CENTRIOLES AND THE FORMATION OF RUDIMENTARY CILIA BY FIBROBLASTS AND SMOOTH MUSCLE CELLS

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ABSTRACT

Cells from a variety of sources, principally differentiating fibroblasts and smooth muscle cells from neonatal chicken and mammalian tissues and from organ cultures of chicken duodenum, were used as materials for an electron microscopic study on the formation of rudimentary cilia. Among the differentiating tissues many cells possessed a short, solitary cilium, which projected from one of the cell's pair of centrioles. Many stages evidently intermediate in the fashioning of cilium from centriole were encountered and furnished the evidence from which a reconstruction of ciliogenesis was attempted. The whole process may be divided into three phases. At first a solitary vesicle appears at one end of a centriole. The ciliary bud grows out from the same end of the centriole and invaginates the sac, which then becomes the temporary ciliary sheath. During the second phase the bud lengthens into a shaft, while the sheath enlarges to contain it. Enlargement of the sheath is effected by the repeated appearance of secondary vesicles nearby and their fusion with the sheath. Shaft and sheath reach the surface of the cell, where the sheath fuses with the plasma membrane during the third phase. Up to this point, formation of cilia follows the classical descriptions in outline. Subsequently, internal development of the shaft makes the rudimentary cilia of the investigated material more like certain non-motile centriolar derivatives than motile cilia. The pertinent literature is examined, and the cilia are tentatively assigned a non-motile status and a sensory function.

Centrioles have long been regarded as organizers of kinetic activity in the cytoplasm of both somatic and germinative animal cells. However, as the dimensions of a centriole are typically but 300 to 500 mμ in height by 150 mμ in diameter (8), knowledge of its detailed structure has awaited development of the electron microscope. Affected by studies employing such a device (2, 4, 8, 14), the term *centriole* has become restricted from previous wider application to the nearly cylindrical organelle composed of a set of nine parallel triple-fibers radially arranged about a space of lesser density. However, current ideas on the functions of centrioles have resulted from earlier studies by light microscopists.

Centrioles usually lie at the cell center adjacent to the nucleus but in columnar epithelium (38) they lie more frequently near the apical surface. During mitosis they occupy the poles of the spindles. It is not certain to what extent centrioles normally are essential to mitosis, for many cells, notably those of the higher plants, regularly divide in their absence. After experimental alteration, even cells normally possessing centrioles, such as the spermatocytes of crane-flies, may divide without their participation (10).

In certain cells, centrioles take part in the formation of cilia and flagella. In ciliated epithelium they first divide repeatedly to form many basal bodies, which subsequently sprout the motile

processes (17, 31). The fine structure of the basal bodies differs only in minor details from that of the parent centrioles. The development of flagella during spermatogenesis has been studied in a variety of forms (33), especially insects (3, 13–15, 20) and mammals (6). One centriole of the pair present in the postnuclear region of the spermatid sprouts a flagellum, but many details of the intermediate steps of this process remain unclear. Centrioles, basal bodies, and cilia have been shown to form part of certain sensory structures of cells, particularly photoreceptors. These range in complexity from the simple association of eye spot and basal bodies in certain Protophyta to the extensively modified lamellar systems in rods and cones of mammalian retinas (12).

Recent studies in electron microscopy have produced some rather interesting observations on centrioles in cells of various embryological origins. In mesodermal cells a centriolar derivative resembling a cilium has occasionally been found. Bernhard and de Harven (2) described such an incomplete cilium in connective tissue cells of chick spleen and mouse ovarian tumor. Mannweiler and Bernhard (25) wrote of the formation of a complete ciliated border in cells of a renal tumor. A single ciliary process had been described in epithelial cells of the loop of Henle and collecting tubule of the kidney of rabbits by Zimmermann (38). With the electron microscope such cilia have been seen in all segments of the nephron (24). A similar solitary cilium may also protrude from cells of endodermal and ectodermal origin. Zimmermann saw this appendage in thyroid epithelium of man (38). Munger (28) found solitary cilia in differentiating β-cells of the pancreatic islets of the mouse. Palay (29) referred to such cilia in neurosecretory cells of the preoptic nucleus of the goldfish. To date the most complete description of these centriolar derivatives is given by Barnes (1) in the hypophysis of the mouse.

Among unpublished observations by members of the Department of Anatomy at Harvard Medical School, several might be mentioned here. D. W. Fawcett has encountered solitary cilia in centroacinar cells of the bat's pancreas; E. D. Hay, in a rare precartilage or blastema cell from a regenerating amphibian limb; S. R. Hilfer, in colloid cells of the young chick's thyroid gland; and J. P. Revel, in the glycogen body of the newly hatched chick.

This paper reports the normal occurrence of these cilium-like processes in developing fibroblasts and smooth muscle cells, principally from the lamina propria, submucosa, and muscularis of the duodenum of both chick and rat, and from organ cultures of the chick's duodenum. The development of the cilia from their parent centrioles is described in detail, and some consideration is given to their possible functional roles in these cells. An account by Sotelo and Trujillo-Cenóz (34) of the formation of somewhat similar solitary cilia in neural epithelium of developing chick is of particular interest in relation to this study.

MATERIALS AND METHODS

The biological materials which form the basis of this study represent both birds and mammals. Tissues from the small intestine of newly hatched chicks and week-old rats, as well as from organ cultures of developing chick duodenum, formed the principal materials. Corresponding tissues from the hen and adult rat were also examined. A more cursory examination was given tissues from the small intestine of other mammals, including the adult deer mouse (*Peromyscus maniculatus gracilis*) and the short-tailed shrew (*Blarina brevicauda*). In addition, centrioles or their derivatives were studied in the lungs and kidneys of all these mammals, as well as in the kidneys of a day-old kitten.

