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# Cilia and centrioles of the rat adrenal cortex

D. N. WHEATLEY

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

## INTRODUCTION

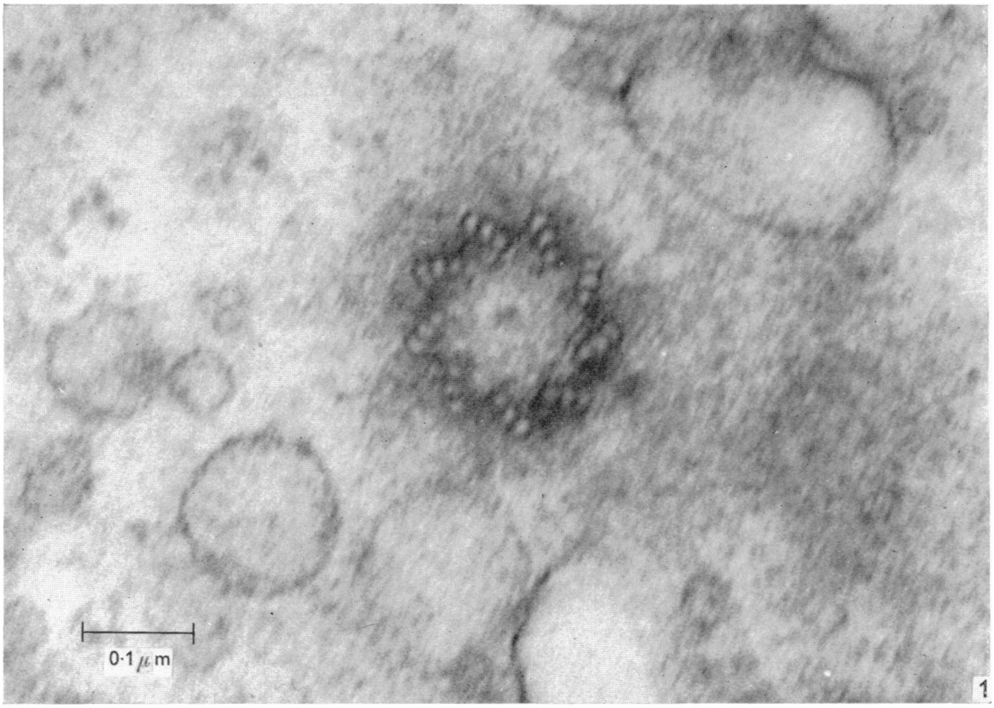
Cells with single cilia associated with two centrioles have been observed in a number of endocrine organs. Munger (1958) noted cilia in the $\beta$-cells of the mouse pancreatic islets and later Eisterhuizen & Lever (1961) reported their presence in the $\alpha$-cells. The adenohypophysis of various mammals, including the mouse (Barnes, 1961), the foetal rat (Maillard, 1963), and the rabbit (Salazar, 1963) possess ciliated cells. Coupland (1965) described cilia in chromaffin cells of the rat adrenal medulla. Fujita (1963) mentioned their presence in the thyroid follicular cells of the domestic fowl; Munger & Roth (1963) demonstrated them in the parathyroid of man and deer. Certain cells in the gonads also possess these cilia, for example Leeson (1962) described them in the rete testis of the rat and Adams & Hertig (1964) in the ovary of the guinea-pig. Apart from endocrine tissues, cells of various neural tissues have single cilia, e.g. rat cerebral cortex (Dahl, 1963), Schwann cells of the rat autonomic system (Grillo & Palay, 1963), and retinal epithelia of several different species of mammal (De Robertis, 1956; Allen, 1965). Although endocrine and neural tissues are particularly well represented, cilia associated with diplosomes have been found in other tissues. These include fibroblasts and smooth muscle cells of the lamina propria of the duodenum in chick and rat (Sorokin 1962), tubule cells of the rat kidney (Latta, Maunsbach & Madden, 1961), basal cell layer and squamous carcinoma of human skin (Wilson & McWhorter, 1963), human foetal skin (Wheatley, unpublished observations) and exocrine pancreas of chick (Zeigel, 1962).

In this paper, cilia are described in the cells of the rat adrenal cortex under normal and abnormal conditions.

## MATERIAL AND METHODS

Female Sprague-Dawley rats 50–60 d of age were used. Some rats received a 5 mg dose of 9,10-dimethyl-1,2-benzanthracene (DMBA) intravenously 3 d before killing in order to produce adrenal necrosis (Huggins & Morii, 1961). Animals were killed by stunning and within 30 s adrenal glands were excised, placed in a drop of fixative and bisected with a razor blade. Each half adrenal was cut into thin wedges and fixation continued in the cold for 1–2 h. Fixatives used were veronal buffered $OsO_4$ (Palade, 1952) and glutaraldehyde followed by osmification (Sabatini, Bensch & Barrnett, 1963). Tissues were dehydrated in absolute ethanol for 2 h and finally embedded in Epon 812 (Luft, 1961).

