole	mt	microtubule
ci	cilium	n	nucleus
d	daughter centriole	pdb	pericentriolar dense bodies
f	myofibrils	s	satellite arm
g	Golgi apparatus	tf	transitional fibres
m	mitochondrion	v	vesicle

Fig. 1. Centriole of a normal BHK fibroblast with a satellite arm. Transitional fibres seem to be present at the distal end but in the absence of a membrane component this centriole does not satisfy the criteria for basal bodies. Two pericentriolar dense bodies are present and a microtubule appears to run between the satellite arm and nuclear membrane. × 72 000.

Fig. 4. Early stage of basal body development in a cell incubated for 5 h after removal of Colcemid. The distal end of the centriole has become associated with a small vesicle. Satellite arms present. × 40 000.

Fig. 5. Ciliary bud (?) formed from one of a pair of centrioles. It could, however, be an oblique section of the base of a more fully differentiated ciliary structure. Note the relationship of the Golgi to the vesicle. × 46 000.

Fig. 6. Cilium in a paranuclear position. The basal body is undergoing replication at its proximal end and the daughter centriole is about two-thirds complete. × 27 000.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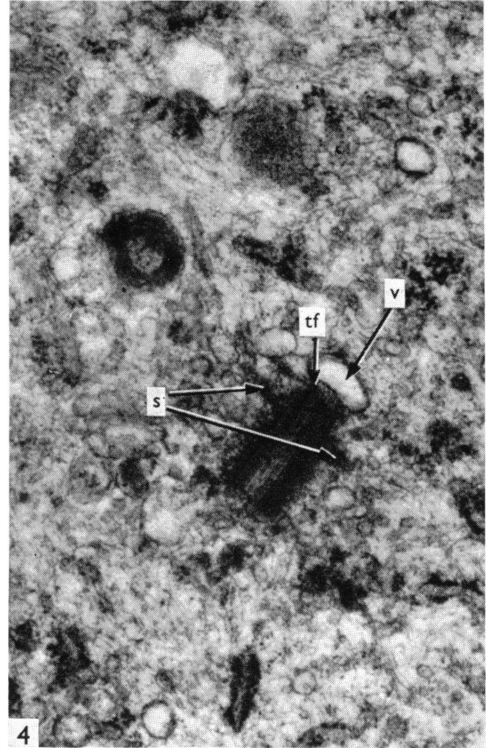
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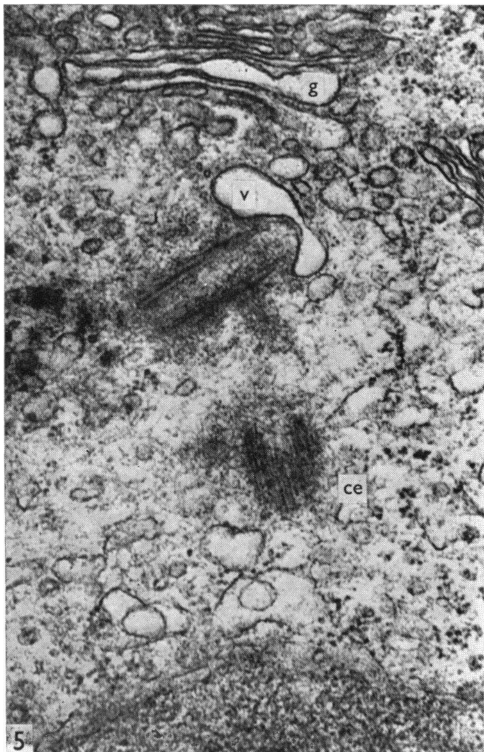
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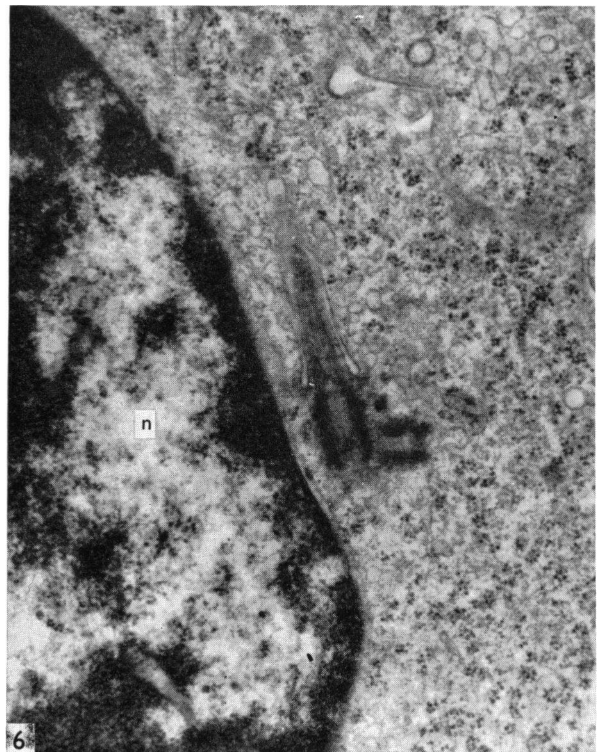
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at 4 °C (Sabatini, Bensch & Barrnett, 1963). The material was dehydrated in absolute ethanol and embedded in Epon 812 (Luft, 1961). Sections were cut with glass knives at 60–80 nm, mounted on uncoated copper grids and stained with 2% aqueous uranyl acetate and lead hydroxide (Karnovsky, 1961). They were examined with a Siemens Elmiskop I or a Phillips 200 electron microscope at 80 kV.