The organ cultures, grown for another purpose, nevertheless provided material suited to this study of centriolar derivatives. The first centimeter of small intestine in the 13-day developing chick was removed, slit longitudinally, and cut into two or more rectangular pieces, which were then placed on solid nutrient medium in culture flasks and incubated at 37.5°C. The medium contained 20 per cent chicken serum, 0.3 per cent glucose, and 100 units of penicillin G per milliliter, mixed into balanced salt solution and solidified with addition of 1.5 per cent agar. The cultures were transplanted to fresh medium every 2 days and were fixed for electron microscopy after 9 days *in vitro*. Further details of the technique of culture are given elsewhere (32). The method employed is such that virtually no outgrowth proceeds from the explants.

Tissues were prepared for electron microscopy by essentially routine methods, which included fixation for 1 hour in cold 1 per cent osmium tetroxide buffered with barbiturate at pH 7.7, followed by rapid dehydration in graded ethyl alcohols, and embedding in Epon. The thin sections were placed on coated grids and stained with lead plumbite (22), using unbuffered solutions, after which they were coated with a carbon film. The sections were examined and photographed in both RCA EMU 3E

and Siemens Elmiskop I electron microscopes, using accelerating voltages of 50 kv and 60 kv, respectively.

OBSERVATIONS

Cytological Characteristics of Participating Cells

The stages of ciliogenesis about to be described occur most frequently in the mesodermal derivatives while they are actively differentiating. The duodenal wall of the newly hatched chick or of the week-old rat is well demarcated into the various mucosal and submucosal layers. Fibroblasts are basophilic and actively engaged in the production of collagen. Smooth muscle cells contain many myofilaments but lack their full complement. Nerve cell processes range widely through the muscular layers. Much younger material, although studied far less thoroughly, provided no evidence of ciliogenesis in fibroblasts or smooth muscle cells. Adult tissues give evidence of continued ciliogenesis, although end stages are rarely seen. The organ cultures furnish material in which centrioles and their derivatives can be seen with the greatest clarity. This appears to result from the looser arrangement of cells and fibers in the duodenal wall of the culture as compared to its counterpart in the newly hatched chick.

Electron micrographs taken at the developmental stage most favorable for the study of ciliogenesis depict the fibroblasts with highly irregular outlines and many thin cytoplasmic processes extending into the intercellular spaces (Fig. 23). The cytoplasm has numerous rod-shaped mitochondria. It is rich in ribosomes (Fig. 9), which are scattered widely as single units or grouped into rosettes. A sizable but smaller proportion of the ribosomes is associated with membranes of the ergastoplasm. The vesicles of the Golgi apparatus are prominent (Fig. 9) and usually surround a centrosome which occupies the hof of the nucleus (Fig. 4). It contains a pair of centrioles and numerous fine interwoven filaments in its matrix (Fig. 4).

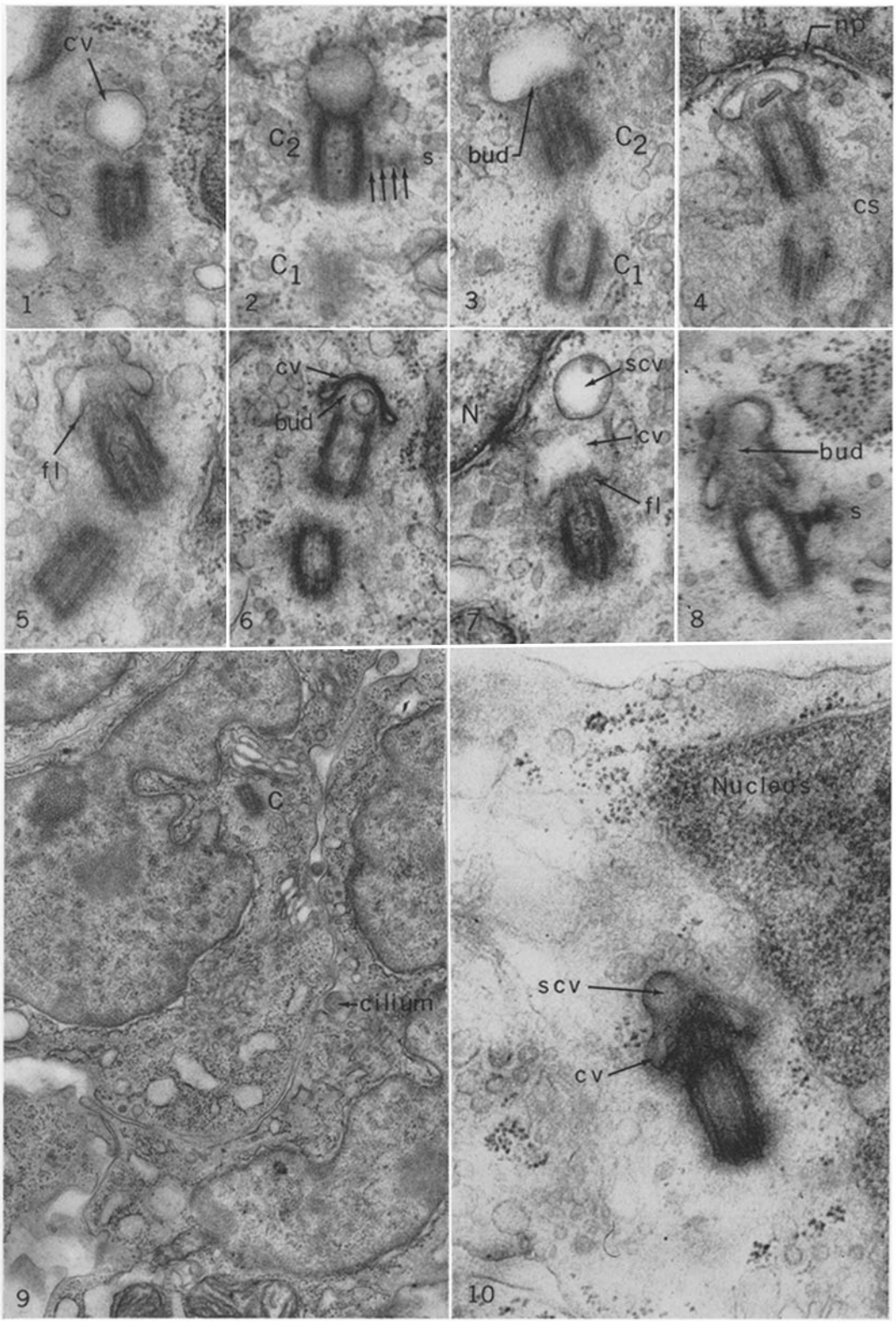
The myofilaments of the smooth muscle cells are for the most part organized into parallel bundles and arranged along the long axis of the cells (Fig. 19). Some areas of cytoplasm in these cells are rich in free ribosomes that are most commonly aggregated into small clusters. Others are associated with membranes. The vesicles of the Golgi apparatus are prominent. Mitochondria tend to be longer and thinner than those present in fibroblasts.