Much of the work reported here concerns the zona glomerulosa. After trimming the wedges of embedded adrenal glands to obtain block faces which included the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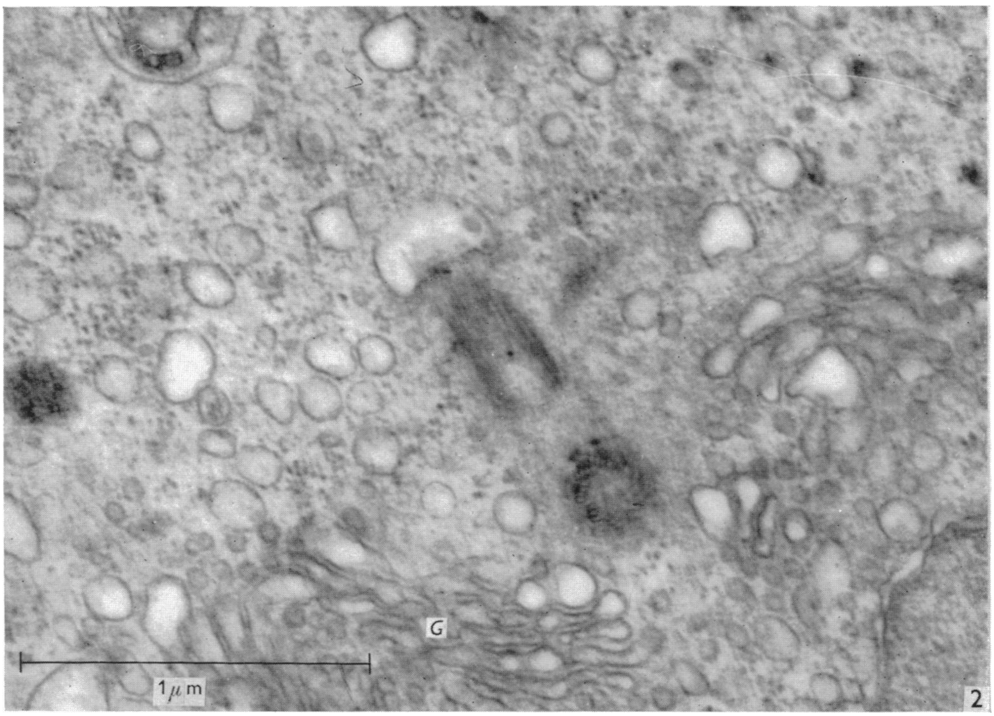
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arcuate capsular profile, sections were cut on a Cambridge Huxley ultramicrotome. They included the capsule, the zona glomerulosa and sometimes cells of the outermost zona fasciculata. After floating on to uncoated copper grids, sections were stained with lead (Karnovsky, 1961), and examined with a Zeiss EM9 electron microscope at an accelerating voltage of 65 kV.

In this investigation eleven pairs of untreated rat adrenals (seven pairs fixed in glutaraldehyde and four pairs in osmic acid) and twelve pairs of adrenals from DMBA-treated rats (all fixed in osmic acid) were studied.

## OBSERVATIONS

During an electron microscopic study of the rat adrenal undergoing necrosis after an intravenous injection of DMBA, large juxta-nuclear vacuoles were found in many of the zona glomerulosa cells which do not undergo necrosis after DMBA. A number of these vacuoles were seen to enclose cilia. This finding prompted a re-examination of the *normal* rat adrenal cortex. The zona glomerulosa cells of untreated rats did not have juxta-nuclear vacuoles but the presence of cilia was confirmed. Since cilia of the rat adrenal zona glomerulosa are associated with diplosomes; a brief description of the latter's appearance is presented.

### *Centrioles and diplosomes*

Centrioles are frequently seen in the endocrine cells of the adrenal cortex. They are also seen in the fibroblasts and endothelial cells of the cortex. Basically, their structure is similar to centrioles previously described (De Harven & Bernhard, 1956). They measure 180–230 m$\mu$ in diameter and 300–350 m$\mu$ in length. Centrioles in transverse section are seen in Figs. 1 and 2.

Centrioles in rat adrenal cortical cells are usually found in pairs. They lie some 200–250 m$\mu$ apart in a particular orientation to one another (Fig. 2), the axis of one being at right angles to the axis of the other. Each pair constitutes a diplosome.

### *Centriole-cilium relationships*

As reported by Barnes (1961) for the mouse adenohypophysis, one centriole of a diplosome often forms the basal body of the cilium (Fig. 3); this centriole, usually lying near the cell membrane, has been called the distal centriole (Barnes, 1961). The two centrioles of the diplosome are often connected by very fine filaments which have periodic bandings at intervals of 550 Å. These filaments resemble young collagen fibres seen in a high intensity electron beam. Similar periodic bandings have also been seen in filaments emanating from centrioles where no connexion is made between two centrioles. These structures are similar to the banded pericentriolar filaments described by Sakaguchi (1965) and are reminiscent of the rootlet filaments found in many ciliated cells, e.g. mussel gill cilia (Gibbons, 1961).