Counting of cilia and basal bodies

The method of quantitatively evaluating the incidence of cilia and basal bodies was the same as that used in the previous study (Wheatley, 1969). All cell profiles in a series of grids were systematically searched and the total number of *centriolar complexes* was recorded, a centriolar complex being defined as the centrosome region of the cytoplasm containing any number or combination of centrioles, basal bodies or cilia. The combined number of basal bodies and cilia found was compared to the total number of centriolar complexes, and this ratio designated as the *cilium index*. Each ratio expressed in the text or tables represents at least 100 centriolar complexes examined in the designated cell population. The method is based on the assumption that all basal bodies and cilia in this cell line are formed in relation to the centrioles and that this spatial relationship is maintained. It is unlikely that the occasional inclusion of more than one section through the same centriolar complex would introduce a significant error in the final ratio. The method eliminates the need for examining maximum diameters of cell profiles, etc., and we believe the accuracy is comparable to that of more complex statistical approaches (Stubblefield & Brinkley, 1966; Mullins & Wette, 1966).

The concept of the cilium index, as used here, also assumes that the significance of a basal body is the same as that of a cilium, in the sense that the basal body represents definitive differentiation toward cilium formation, whether or not a cilium would, in fact, have been formed. Therefore morphological criteria for basal bodies were strictly drawn, and required the presence of *transitional fibres* extending from the distal end of the centriole to the *basal rim* of thickened membrane at the near pole of an adjacent *vesicle*. The presence of satellite arms or basal feet, without the other structures, was not considered sufficient to identify a basal body (as in Fig. 1), since satellite arms have been seen in the centrioles of leucocytes of the blood (Fawcett, 1966), one of the few mammalian cell types in which primary cilia have not been observed. For a cilium to be scored, the presence of a shaft with filaments was required.

RESULTS

Cilia in randomly growing cultures of BHK 21/C13 fibroblasts

The incidence of cilia and basal bodies in normal BHK 21/C13 cells growing in random cultures remained at approximately one in every three centriolar complexes observed (Table 1). This is a lower incidence than that found in samples of the same cell line studied previously (Wheatley, 1969), and is probably due to many factors such as different observers, prolonged BHK cell culture, more rigid criteria for basal bodies, etc. Moreover, after approximately 100 consecutive passages of these cells, made during the 1-year period over which the present study extended, the incidence

remained the same, indicating that cilia are a regular ultrastructural feature of interphase cells of this line.

Cilia in mitotic BHK 21/C13 cells

By contrast, no cilia were seen in cells in mitosis, either in untreated BHK cells or in cells arrested chemically in mitosis. A total of 224 centriolar complexes was studied in mitotic cells collected by all methods, without finding a cilium. Full details are given in Table 1. Five basal bodies seen in mechanically collected mitotic cells were found in very late telophase stages, suggesting that under these conditions the onset of ciliogenesis occurs as the cells complete mitosis.

Table 1. *Comparative incidence of cilia and basal bodies in normal interphase cells and in mitotic cells of the BHK 21/C13 line*

	Total centriolar complexes	No. of cilia, with or without basal bodies	No. of basal bodies only	Cilia + basal bodies	Ratio of: cilia + basal bodies/ total complexes
Normal interphase cells (from Wheatley, 1969)	102	37	11	48	0·47
Normal interphase cells	320	45	51	96	0·30
Totals – interphase cells	422	82	62	144	0·34
Normal mitotic cells	72	0	5*	5	0·07
Vinblastine-arrested mitotic cells	86	0	0	0	—
Colcemid-arrested mitotic cells	66	0	0	0	—
Totals – mitotic cells	224	0	5	5	0·02**

* All 5 cells were in the telophase stage of mitosis.
** Significance of difference from normal interphase cells: $P < 0{\cdot}0001$.

Recovery of Colcemid-arrested mitotic cells (Cm)

When cells arrested in mitosis by 2 h exposure to Colcemid were collected by the method described and inoculated into fresh medium at a concentration of 2×10^5 cells/ml, about 50 % of the cells completed mitosis by the end of 30 min incubation, and by 1 h all viable cells had entered interphase and had become firmly attached to the glass surface. Spreading of cell cytoplasm and formation of cytoplasmic processes were well advanced by 5–7 h. After completion of the initial division mitotic activity was absent until after 9 h, when the mitotic index began to rise to several times the normal levels for BHK cells at 12 h (Fig. 2). Thus the cells complete the initial mitosis synchronously but the absence of a subsequent high peak of mitosis indicates a rather rapid loss of synchrony by the end of the first 12 h cycle. Cell counts from a series of cultures of Cm cells during the first 48 h after inoculation into fresh medium reveal that subsequent proliferation follows a complex pattern, at a considerably slower rate than normal BHK cells. The comparison is illustrated in Fig. 3.

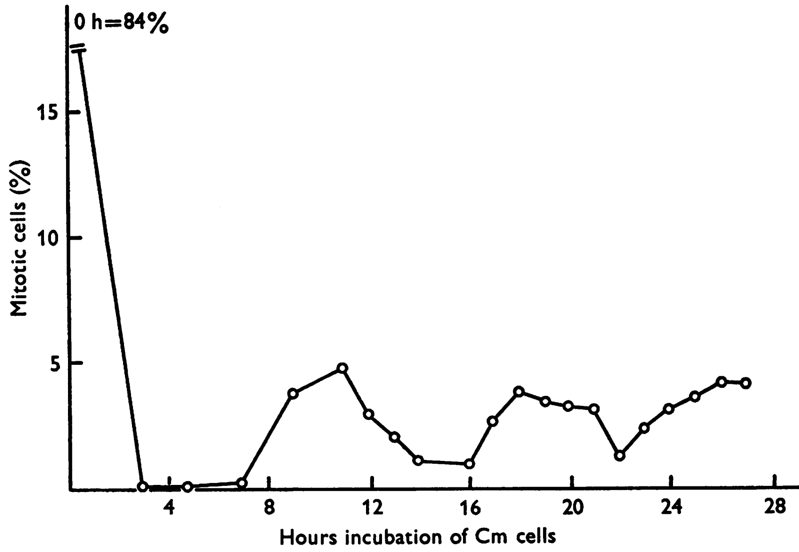


Fig. 2. Mitotic activity of Cm cells inoculated into fresh medium.