It is relevant to add that each epithelial cell of both intact and cultured duodenum contains a pair of centrioles. At the time when cilia are forming in the connective tissues none of the epithelial centrioles are similarly engaged. Light and electron microscopic sections of this material reveal an abundance of mitotic figures present in both connective tissue and adjacent epithelium.

The Formation of Cilia

The process of ciliogenesis is similar in all cells included in this study of avian and mammalian material. Although the noun *cilium* is used freely in the following account, it is to be understood as preceded by the silent adjectives *rudimentary*, *vestigial*, or *modified*. Attention is principally given the intestinal material; but similar stages of ciliogenesis are also encountered along the nephrons of the mammalian kidneys examined, as well as in occasional endothelial cells of the lung. In many respects, formation of cilia in the pulmonary epithelium is like that reported here, but it merits separate attention in another article. The reconstruction of ciliogenesis offered here has been made from stages pictured in a large collection of electron micrographs. The scheme is divided into three phases for convenience in presentation. It verifies the classical accounts of ciliogenesis in general but adds some new particulars.

PHASE I. CENTRIOLE, PRIMARY VESICLE, AND CILIARY BUD: The first indication of the formation of a cilium is given by the appearance of a solitary vesicle at that end of one centriole which faces away from the other centriole of the pair. That end will be called the *distal end* of the centriole. For convenience the vesicle will be called the *primary ciliary vesicle*. As shown in a micrograph (Fig. 1), the contents of the vesicle appear homogeneous and electron permeable. It is not certain whether or not the primary ciliary vesicle is produced by the cilium-forming centriole, but in any case it is clear that the vesicle becomes attached to its distal end (Fig. 2). As this is done a hillock of material having a density comparable to that of the centrosome appears at the distal end of the centriole and grows against the limiting membrane of the primary vesicle (Fig. 3). The hillock is the rudiment of the cilium, or *ciliary bud*. It sometimes contains small vesicles or granules, of unknown significance (Figs. 4, 6). The primary


cv
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C
cilium
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Nucleus
scv
cv
10

FIGURES 1 TO 10

Centriole, bud, and ciliary vesicles. Figs. 1 through 8 show centrioles in fibroblasts from the lamina propria and submucosa in 9-day organ cultures of chick duodenum.

FIGURE 1

The primary ciliary vesicle (*cv*) hangs over the distal end of the centriole in a somewhat tangential section. × 36,000.

FIGURE 2

A stage similar to that of Fig. 1. The cilium-forming centriole (C_2) is cut nearly through the center. The primary ciliary vesicle is connected to its distal end. A satellite (*s*) exhibits cross-banding (arrows). The other centriole (C_1) is visible only as a gray shadow. × 36,000.

FIGURE 3

A later stage in the development of the cilium. The primary vesicle is distorted by ingrowth of the ciliary bud from the distal end of the centriole (C_2). The other centriole (C_1) does not contribute to the emerging cilium. × 36,000.

FIGURE 4

A "mushroom" stage similar to that of Fig. 3. The cilium-forming centriole is cut in mid-sagittal plane so that the ciliary bud is readily seen. The centrioles lie within the fibrillar matrix of the centrosome (*cs*), which communicates with the nucleus through a nuclear pore (*np*). × 36,000.

FIGURE 5

Continued ingrowth of the ciliary bud flattens the primary ciliary vesicle. The membrane of the vesicle becomes fluted (*fl*) where vesicle joins centriole. × 37,000.

FIGURE 6

A mid-sagittal section through the cilium-forming centriole, showing the ciliary bud and flattened ciliary vesicle (*cv*). The bud contains a small vesicle. × 34,000.

FIGURE 7

Centriole, bud, and primary vesicle (*cv*) with its membrane fluted (*fl*) at the junction with the centriole. A second vesicle (*scv*) appears to be associating with the developing organelle. The nucleus (*N*) of the cell is adjacent. × 36,000.

FIGURE 8

Centriole with satellite (*s*), lengthened ciliary bud, and asymmetric ciliary vesicle, or sheath, apparently formed by fusion of the primary with a secondary vesicle. × 39,000.

FIGURE 9

A view of several fibroblasts from the lamina propria in an organ culture of chick duodenum. Cytological characteristics of cells undergoing ciliogenesis are shown. The cell on the left displays one of its centrioles (*C*) within a centrosome, which is surrounded by a well developed Golgi apparatus. The cell on the right extends a cilium into the intercellular space. Mitochondria are shown below, and the disposition of the ribosomes can be seen in all cells. × 12,000.