'Satellites' come off the distal centriole with their axes usually subtending an

Fig. 1. Transverse section of a centriole of a zona glomerulosa cell. $OsO_4$, ×140000.

Fig. 2. Paired centrioles (diplosome) of a zona glomerulosa cell. The proximal centriole is seen in transverse section, the distal in longitudinal section. Golgi membranes are seen at *G*. $OsO_4$, ×46500.


Fig. 3. Zona glomerulosa of a DMBA-treated rat. An extravasated erythrocyte is present at *H*. A large vacuole is seen (*R*) with a cilium projecting into it. Golgi membranes (*G*) lie close to the proximal ($C^1$) and distal ($C^2$) centrioles. $OsO_4$, $\times 24000$.

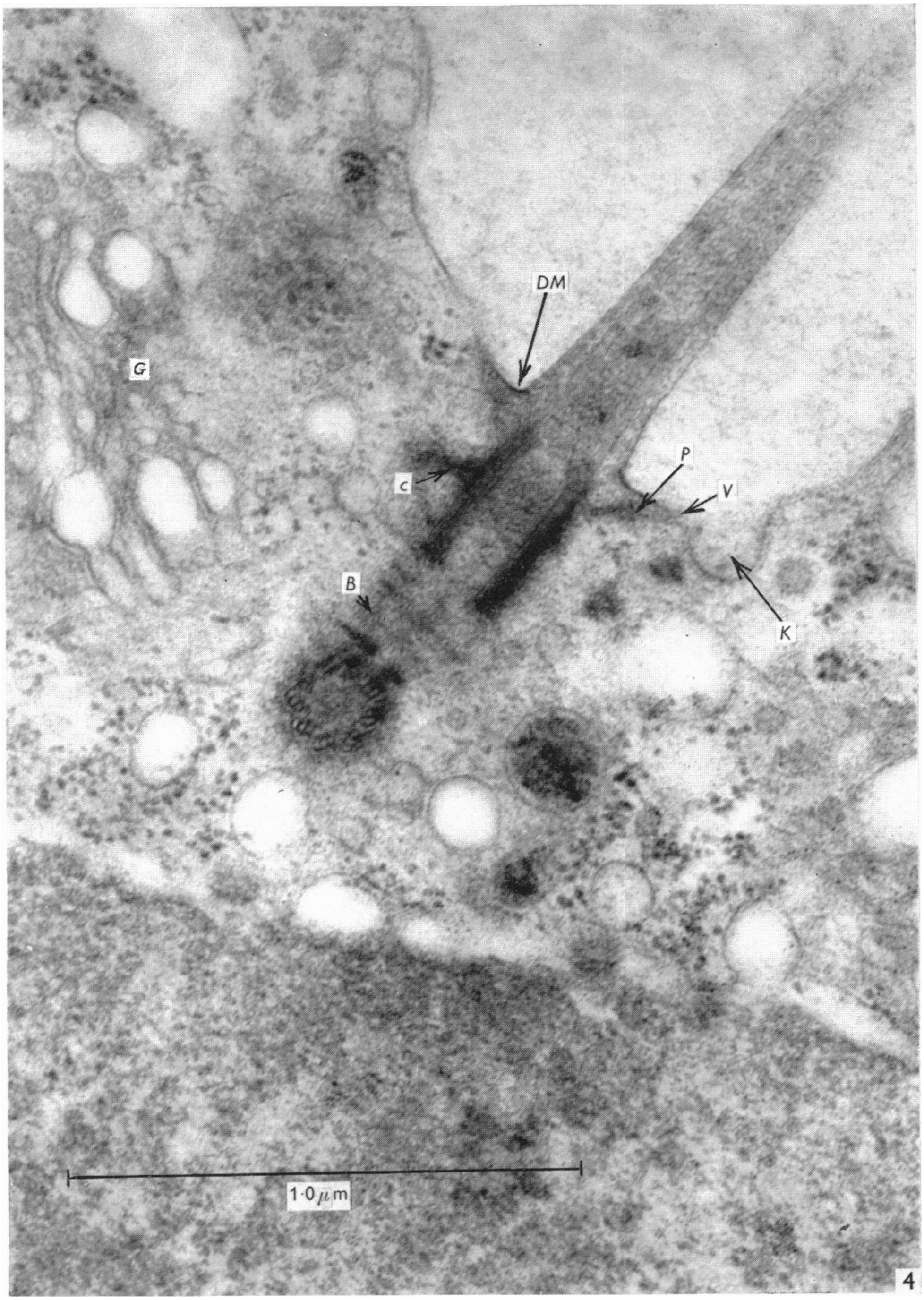
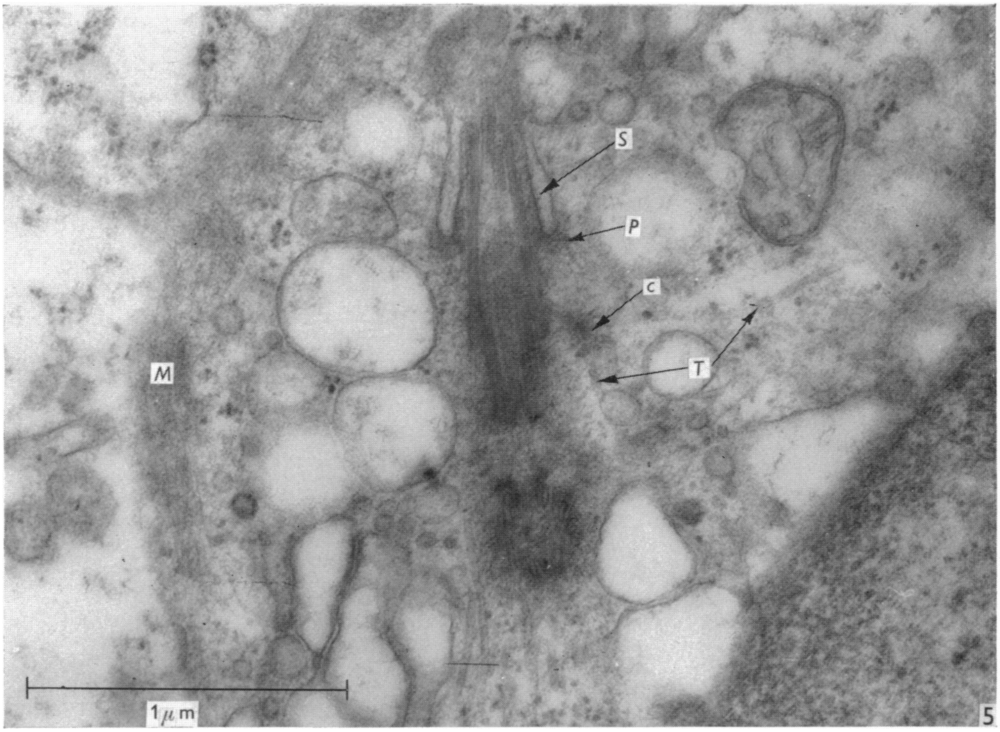