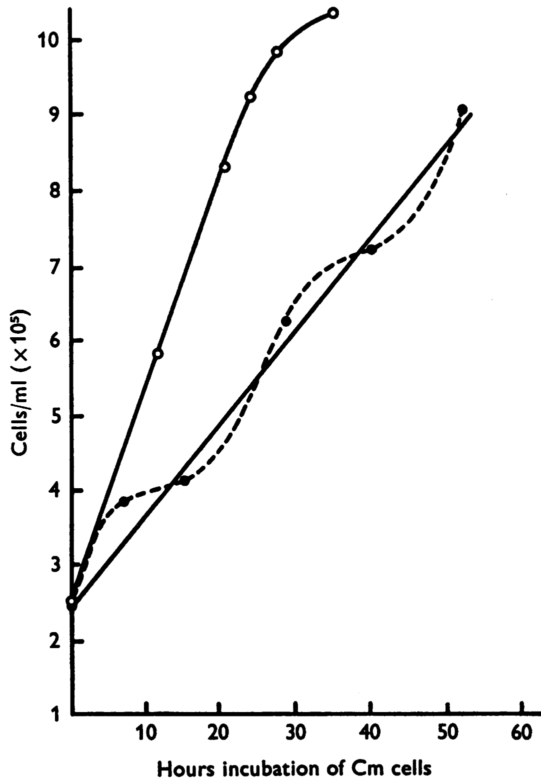


Fig. 3. Growth of Cm compared with control BHK cells following inoculation into fresh medium. ●——● Cm, ○——○ control.

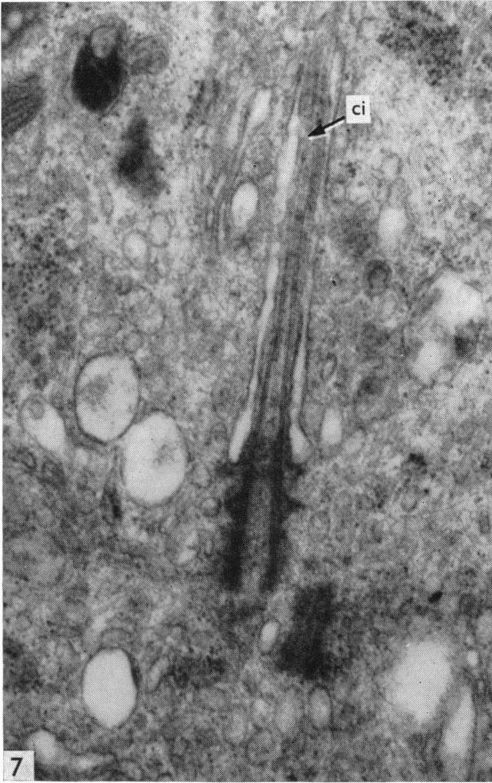
Reappearance of cilia in recovering cells

Following inoculation of cells into fresh medium after the Colcemid collection technique, a succession of cultures were trypsinized and fixed for electron-microscopic examination at intervals of 1, 3, 5, 7, 9, 11, 33 and 45 h. During the first 5 h of post-Colcemid incubation the cilium index was very low, only 5 % of the centriolar complexes of interphase cells containing cilia or basal bodies, compared to the 0·3 index for normal BHK interphase cells. During subsequent incubation there was progressive recovery of the ciliated condition, but even at 33 to 45 h (i.e. after about 2 generation cycles at the slower growth rate) the normal incidence of cilia was not fully restored, reaching an index of only 0·17 to 0·18 (Table 2).

Table 2. *Cilia and basal body incidence related to total centriolar complexes seen in normal and post-mitotic interphase BHK 21/C13 fibroblasts recovering from Colemid synchronization*

Time (h) after inoculation of Cm cells	Total no. centriolar complexes observed	No. of cilia	No. of basal bodies	Total no. of cilia and basal bodies	Ratio of: total cilia + basal bodies/ total centriolar complexes
0	76	0	0	0	0·0
1	236	4	5	9	0·017
3	232	0	11	11	0·047
5	185	2	7	9	0·049
7	123	7	4	11	0·090
9	122	3	6	9	0·074
11	219	13	9	22	0·100
15	142	7	13	20	0·141
33	161	20	9	29	0·180
45	151	11	14	25	0·166
Normal interphase BHK cells	320	45	51	96	0·300