FIGURE 10

A stage similar to Fig. 8, as seen in a fibroblast from a week-old rat. The asymmetric ciliary sheath is evident as is the juxtanuclear position of the centriole. The sheath appears to be formed by fusion of primary (*cv*) and secondary (*scv*) vesicles. × 45,000.

ciliary vesicle, being fixed in position by its attachment to the rim of the centriole (Fig. 2, *cf.* Fig. 17), becomes invaginated by the elongation of the ciliary bud. The primary vesicle accordingly becomes flattened, so that the complex formed by centriole, ciliary bud, and primary vesicle comes to resemble a mushroom (Figs. 3 to 6). As a result of this process two layers of membrane from the primary vesicle surround the bud except where it remains attached to the centriole. In this manner the *ciliary sheath* seems first to be formed. The sheath covers the developing cilium until it emerges from the cell. The outer wall of the sheath then becomes continuous with the plasma membrane and the inner wall forms the ciliary membrane. The membrane of the primary vesicle (or sheath) becomes fluted, or thrown into regular folds, near its junction with the centriole (Figs. 3, 5, 7, 12). The fluting is first seen during the "mushroom" stage and is visible until the cilium emerges from the cell.

While the cilium is forming, the centriole frequently extends an arm or "satellite" from some point along its side into the matrix of the centrosome, at right angles to its own axis. In sagittal sections of the centriole the arm appears as a wedge-shaped projection which is widest at its junction with the centriole. The tapering process is cross-striated with bands of high density separated by bands of lesser density (Figs. 2, 8,

FIGURES 11 TO 17

Development of stalk and sheath of the cilium. All figures are from fibroblasts in 9-day organ cultures of chick duodenum, except Fig. 14 which is from a week-old rat.

FIGURE 11

Mid-sagittal section through a developing cilium, showing a well formed ciliary bud, the parent centriole, and the ciliary sheath formed from the ciliary vesicles. × 55,000.

FIGURES 12, 13

Continued growth of the bud. Portions of the nuclei (*N*) are close to the centrioles. × 36,000.

FIGURE 14

A row of small secondary vesicles (*scv*, arrows) extends away from the primary ciliary vesicle (*cv*) of the centriole (C_2). Other centriole, C_1 . × 37,000.

FIGURE 15

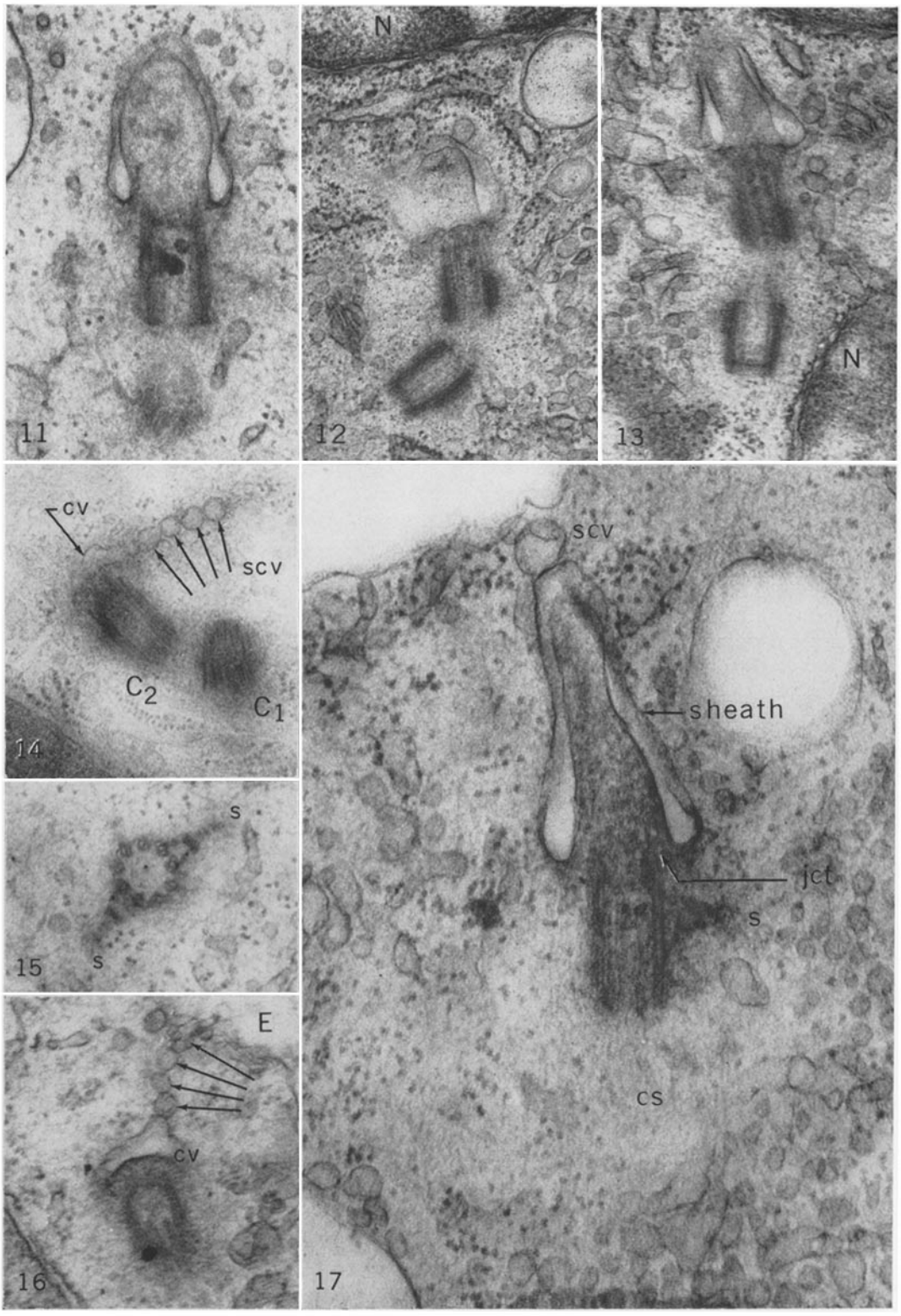
A cross-section of a centriole having two arms, or satellites (*s*), in view. The arms connect to the outer wall of the centriole at the middle subfiber of each of two adjacent triplet fibers. × 55,000.