Fig. 4. High-power electronmicrograph of the cilium base of Fig. 3. Banded filaments between the two centrioles are shown at *B*. One of the paracentrioles of the distal centriole is found at *c*. A process connecting the distal centriole to the membrane has a row of small particles attached to it at *P*. Distal to this a cluster of small vesicles (*V*) is found. At the base of the cilium the membrane is very dense (*DM*) with a depression seen to one side (*K*). Golgi membranes (*G*) are to the left of the centrioles. $OsO_4$, ×62000.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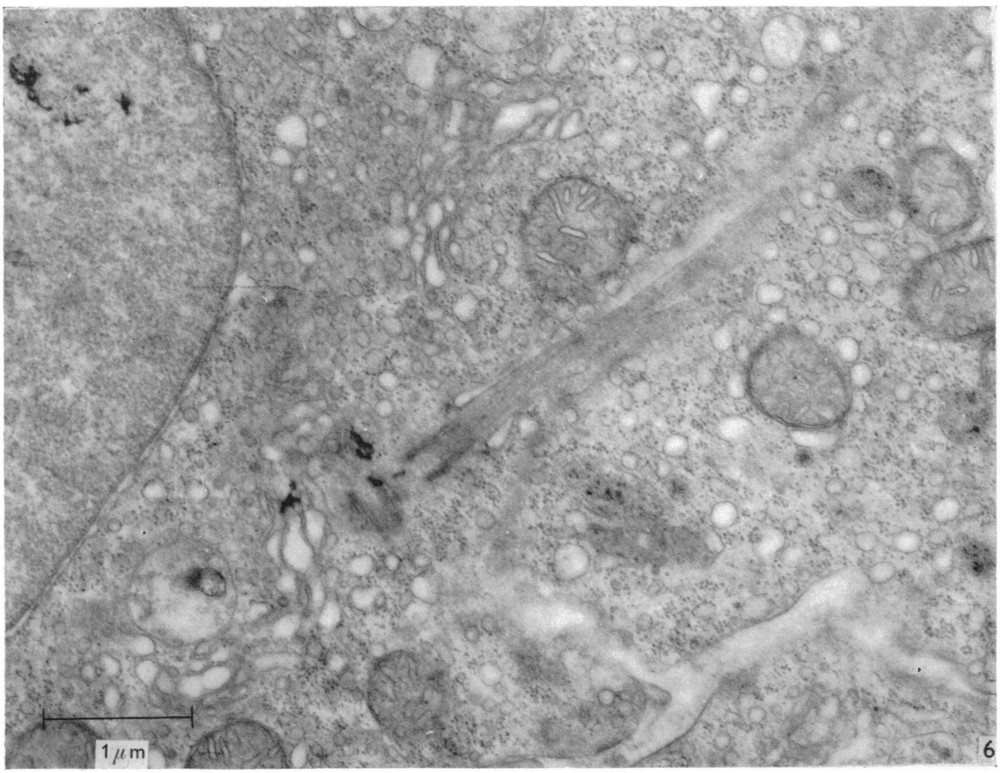
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angle of about 50° to the centriolar axis. Processes similar to these satellites connect the triplet fibres of the distal centriole to the cell membrane at the base of the cilium. At the centriolar end, these processes point away from the membrane; a second segment then joins the end of the first segment to the cell membrane. Distally the second segment fans out to form a row of small particles lying close to the membrane. These particles (*P* in Fig. 4) are smaller than ribosomes. Around this region, in particular, numerous small vesicles (*V*) of about 200 Å in diameter are found. The cell membrane near these vesicles is not as clearly defined as the membrane at the base of the cilium where it is very dense and thick (*DM* in Fig. 4). Vesicles are also found dispersed throughout the matrix of the centriolar region and in matrix surrounding the ciliary fibres. In Fig. 5 another diplosome is shown in which the invaginated cell membrane forms a circular sac surrounding the base of the cilium. The membrane is very electron dense and again shows a row of very small particles (*P*). Of particular interest in this case is the presence of microtubules (*T*) similar to those described by De-Thé (1964). Some of these can be seen passing close to the proximal centriole whilst in the distal centriole they appear to impinge upon a satellite.