Except for their relative numbers, most cilia seen in post-mitotic interphase cells arising from Colcemid-treated cells did not differ recognizably from those seen in normal interphase BHK cells. All stages in apparent ciliary development were seen. The earliest recognizable stage was the basal body, in which transitional fibres could be identified extending from one end of a centriole to a thickened basal rim at one pole of an adjacent vesicle (Fig. 4). The vesicle was bound by a single unit membrane and was frequently associated with the larger vesicular components of the adjacent Golgi apparatus. In other examples of 'early' forms the basal body fibrils extended into a small projection or ciliary bud which protruded into the lumen of the enlarged vesicle (Fig. 5). In still other forms the bud and its enclosing vesicle were elongated, and approached the shape and appearance of the fully formed ciliary shaft. The great majority of basal bodies and ciliary forms originated deep in the cell cytoplasm close to the nucleus, and the cilia appeared to remain largely isolated within the cell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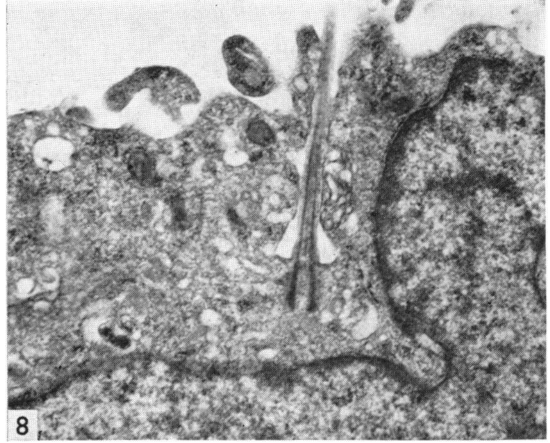
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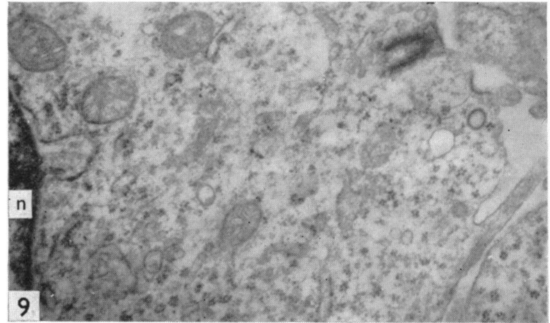
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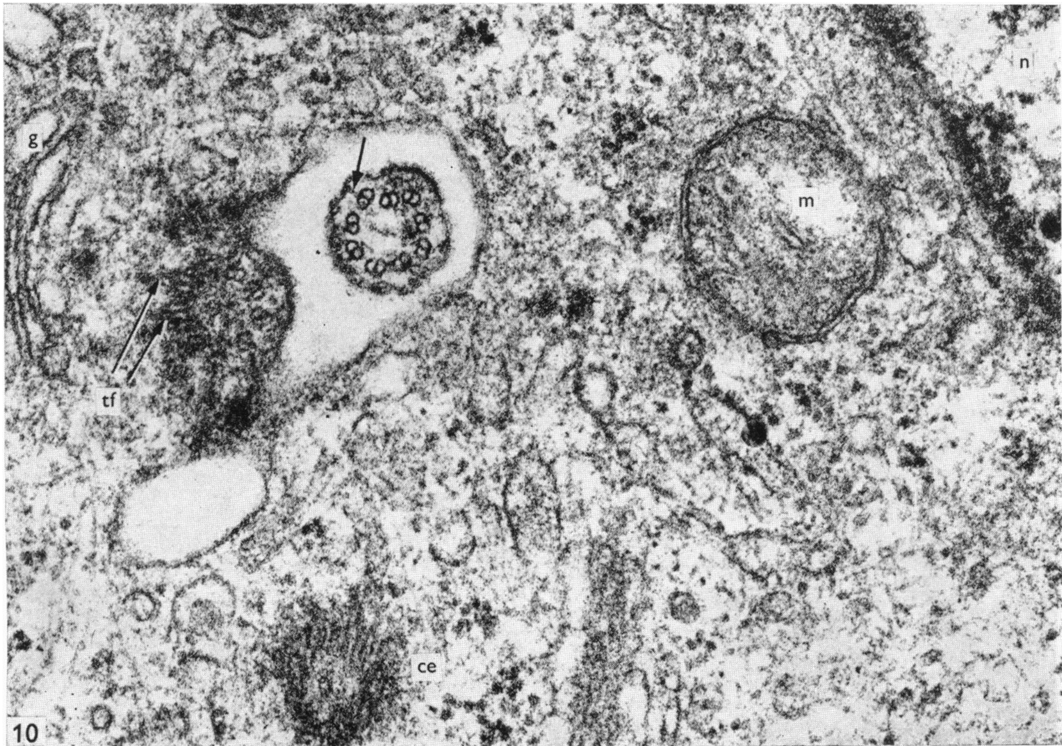
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(Figs. 6, 7), but in some instances the vesicle formed a channel which completed communication with the extracellular space (Fig. 8). It remains possible, though not demonstrated in our material, that serial sections would have shown similar invaginations of the plasma membrane around most mature cilia, although this is less likely in the case of the early ciliary buds. In rare instances cilia originated from basal bodies located peripherally, in apposition to an uninvaginated cell membrane (Fig. 9). In all of the transverse sections which were encountered through ciliary shafts, the 9+0 pattern of fibres was evident (Fig. 10). Several cilia in Colcemid-treated cells had ciliary shafts which were expanded to about twice the usual diameter, and contained distinct transverse striations with about 10 nm periodicity (Figs. 11, 12). The significance of the striation, which is almost certainly not due to sectioning artefacts, is not understood.

Pericentriolar dense bodies, which were inconspicuous in the majority of normal BHK cells and in the early interphase of recovering Cm cells, were especially numerous and conspicuous in many of the latter cells after 9 h incubation (Fig. 13). They probably represent precursor material involved in the formation of cilia as described by Lin & Chen (1969).

Morphologic features of Colcemid-treated BHK cells

Many Colcemid-treated cells, both mitotic and daughter interphase cells, displayed structural abnormalities, affecting principally the nucleus. In cells in metaphase there was displacement of centrioles towards the centre of chromosome aggregates, in contrast to their peripheral location at the spindle poles in normal metaphase BHK cells (Figs. 14, 15). The displacement was similar to that noted by Brinkley & Stubblefield (1967) in Colcemid-arrested metaphase cells. It was also a regular feature of vinblastine-arrested metaphases.