FIGURE 16

Oblique view of a centriole. The primary vesicle (*cv*) is linked to the extracellular space (*E*) by a row of secondary vesicles (arrows). × 45,000.

FIGURE 17

A developing cilium within the centrosome (*cs*) about to emerge from the cell. The satellite-bearing centriole (*s*) remains attached. The ciliary sheath has lengthened in pace with the ciliary shaft, apparently by fusion of primary vesicle with secondaries such as shown in Figs. 14 and 16. An as yet unfused secondary vesicle (*scv*) separates the apex of the sheath from the outside of the cell. Granular "morphogenetic material" is present at the swollen base of the cilium, possibly indicating early stages in the internal organization of the appendage. The point of junction (*jct*) between an outer fiber of the centriole and the sheath is seen on the right. × 62,000.


11
N
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17

17). The satellites may occur in varying numbers, but often two or three are seen in the same cross-sectional plane of the centriole (Fig. 15). In such sections it can be seen that each process or arm makes contact at its base with two adjacent outer fibers of the centriolar wall. Arm and fiber are joined near the middle component of each triplet fiber, so that the arm has more the shape of an Eiffel Tower than of a club, to which it had previously been compared (4). It is possible that, whatever may be their primary function, the arms of the cilium-forming centriole may assist in stabilizing the centriole while the cilium is emerging from it. The other centriole, lacking such arms, appears to change its position during ciliogenesis (Figs. 5, 12, 13, 18) and may move between extreme positions at right angles to the cilium-forming centriole and in line with it.

Beginning with the earliest stages of formation, cilia are developed within the centrosome, which usually occupies a position next to the nucleus (Figs. 4, 10, 13, 20, 23, 24). Centrioles evidently are not required to migrate to the surface of the cell before sprouting cilia.

PHASE II. DEVELOPMENT OF THE SHAFT AND SHEATH OF THE CILIUM: The shaft of the cilium develops by elongation of the original ciliary bud. At first it appears free of characteristic structural contents. As the shaft elongates, however, granular material of greater density appears at the base. The granular material is contiguous with similar material located in the distal region of the centriole (Fig. 17). Indeed, granules and vesicles of various sizes are seen at times within the walls of centrioles, whether they are producing cilia (Figs. 5, 7, 13, 17, 20), or not (Figs. 3, 6). Similar granules have been described in amphibian material (7) but their significance is not known.

The sheath of the developing cilium lengthens concurrently with the elongation of the ciliary shaft. As the primary ciliary vesicle becomes compressed by the growth of the ciliary bud a second vesicle identical in appearance to the primary vesicle comes into being distal to the first (Fig. 7). This secondary vesicle evidently fuses with the primary vesicle to produce an enlarged spheroid, which the lengthening cilium invaginates. At first the newly enlarged sheath is asymmetrical (Figs. 8, 10), but it later regains a radial symmetry (Fig. 11). Further enlargement of the ciliary sheath appears to involve repeated formation of discrete *secondary ciliary vesicles*, which subsequently fuse together to form a still larger sheath (Figs. 14, 16, 17).

The source of the membranes of the sheath is

FIGURES 18 TO 21

Emergence of the cilium. All figures are from organ cultures of chick duodenum, except for Fig. 19 which is from the duodenum of a day-old chick.

FIGURE 18

View of an emerged cilium before the ciliary shaft has developed internal fibers. Both centrioles (C_1 , C_2) are often nearly in line at this stage. × 37,000.

FIGURE 19

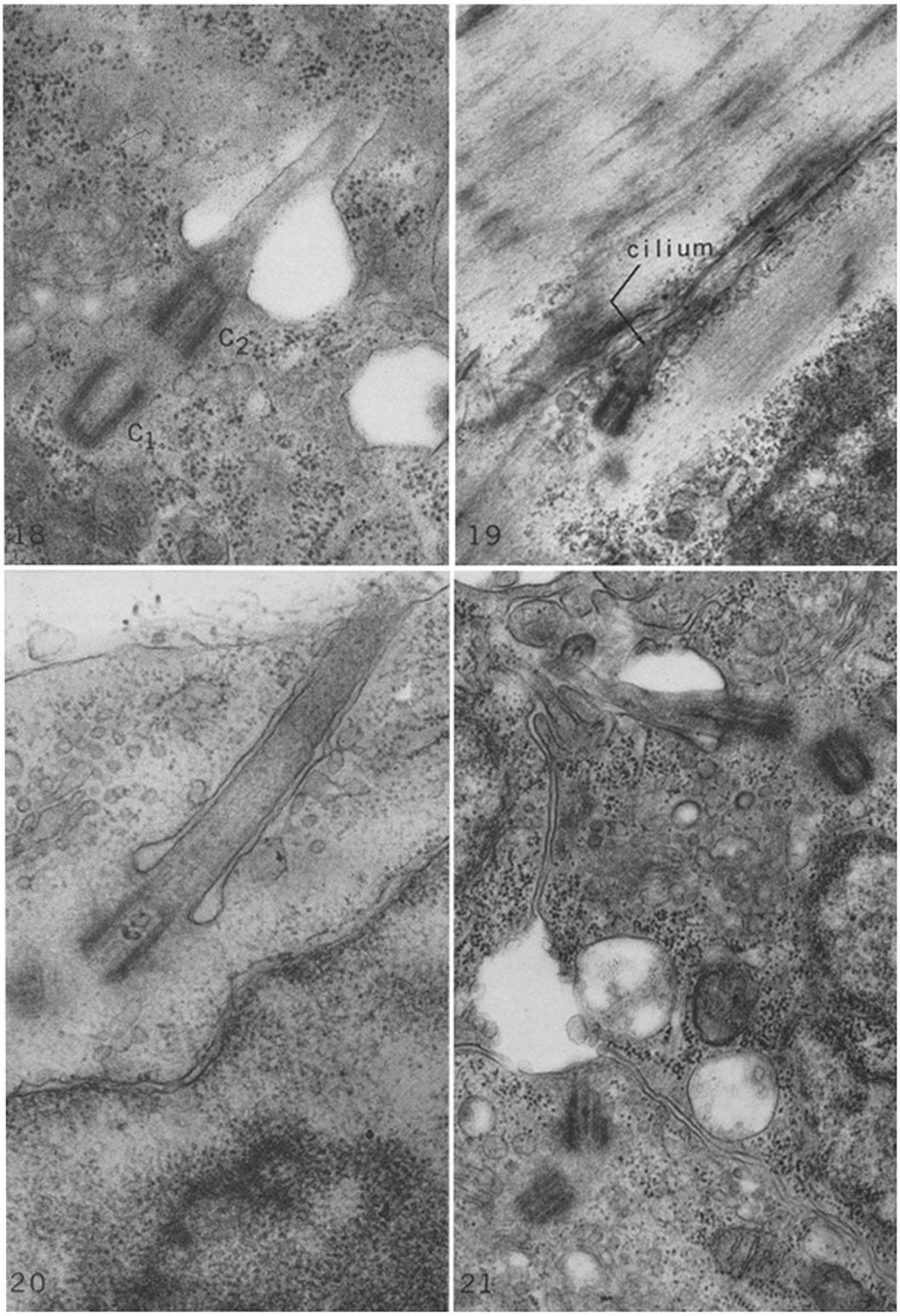
A newly emerged cilium extending into the space between two smooth muscle cells. The cilium is formed in a manner similar to that shown in the fibroblasts. Where cells are closely apposed the cilium is often inconspicuous. × 20,000.