### *Incursion of cell membrane*

In the normal zona glomerulosa, cilia are more commonly found surrounded by narrow sheaths of cell membrane similar to those described by Barnes (1961). These invaginations of the cell membrane may be of the order of several microns or more, as seen in Fig. 6. In Fig. 7 a cilium is shown in a large vacuole of a zona glomerulosa cell of a rat which had been treated with 5 mg DMBA intravenously 3 d prior to adrenal excision. By serial sectioning, it was found that a narrow gully gave continuity between the vacuole and the extracellular space. One not uncommon feature at the base of a cilium within a vacuole is a depression on either side of the thick electron-dense membranous region (see *K* in Fig. 4). The inner lip of the depression is often the site of a cluster of 200 Å vesicles.

Cilia are not always in deep invaginations but can be found in a superficial position as in Figs. 8 and 9. Even so, some retraction of the membrane around the base of the cilium is often seen.

### *Fibre arrangement of adrenal cilia*

In transverse section adrenal cilia are seen to lack a central pair of fibres (Figs. 10, 11). Thus the fibre formula is $9+0$. Fig. 12 shows nine fibre pairs in all, but two pairs do not fall on the circumference of the circle formed by the others. From serial sections it was found that cilia taper over the greater part of their length.

Fig. 5. Diplosome of a zona fasciculata cell of an untreated rat. Only the base of the cilium is seen in this section. Invagination of the cell membrane has formed two sacs (*S*). Note the density and thickness of the membrane at the bottom of these sacs. A fine row of small particles can be seen at *P*. The satellite at (*c*) has several tubules impinging upon it (*T*). More tubules are seen running close to the proximal centriole. Filamentous cytoplasmic matrix (*M*) surrounds the diplosome. $OsO_4$, $\times 42500$.

Fig. 6. A long cilium (over $2\mu$) of a normal zona glomerulosa cell. The sheath surrounding the cilium is very narrow. Compare with Fig. 7. $OsO_4$, $\times 18500$.


Fig. 7. A cilium of a zona glomerulosa cell from a rat pretreated with DMBA. The nucleus (*N*) has a flattened surface near the cilium and irregularities of the nuclear membrane are found. The Golgi region is prominent at *G*. $OsO_4$, $\times 25000$.

Therefore fibres of a cilium probably adopt arrangements like that seen in Fig. 12 in order to be accommodated in a cilium of narrowing diameter.

Ciliary fibres are about 200 Å in diameter. In appearance they resemble cytoplasmic microtubules. The diameter of cytoplasmic microtubules seen in Fig. 5 and in other micrographs taken during this work has been consistently 210–220 Å. Since both ciliary fibres and cytoplasmic microtubules impinge upon or arise from the centrioles or their satellites, the two structures may be identical apart from the double nature of the former.

### *Relationships of cilia to other cell structures*

Cilia are invariably found close to, or within the confines of, the Golgi apparatus. The nucleus, sometimes with a hilar region, often lies in close proximity to these cell organelles. Sometimes fine filamentous regions of cytoplasm surround the centrioles and cilia, as in Fig. 5 (*M*). Similar filamentous regions have been reported by De Petris, Karlsbad & Pernis (1962) and De-Thé (1964).