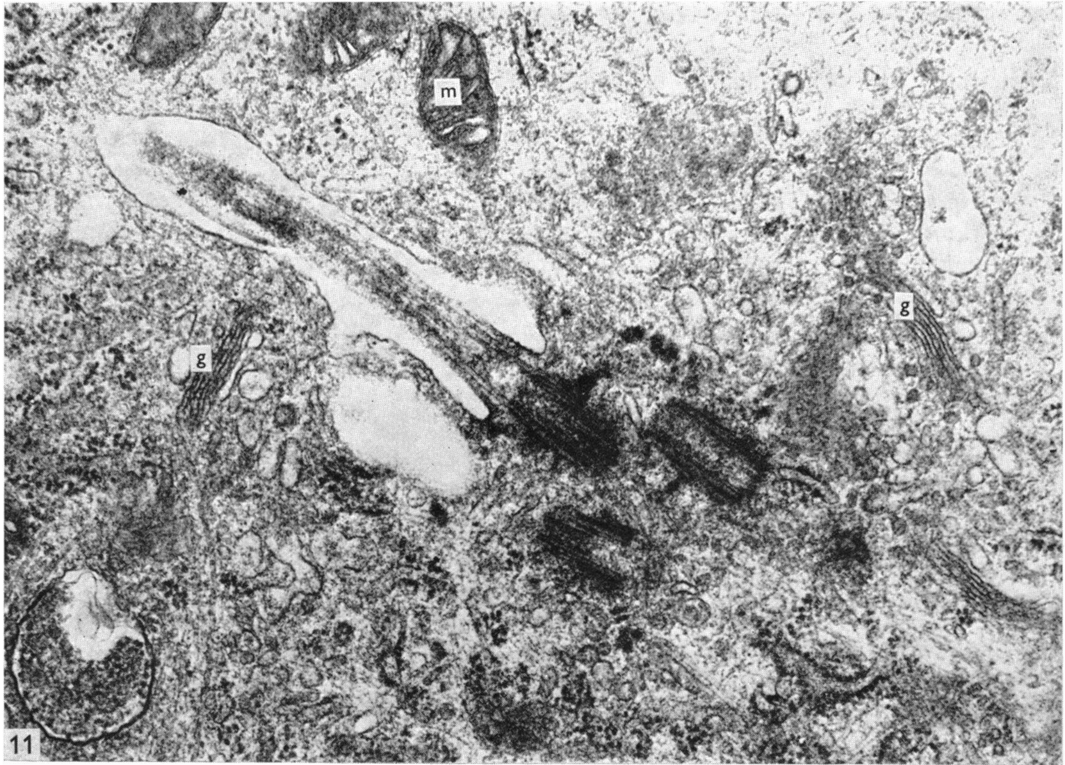
A hitherto unreported observation, however, was the presence in several daughter cells of centrioles *within* reformed interphase nuclei (Fig. 16). In addition, many telophase and interphase cells contained fragmented or convoluted nuclei, as well as massive accumulations of cytoplasm in which were many autophagic lysosomes and increased numbers of smaller than average mitochondria.

Fig. 7. Well-developed cilium deep within the cytoplasm of an interphase BHK 21/C13 cell 45 h after recovery from Colcemid collection. Ciliary fibres are clearly differentiated. × 30000.

Fig. 8. Cilium arising from basal body deep in the cytoplasm of a recovered Cm BHK cell and protruding into the medium. × 16 000.

Fig. 9. Basal body in a rare location at the plasma membrane. No obvious ciliary shaft development can be seen in this plane of section. × 42000.

Fig. 10. Centriolar complex in what is probably a biciliate BHK cell. One ciliary shaft is cut transversely showing the 9+0 fibre pattern, slight displacement of one fibre pair towards the centre and linkage of the fibre pairs to the outer membrane (arrow). The other cilium has been sectioned at the transition between basal body and shaft. It shows the radiating transitional fibres. Another centriole is close by. × 72000.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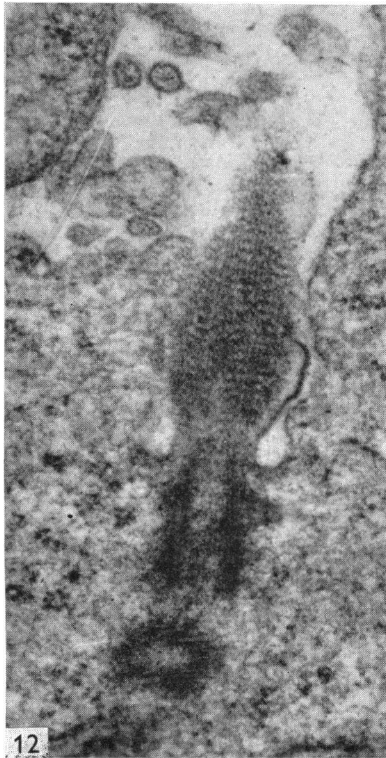
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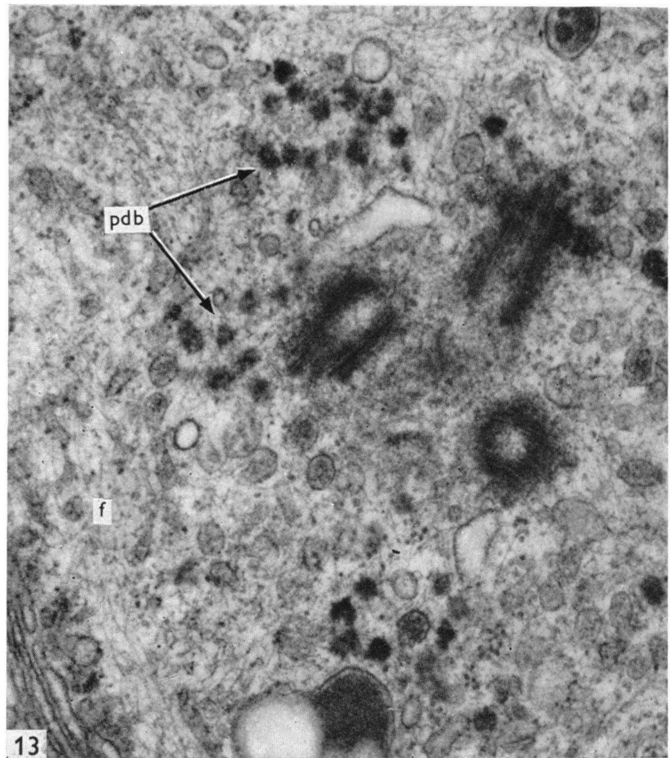
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DISCUSSION

The absence of cilia during mitosis

The absence of cilia or basal bodies in BHK 21/C13 cells in mitosis confirms an impression gained from a considerable experience of primary mammalian cell cultures, established cell lines and a number of mammalian tissues (Currie & Wheatley, 1966; Wheatley, 1967*a*, *b*, 1969; Wheatley & Archer, unpublished observations), as well as the findings of others, either from their general experience (Boquist, 1968; Dingemans, 1969; Rash, Shay & Biesele, 1969) or by analysis of mitotic cells (Stubblefield & Brinkley, 1966). The absence of cilia in mechanically collected mitotic cells as well as in Cm cells excludes the possibility that anti-mitotic agents themselves were the cause of the disappearance of pre-existing cilia.