FIGURE 20

The emerged cilium surrounded by its sheath. The base of the cilium remains attached to the centriole of the fibroblast. Both centrioles lie in their customary position near the nucleus. × 37,000.

FIGURE 21

Formation of cilia is taking place in two adjacent fibroblasts. The micrograph conveys some idea of the relatively common occurrence of the phenomenon. × 28,000.


C2
C1
18
cilium
19
20
21

undetermined. The secondary vesicles appear distal to the primary ciliary vesicle and in contact with it. It may be that both primary and secondary vesicles are produced by the centriole, but it is also possible that they are formed *de novo* in the Golgi body of the cell which surrounds the centrosome (Fig. 9). In support of the former idea, there is evidence in orthopteran spermatids that the cilium-forming centriole contributes to formation of the flagellar sheath (3, 14).

Whatever may be the precise method of the formation of the ciliary vesicles, they ultimately form a path which leads to the outside of the cell (Fig. 16). As the cilium grows in length the secondary ciliary vesicles become part of the sheath (Fig. 17), until cilium and sheath reach the surface of the cell.

PHASE III. EMERGENCE AND INTERNAL DEVELOPMENT OF THE CILIUM: Once the shaft and sheath of the cilium have reached the surface of the cell the membranes of the sheath fuse with the plasma membrane and the developing cilium is exposed to the extracellular environment. The sheath persists as a more or less conspicuous structure associated with the cilium, being more prominent in those fibroblasts whose centrioles lie deep in the cytoplasm (Fig. 20) and less prominent in cells whose centrioles lie close to the surface (Figs. 19, 22, 23). In some cells the cilium continues to lengthen appreciably. In others it protrudes as a stubby appendage (Fig. 23). Several stages of ciliary morphogenesis can often be seen in neighboring cells (Fig. 21).

Even among the more highly developed of the

FIGURES 22 TO 27

Typical products of the morphogenetic process. The illustrations are of fibroblasts from the organ cultures.

FIGURE 22

An emerged cilium whose tip has been bent over by growth against an adjacent cell. At the base of the shaft the presence of rod-like fibers likens the appendage to a developing motile cilium. However, the fibers do not reach the tip of the shaft but terminate part way up with clubbed ends. × 34,000.

FIGURE 23

A short club-shaped cilium filled with many small vesicles and short rods. The cilium itself is inconspicuous among the other cytoplasmic processes extending into the extracellular space (*E*). Its centriole remains surrounded by centrosomal material (*cs*). × 39,000.

FIGURE 24

A sheathed cilium similar in form to that in Fig. 23. One centriole (C_1) is seen nearly in cross-section. The nucleus (*N*) is nearby. × 39,000.