In the cytoplasm immediately surrounding the diplosome, ribosomes, mitochondria and other structures are almost entirely excluded so that the cytoplasm appears as a fine granular, amorphous region of slightly greater electron density than the general cytoplasmic matrix, especially between the fibres of the centrioles and the fibres of the cilium.

### *Inner cortical zones: zona fasiculata and zona reticularis*

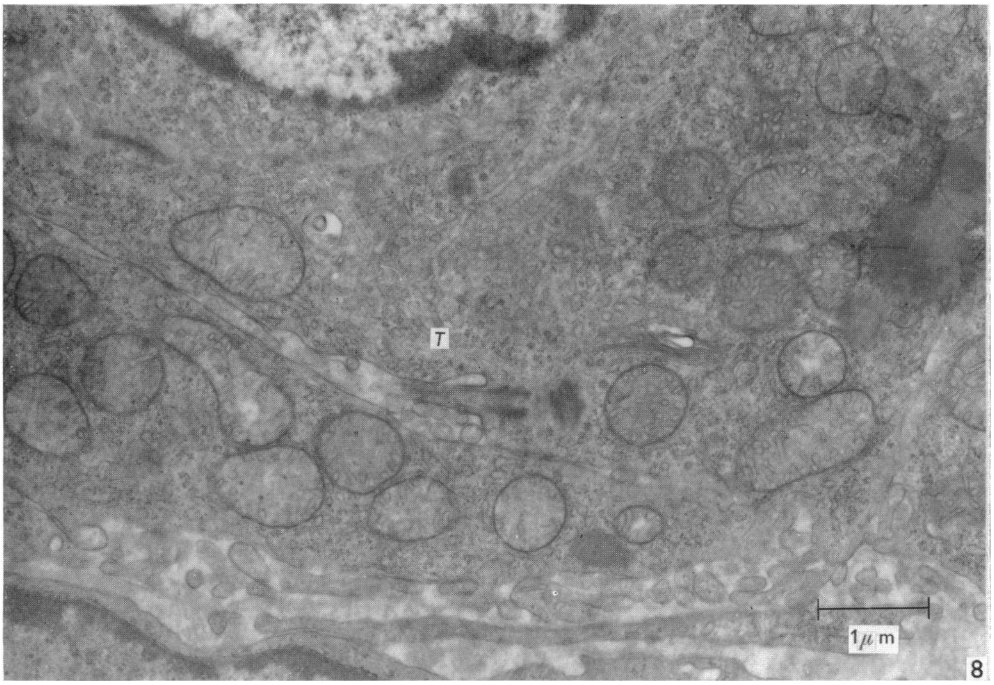
Investigation of the fine structure of the inner cortical zones of the normal rat adrenal has been as intensive as that of the zona glomerulosa. However, no evidence of cilia has yet been found in the cells of the zona fasciculata or zona reticularis of the rat adrenal gland. Centrioles are frequently seen in these cells. A diplosome of a zona reticularis cell is shown in Fig. 13. From the centriole seen in longitudinal section a process can be seen radiating towards the cell membrane, suggestive of this centriole being a basal body of a cilium but there was no evidence of a cilium. Even more suggestive of a centriole being a basal body is the example shown in Fig. 14. In this case there appears to be a thickened rim of cell membrane close to the centriole. Since the centriole is cut in median longitudinal section, it seems likely that if a cilium was present, its base at least would have been cut; again there is no evidence of one.

## DISCUSSION

Cilia have not been described previously in the adrenal cortex. In this paper their presence is demonstrated in the zona glomerulosa of the rat adrenal gland. Although centrioles which seem to have the appearance of basal bodies are found in cells of the inner cortical zones, no cilia have been found issuing from them.

In general morphology, cilia of the zona glomerulosa are similar to those seen in other endocrine organs, being associated with two centrioles and having a 9+0 fibre formula. In the rat adrenal zona glomerulosa detailed descriptions of the intricate centriole-centriole and centriole-cilium relationships have been possible.