These observations suggest that the *disappearance of primary cilia from cells entering mitosis is a general phenomenon of mammalian cells.* It is not certain, however, that absence of primary cilia from mitotic cells is a feature which extends to cells of other phyla since Roth, Wilson & Chakraborty (1966) have illustrated the occurrence of ill-formed ciliary-like appendages of the centrioles in a dividing insect spermatocyte.

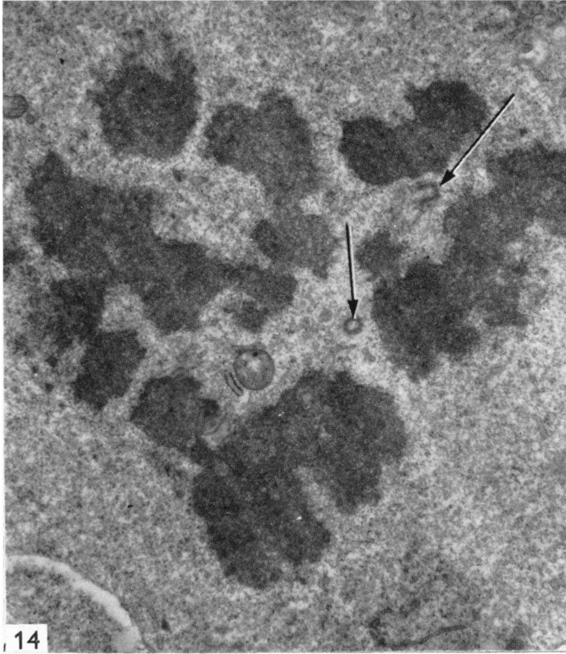
The data obtained here are insufficient to establish with certainty the exact time of disappearance of cilia in the cell cycle. But since cilia were several times seen in centriolar complexes comprised of two sets of mature centrioles – a situation which, according to the studies of Robbins *et al.* (1968) in HeLa cells, does not obtain until late S phase or the beginning of the G_2 phase – and since cilia were never observed in any BHK cells which had reached middle or late prophase, it can be deduced that cilia are lost during G_2 or early prophase of mitosis. This deduction rests on the assumption that the findings of Robbins *et al.* (1968) also apply to BHK 21/C13 cells, for detailed studies of time relationships of centriolar replication during the cell cycle are not available for mammalian cell lines other than HeLa.

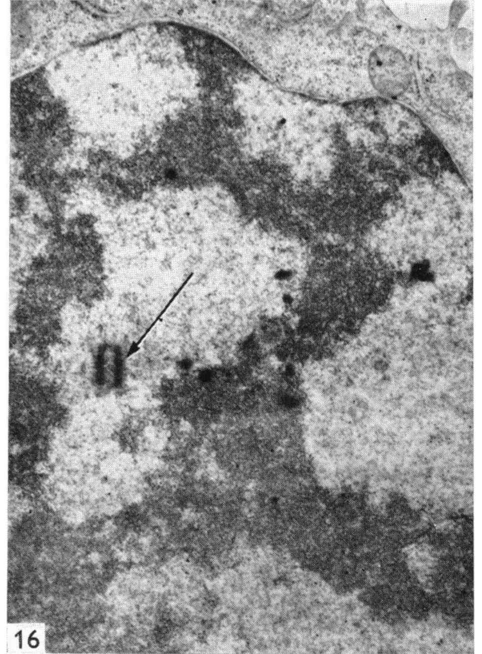
The significance of the loss of cilia at this stage is problematic. It seems unlikely that primary cilia contribute significantly to the organization or structure of the spindle apparatus of the mitotic cell, since cells which are devoid of primary cilia readily undergo mitosis; for example, L-929 fibroblasts (Wheatley, 1969), liver cells after partial hepatectomy (Wheatley, 1968), HeLa cells (Wheatley, unpublished; Robbins, Jentzsch & Micali, 1968), and ML-2 epithelial cells (Wheatley & Archer, unpublished). Some authors (Boquist, 1968; Pehlemann, 1968) have suggested that the resorption of cilia may provide extra reserves of microtubule protein for mitosis. Although resorption is the more probable fate of a cilium we are unable to determine from our studies whether loss of cilia occurs by resorption or by shedding.

Fig. 11. Centriolar complex and cilium in a recovered Cm cell. A few pericentriolar dense bodies are present. The ciliary shaft appears slightly dilated distally and has suggestions of cross striations. × 39000.

Fig. 12. More obvious striation can be seen in this thicker section of a short dilated ciliary shaft. Colcemid-collected BHK cell after 5 h incubation in fresh medium. × 42000.

Fig. 13. Prominent pericentriolar dense bodies in a centriolar complex. Fine myofibrils form a meshwork in the cytoplasmic matrix. No evidence of basal body formation but several vesicles lie near the centrioles. 33 h recovery after Colcemid collection. × 33000





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The reappearance of cilia in Colcemid-treated cells

The low incidence of basal bodies and ciliary shafts in Colcemid-treated cells during the first few hours following completion of mitosis is open to several possible interpretations. The finding of basal bodies in several post-Colcemid cells in telophase indicates that at least some of the recovering cells are able to begin ciliogenesis immediately, and it is known from studies of other organisms (e.g. *Naegleria*) that the complete process of cilium or flagellum formation may take place in a matter of minutes (Dingle & Fulton, 1966). However, during the first 7 h of incubation the number of cilia and basal bodies present in the incubated cells is not greater than might be expected from contamination of the original inoculum with interphase cells containing cilia in the usual proportion.