FIGURE 25

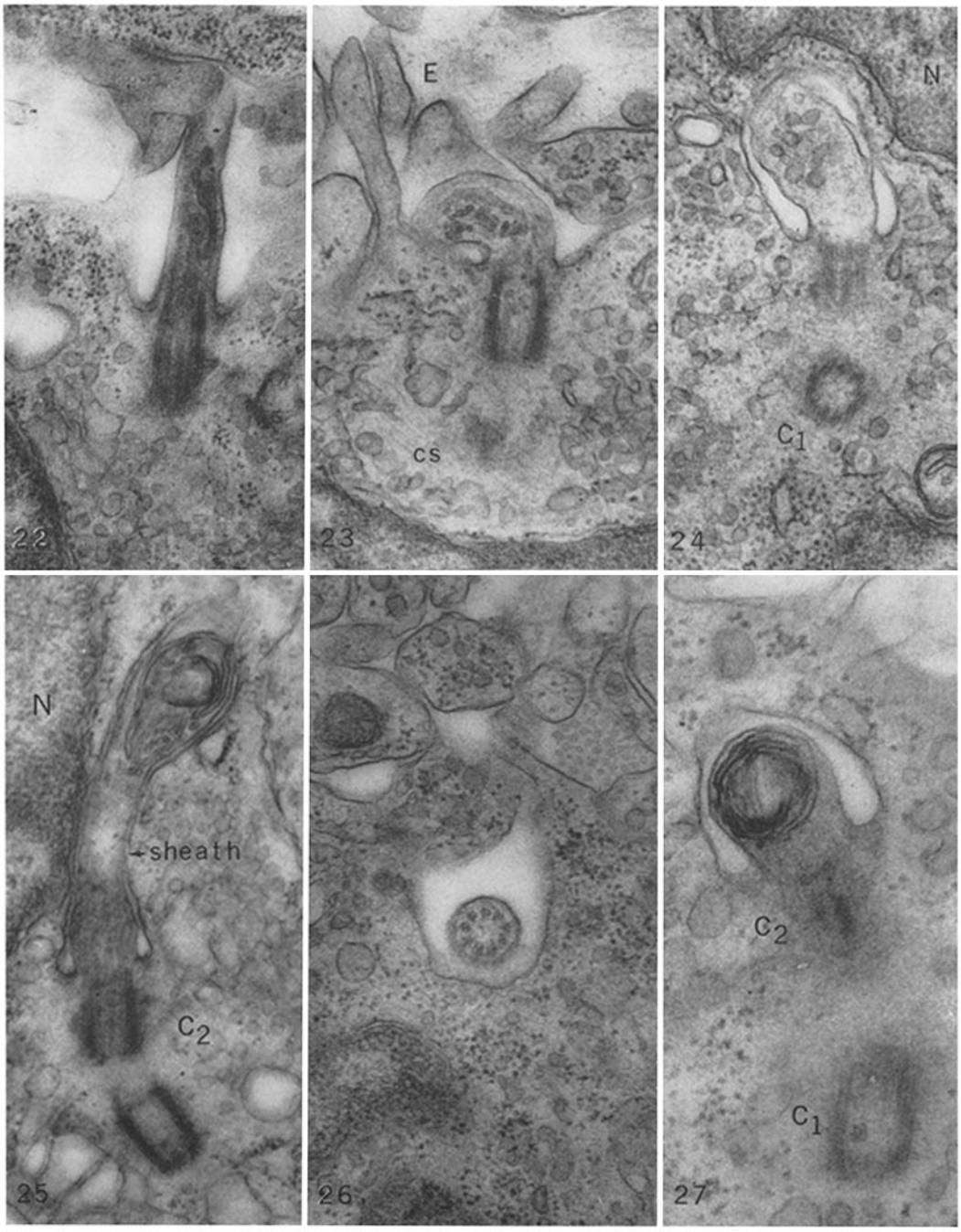
The cilium is within its sheath and lies near the nucleus (*N*). Its distal end is transformed into a lamellar structure, possibly by coalescence of small vesicles found in prior stages (Figs. 23, 24) and still present among the layers at the tip. The base of this cilium is swollen with material similar to that seen in Fig. 17, of possible significance in the formation of ciliary fibers. × 37,000.

FIGURE 26

A cross-section through the cilium of a fibroblast. The cilium lies in a tunnel formed by the ciliary sheath and is sectioned near its base. It contains nine radially arranged doublet fibers but lacks a central pair. × 40,000.

FIGURE 27

An oblique section through the tip of a cilium like that in Fig. 25. Membranes are arranged concentrically like leaves in a head of cabbage. Both centrioles of the cell (C_1, C_2) are shown. × 58,000.


22
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C1
24
N
sheath
C2
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26
C2
C1
27

cilia observed, no structures exactly comparable to mature, motile cilia are seen. Instead, club-shaped processes of various lengths are typical. They contain an assortment of small vesicles and short hollow rods in some specimens (Figs. 23, 24), and filaments in the basal regions of others (Fig. 22). The most elaborate and presumably most highly developed of these centriolar derivatives contain layered membranes at their tips (Fig. 25). Cross-sections through proximal segments of such modified cilia reveal nine doublet fibers arranged in a circle, as in motile cilia and flagella. However, in these cilia, unlike motile cilia, the central pair of fibers is missing (Fig. 26). The distal portions of the cilia contain no doublets. There the dense layers of membranes exhibit a vernation like leaves in a head of cabbage (Fig. 27).

Adult intestinal tissues provide few examples of these solitary cilia. It is not yet clear whether these cilia are rarely seen because they become obscured by dense depositions of intercellular fibers, or whether they are shed.

DISCUSSION

The observations presented in the results may profitably be discussed in relation to two questions, namely, the nature of the cilia formed and the process of ciliogenesis.

The small size of the single cilia of developing smooth muscle cells and fibroblasts and the tendency for them to be obscured by superimposition of structures in histological sections preclude an effective study of them in the light microscope. In the ultrathin sections and limited fields scanned in electron microscopy numerical estimations of their abundance would be unreliable. However, these single cilia seem to be formed too frequently to be considered rare anomalies of development. Notwithstanding, it is still prudent to inquire whether such cilia in metazoan cells are merely vestiges of a free-swimming unicellular heritage, or whether they meet some current need.

In the material used for this reconstruction of ciliogenesis, it was noted that examples of the more advanced stages lacked the central pair of axial fibers possessed by motile cilia. They also exhibited greater morphological variation than did the examples of earlier stages. These observations would lead to an interpretation of these structures as atavistic or degenerate cilia, were it not for knowledge of the existence of several types of non-motile modified cilia (12). Barnes (1) collected the scattered reports of solitary non-motile cilia in various somatic cells and noted the absence of the central fibers from their shafts. She assigned speculatively to all such cilia a sensory function. Following a similar train of thought, Inoué (19) conjectured that the nine outer doublet fibers of all ciliary shafts were concerned with conduction, while the central pair of motile cilia had to do with contraction. Munger (28) also suggested a sensory role for the single cilia which he found on β-cells of the pancreas. However, it must be admitted that evidence in favor of a sensory function for all such unusual cilia is purely circumstantial. It is based upon structural similarities between the more modified of these cilia and the centriolar derivatives with known sensory function. Such similarities could as well be explained by common features of their development.