The function of these cilia has not been determined. Barnes (1961) considered they were probably sensory since cilia of a 9+0 form have been found in a number of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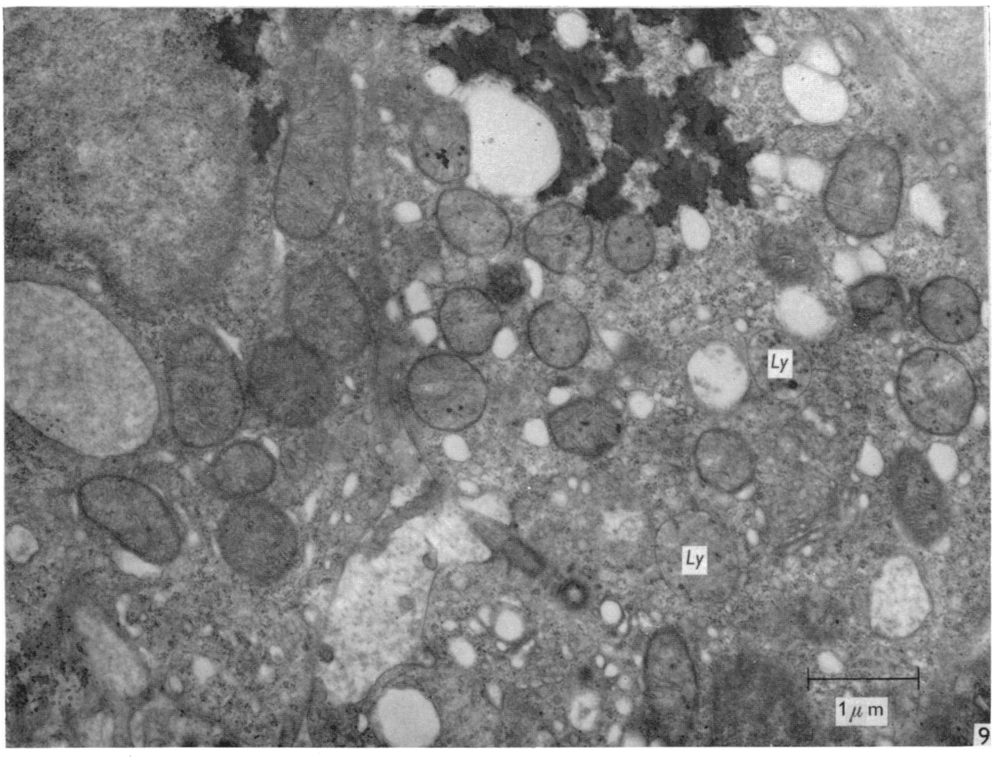
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sensory organs. On the grounds of their modification for a sensory function she suggested they lacked motility. Since it is thought that the central fibres of a 9+2 cilium determine the plane of beat (Afzelius, 1962) there is no reason to suppose 9+0 cilia are incapable of moving. Indeed Afzelius (1962) described a 9+0 flagellum of gametes of a myzostomid worm which was undoubtedly motile. Coupland (1965) reported fibre patterns of 6+2 to 8+2 in cilia of the rat adrenal medulla. He considered that they were modified 9+2 cilia which were motile, stirring up 'stagnant backwaters' of intercellular fluid. On structural grounds alone, fibres seen within the central core of the cilium by Coupland (1965) are not similar to the central fibre pair seen in 9+2 cilia. It is more probable that the unusual fibre arrangements he reported were due to displacement of peripheral fibres into the ciliary core in the same fashion as seen in Fig. 13 and as described by others (Dahl, 1963; Allen, 1965).

Cilia are described in this paper in both normal and abnormal adrenal glands. It is by this kind of comparison that some indication of their function may eventually be found. DMBA causes necrosis of the zona fasciculata and zona reticularis of the rat adrenal gland. Cells of the zona glomerulosa are not destroyed although their environment is markedly altered. They become very active mitotically at about the time adrenal glands were fixed after DMBA treatment and, left *in situ*, they would have regenerated the whole cortex within 3–4 weeks. Subjectively cilia appear more prevalent in the zona glomerulosa of DMBA-treated rats. This could be due to easier detection of cilia which are often situated within large vacuoles of extracellular fluid enclosed within the cells; it could also be due to a real increase in the number of cilia present under the altered conditions. The close connexion of cilia to centrioles has obvious implications as far as mitosis is concerned.

Since cilia may be found retracted in a deep invagination or protruding superficially into intercellular spaces, it is possible that their position can be controlled by the cell. The filamentous cytoplasm around the cilium-centriole complex, the rootlets coming from centrioles and the processes extending from the centriole to the cell membrane may be structures involved in their retraction or protrusion.

The presence of cilia in many different tissues poses some obvious problems apart from their function. First, it would be of interest to know whether every cell of a tissue such as the zona glomerulosa possesses a cilium. Some evidence has been presented by Dahl (1963) which suggests that cilia in the rat cerebral cortex are found in an incidence approaching one per cell. Secondly, since one centriole of a diplosome can produce a cilium, the possibility exists that the other centriole also produces a cilium. Barnes (1961) saw two ciliary transverse sections in a single invagination of a cell of the mouse adenohypophysis, suggesting two cilia arose from the same cell. Definite evidence of two cilia issuing from a single cell has been found in the rat adenohypophysis (Wheatley, to be published). Thirdly, it is of interest to know what happens to a cilium when the cell bearing it enters division.