The most obvious factor which may cause the failure to form cilia during these first few hours is the continuing influence of Colcemid. It is well known that colchicine binds to microtubule protein (Borisy & Taylor, 1967), and that after equilibrium has been reached binding can persist for some hours. Although colchicine has no discernible effect on pre-existing ciliary structures (Tilney & Gibbins, 1968), regeneration of cilia involves polymerization of microtubule protein, and this process is strongly inhibited by colchicine (Rosenbaum & Carlson, 1969). Our findings probably represent the efforts of the cell to regenerate cilia in the presence of a declining intracellular concentration of bound Colcemid. This hypothesis requires the assumption that ciliary microtubule assembly is more sensitive to Colcemid than spindle microtubule assembly, since the incubated Cm cells were able to complete the formation of the achromatic figure and the initial mitosis. However, the displacement of centrioles during the metaphase stage and their subsequent entrapment within interphase nuclei are evidence that in many cells the spindle apparatus does not function normally under these experimental conditions. Cells may have re-entered interphase by devious methods.

The main factor responsible for the late appearance of cilia in incubated Cm cells is probably the poor recovery of the cell population, which must contain large numbers of damaged or dying cells, blocked cells failing to recycle, and deranged cells in which recycling occurs at slower and varying rates. Nevertheless, the increase in cell number with time indicates a substantial population of viable cells in which the cilium index rises towards normal interphase levels.

Another view of the significance of the appearance of cilia after Colcemid exposure was proposed by Stubblefield & Brinkley (1966). They reported that cilia were absent from the Don/C strain of hamster fibroblasts, but noted the formation of cilia following exposure to Colcemid, and interpreted this as an induction of cilia by prior

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Fig. 14. 2 centrioles (arrows) misplaced among 'metaphase' chromosomes of a Colcemid-arrested mitotic BHK fibroblast. × 12000.

Fig. 15. Normal metaphase – early anaphase arrangement of chromosomes in a BHK cell in mitosis 33 h after recovery from Colcemid collection. However, there appear to be extra centrioles toward the right-hand pole (arrow). × 13500

Fig. 16. Centriole within a reformed nucleus 11 h incubation after collection of BHK cell in Colcemid. × 14000.

exposure to the drug. A comparison of their results with those in the present study of BHK cells is of particular interest and importance. There is agreement that cilia are absent from mitotic cells of both cell lines, and that following incubation in fresh medium, Colcemid-arrested mitotic cells enter interphase synchronously, with basal bodies appearing very early and ciliary shafts following. Cilia appeared much earlier in the post-mitotic Don/C cells (30–60 min), possibly because the cells recover from the lower dose of Colcemid (0·06 μg/ml) more rapidly, as evidenced by the excellent synchronization which can be obtained with these cells. In another study of Don/C cells (Wheatley, 1969) cilia were found to be a regular feature of interphase cells, in contrast to the findings of Stubblefield & Brinkley. Moreover, the post-reversal ratios which Stubblefield & Brinkley gave – total basal bodies/total basal bodies + centrioles (0·25), and the total cilia/total basal bodies + centrioles (0·12) – agree closely with the corresponding ratios of 0·30 and 0·12 respectively, independently estimated for interphase cells of the same line by Wheatley (1969); the smaller numbers in the former study do not permit statistical verification of this point. The data of Stubblefield & Brinkley are more readily interpreted as showing the post-mitotic recovery of cilia by Don/C cells to the normal interphase incidence. For reasons already discussed, this interpretation is the more plausible since exposure to Colcemid would be expected to suppress rather than induce ciliogenesis.

Morphological aspects of ciliogenesis

Although our observations on the appearance of 'immature' cilia during the various stages of formation agree in general with descriptions found elsewhere (Sorokin, 1962, 1968; Martinez & Daems, 1968), we would re-emphasize the caution expressed by others (e.g. Rash *et al.* 1969) that inferences about ciliogenesis derived from various forms seen in random sections of centriolar apparatus may not give an accurate representation of the real situation. More systematic analysis by serial sectioning of properly staged material is necessary to resolve the pattern of ciliogenesis for primary cilia.

Further comments

A number of reports have implicated primary cilia in the regulation of mitotic frequency in cell populations, especially during differentiation (Wilson & McWhorter, 1963; Boquist, 1968; Dingemans, 1969; Rash *et al.* 1969); our results to date have not provided any definite evidence in favour of this hypothesis. Primary cilia develop in rapidly dividing cells as well as in highly differentiated cells with little or no mitotic activity (e.g. neurons). In the individual cell in culture, the presence or absence of a cilium seems to make no difference to the mitotic frequency itself or its potential. Cultured L-929 fibroblasts, which have no cilia (Wheatley, 1969), divide as rapidly as BHK 21/C13 cells which have a relatively high cilium index. *In vivo*, hepatocytes can readily be induced to divide by partial resection of the liver, and neither the mature cell type nor the regenerating hepatocyte develops cilia (Wheatley, 1968). However, in the organized tissue *in vivo*, where cell proliferation is under many different restraining influences and regulatory mechanisms are more subtle, primary cilia may play some part. We are at present giving attention to the factors which control the expression of primary cilia, especially during the period of post-mitotic

regeneration, and to the correlation of cilium index with mitotic index in cultured cell systems.

SUMMARY AND CONCLUSIONS

In baby hamster kidney fibroblasts of the strain BHK 21/C13 grown in monolayer culture, basal bodies and primary (or '9+0') cilia were evident in approximately one in every 3 centriolar complexes detected by electron-microscopic examination of random sections. This incidence was not affected by prolonged storage, nor by repeated subculture of cells over a 12- to 18-month period. No cilia were found among 224 centriolar complexes seen in cells in the metaphase–anaphase period of mitosis, including both mechanically collected BHK mitotic cells and those arrested in mitosis by Colcemid or vinblastine. However, a small percentage of late telophase cells appeared to possess basal bodies. It was deduced that cilia must be lost sometime after S phase has been completed and before midprophase has been reached, and that they can begin reappearing soon after mitosis is complete.

Cells arrested in mitosis by 2 h exposure to low concentrations of Colcemid completed mitosis synchronously and continued to proliferate when returned to fresh medium, though at a considerably slower rate than normal BHK cells. Although a few basal bodies were seen in telophase cells during the first 5 h following completion of mitosis, the incidence of cilia and basal bodies in interphase cells was very low. It began to rise at 9 h and reached approximately 60% of the normal incidence by 33 h. It is suggested that the appearance of cilia after a lag period following Colcemid exposure represents the efforts of the cells to regenerate cilia in the face of a declining intracellular concentration of Colcemid.