Optical microscopic studies established the general facts on ciliogenesis: centrioles or basal bodies may produce the cilia. Thus, Renyi (31) found in tracheal epithelium that an originally superficial and centrally located diplosome migrates to one corner of a cell and divides to produce a series of prebasal bodies. These eventually become arranged in a plane just under the apical surface. Cilia grow as protoplasmic extensions from the prebasals, which then take on an oval shape and become basal bodies. The description by Jordan and Helvestine (21) of ciliogenesis in ductuli efferentes is essentially similar. Sotelo and Trujillo-Cenóz (34) studied the formation of solitary cilia in neuroepithelial cells with the electron microscope. Their interpretation of ciliogenesis in the ependyma differs little from their view of flagellar formation in spermatogenesis (33). Essentially, the cilium-forming centriole attaches to the cell membrane, which then bulges to form a ciliary bud. Then, while the ciliary shaft elongates, the centriole withdraws from the surface, bringing the plasma membrane along with it. During this stage the inner structure of the ciliary shaft becomes organized. The centriole then returns to the surface and the whole length of the ciliary shaft becomes evaginated into the lumen, while the axial fibers attain final maturation. Common points in the reconstructions of Sotelo and Trujillo-Cenóz in their material and in that based on fibroblasts and smooth muscle cells include the outgrowth of a ciliary bud from one centriole of a pair, the apparent alignment

of centrioles during some stage of ciliogenesis, and the formation of the axial fibers at least in part through fusion of small vesicles found within the ciliary bud. Sotelo and Trujillo-Cenóz observed no sheath other than that formed by the invaginated plasma membrane and saw no ciliary bud unless the parent centriole were attached to that membrane. However, the idea of centriolar migration and attachment to the cell surface as an obligate precursor to ciliogenesis can be doubted, in view of the evidence from the mesodermal cells. There the cilium may be elaborated while the parent centriole lies deep in the cytoplasm. In the same cells the plasma membrane at first contributes relatively little to the sheath, which appears to be formed by fusion of intracytoplasmic vesicles.

The appearance of rows of secondary vesicles in this material is reminiscent of stages in the development of other continuous cellular membranes from discontinuous vesicular elements. As seen in the electron microscope, elaboration and alignment of vesicles precedes the formation of demarcation membranes, which appear in the mid-zone of the cytoplasm of megakaryocytes during the formation of platelets (37). The formation of plasma membranes between muscle fiber and derivative blastema cell during dedifferentiation of amphibian striated muscle (16), the production of the cell plate in root tips (30), and the development of the cleavage furrow in hematopoietic cells (5) apparently take place in this manner. During cell division the nuclear envelope becomes reconstituted from small vesicles (26). Membranes forming within outer segments of visual cells are preceded by the appearance of vesicles in the same region (36). Something similar has been seen in the tips of the more highly developed cilia of fibroblasts. Possibly the multilamellar structure of chloroplast grana develops in the same way (18) from the primary granum of a proplastid (27).

Subsequent to the emergence of the cilium from the cell the internal development of the shaft appears to differ in motile and presumably nonmotile types. In fibroblasts and smooth muscle cells, granular or fine vesicular material observed at the base of the cilia may be used in forming the axial fibers. Such material has been observed in other immature cilia as well (35). Axial fibers of motile flagella are also thought to develop after the shafts have emerged (15). The fibers of both motile and mesodermal species appear to be first formed near the bases of the shafts, although it is not yet clear whether they are composed in part of expanded centriolar fibers, or whether they are entirely made *in situ*. At the club-shaped tips of certain of the modified cilia, the presence of vesicles in association with layered membranes resemble stages in the formation of the outer segments of the rods and cones of mammalian retinas. The outer segments contain the visual carotenoids and are developed from the distal part of a solitary modified cilium (9, 23, 36). The outer segment is linked to the inner body of the retinal cell through the proximal part of the same cilium. In this basal region the nine outer doublet fibers of the cilium are present, but the central pair is absent. There are also similarities to be seen between the structure of cilia here described and that in degenerating outer segments from rats deprived of vitamin A (11). In the latter, the retinal discs undergo swelling and segmentation in what resembles a reverse of morphogenesis. However, the soundness of the present material has been stressed and contradicts an interpretation of its cilia as degenerate structures. In summary, of the modified cilia that have been described in various somatic cells so far, the cilia of fibroblasts appear to resemble most closely those of developing visual cells. If the solitary cilia are not atavistic or degenerate organelles it indeed remains to be demonstrated that they are functional. Perhaps something about the possible functional capacity of such cilia may be learned from an investigation of the degree of differentiation of ciliary processes at the onset of photoreception in developing eyes. Such a study might give some indication of the minimum structural differentiation compatible with sensory function.

Observations have also been recorded here for which no explanations are yet at hand, such as the changing positions of the two centrioles with respect to each other and the appearance of satellites or arms on the cilium-forming centriole. The other centriole of the pair appears to contribute nothing to the development of the cilium. Perhaps it is reserved for future division and participation in mitotic activities of the cell.

The centrioles continue to be the most enigmatic of the organelles, but the results of this study may heighten our awareness of their versatility in morphogenesis and their varied synthetic capacity.

I should like to thank Professor Don W. Fawcett for his helpful attention to the progress of this work and for his criticism of the manuscript. I am also grateful to Drs. Susumu Ito and Jean-Paul Revel for their patience and kindness while instructing me in the ways of electron microscopy.

Received for publication, October 14, 1962.

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