Several examples of an unusual form of the cilium were observed, characterized by distinct transverse striations of a dilated ciliary shaft. Forms suggesting a progression from basal bodies through to mature cilia were seen, but it remains uncertain to what extent these accurately represent real stages in cilium maturation.

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REFERENCES

Boquist, L. (1968). Cilia in normal and regenerating islet tissue. An ultrastructural study in the Chinese hamster with particular reference to the β-cells and the ductular epithelium. *Z. Zellforsch. mikrosk. Anat.* **89**, 519–532.

Borisy, G. G. & Taylor, E. W. (1967). The mechanism of action of colchicine. Binding of colchicine-^{3}H to cellular protein. *J. Cell Biol.* **37**, 525–533.

Brinkley, B. R. & Stubblefield, E. (1967). The effects of colcemid inhibition and reversal on the fine structure of the mitotic apparatus of Chinese hamster cells *in vitro*. *J. Ultrastruct. Res.* **19**, 1–18.

Currie, A. R. & Wheatley, D. N. (1966). Cilia of a distinctive (9+0) structure in endocrine and other tissues. *Postgrad. med. J.* **42**, 403–408.

Dingemans, K. P. (1969). The relation between cilia and mitoses in the mouse adenohypophysis. *J. Cell Biol.* **43**, 361–367.

Dingle, A. D. & Fulton, C. (1966). Development of the flagellar apparatus of *Naegleria*. *J. Cell Biol.* **31**, 43–54.

Fawcett, D. (1966). *The Cell: Its Organelles and Inclusions*, p. 53. Philadelphia: Saunders.

Karnovsky, M. J. (1961). Simple methods for 'staining with lead' at high pH in electron microscopy. *J. biophys. biochem. Cytol.* **11**, 729–732.

Lin, H-S. & Chen, I-L. (1969). Development of the ciliary complex and microtubules in the cells of rat subcommissural organ. *Z. Zellforsch. mikrosk. Anat.* **96**, 186–205.

Luft, J. H. (1961). Improvements in epoxy resin embedding methods. *J. biophys. biochem. Cytol.* **9**, 409–414.

Martinez, P. & Daems, W. (1968). Les phases précoces de la formation des cils et le problème de l'origine du corpuscule basal. *Z. Zellforsch. mikrosk. Anat.* **87**, 46–68.

Mullins, R. & Wette, R. (1966). On the statistical expectation and evaluation of centriole orientations in cell profiles. *J. Cell Biol.* **30**, 652–654.

Pehlemann, F. W. (1968). Die amitotische Zellteilung. Eine electronenmikroskopische Untersuchung an Interrenalzellen von *Rana temporaria*. *Z. Zellforsch. mikrosk. Anat.* **84**, 516–548.

Rash, J. E., Shay, J. W. & Biesele, J. J. (1969). Cilia in cardiac differentiation. *J. Ultrastruct. Res.* **29**, 470–484.

Robbins, E., Jentzsch, G. & Micali, A. (1968). The centriole cycle in synchronized HeLa cells. *J. Cell Biol.* **36**, 329–339.

Robbins, E. & Marcus, P. (1964). Mitotically synchronized mammalian cells: a simple method for obtaining large populations. *Science, N.Y.* **144**, 1152–1153.

Rosenbaum, J. L. & Carlson, K. (1969). Cilia regeneration in *Tetrahymena* and its inhibition by colchicine. *J. Cell Biol.* **40**, 415–425.

Roth, L. E., Wilson, H. J. & Chakraborty, J. (1966). Anaphase structure in mitotic cells typified by spindle elongation. *J. Ultrastruct. Res.* **14**, 460–483.

Sabatini, D. D., Bensch, K. G. & Barrnett, R. J. (1963). Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. *J. Cell Biol.* **17**, 19–58.

Sorokin, S. (1962). Centrioles and the formation of rudimentary cilia by fibroblasts and smooth muscle cells. *J. Cell Biol.* **15**, 363–377.

Sorokin, S. (1968). Reconstructions of centriole formation and ciliogenesis in mammalian lungs. *J. Cell Sci.* **3**, 207–233.

Stoker, M. & MacPherson, I. A. (1964). Syrian hamster fibroblast cell line BHK 21 and its derivatives. *Nature, Lond.* **203**, 1355–1357.

Stubblefield, E. & Brinkley, B. R. (1966). Cilia formation in Chinese hamster fibroblasts *in vitro* as a response to colcemid treatment. *J. Cell Biol.* **30**, 645–652.

Stubblefield, E. & Klevecz, R. (1965). Synchronization of Chinese hamster cells by reversal of colcemid inhibition. *Exp. Cell Res.* **40**, 660–664.

Terasima, T. & Tolmach, L. J. (1963). Growth and nucleic acid synthesis in synchronously dividing populations of HeLa cells. *Exp. Cell Res.* **30**, 344–362.

Tilney, L. G. & Gibbins, J. R. (1968). Differential effects of antimitotic agents on the stability and behaviour of cytoplasmic and ciliary microtubules. *Protoplasma* **65**, 167–179.

Wheatley, D. N. (1967*a*). Cilia and centrioles in the rat adrenal cortex. *J. Anat.* **101**, 223–237.

Wheatley, D. N. (1967*b*). Cells with two cilia in the rat adenohypophysis. *J. Anat.* **101**, 479–485.

Wheatley, D. N. (1968). Centrioles in hepatocytes. *Experientia* **24**, 1157–1159.

Wheatley, D. N. (1969). Cilia in cell-cultured fibroblasts. I. On their occurrence and relative frequencies in primary cultures and established cell lines. *J. Anat.* **105**, 351–362.

Wilson, R. B. & McWhorter, C. A. (1963). Isolated flagella in human skin. Electron microscopic observations. *Lab. Invest.* **12**, 242–249.