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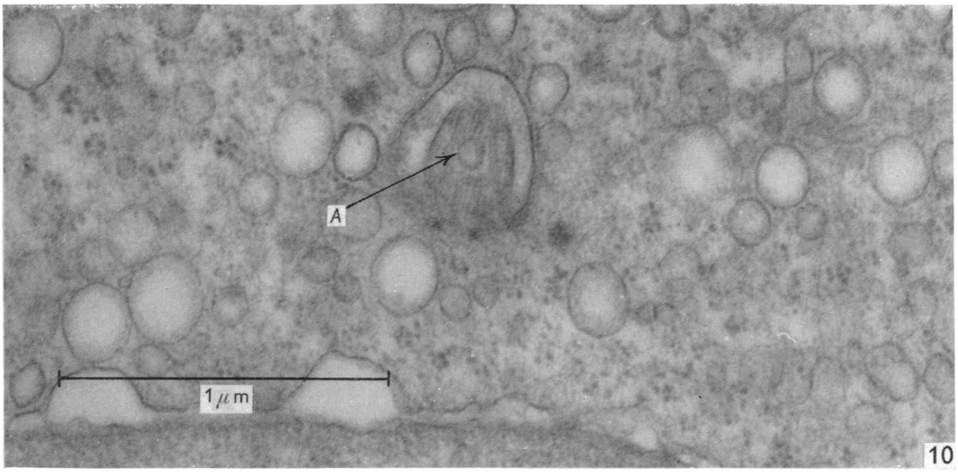
Fig. 8. Zona glomerulosa of an untreated rat showing a superficially situated cilium. Golgi membranes lie near the centrioles. Fine cytoplasmic microtubules run through the cytoplasm into the centriolar region (*T*). Glutaraldehyde, ×14500.

Fig. 9. Zona glomerulosa of a DMBA-treated rat. A superficial cilium protrudes into an enlarged intercellular space, its distal region being buckled against an opposing cell. Banded filaments can be seen connecting the two centrioles. Cytolysosomes are present at *Ly*. $OsO_4$, ×14500.

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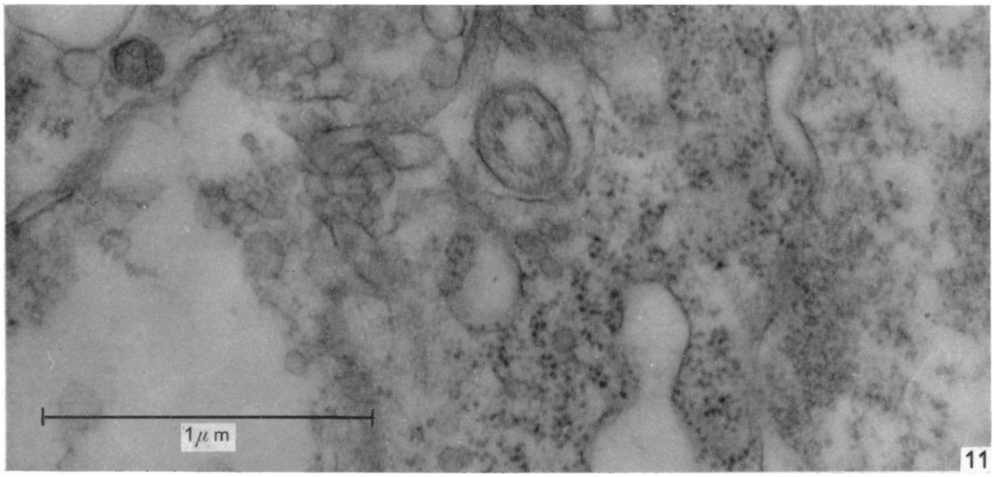


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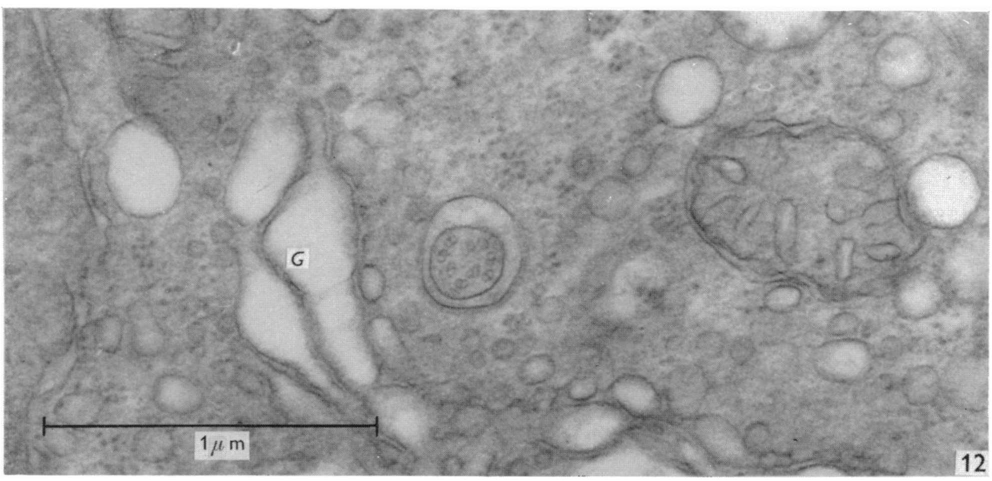


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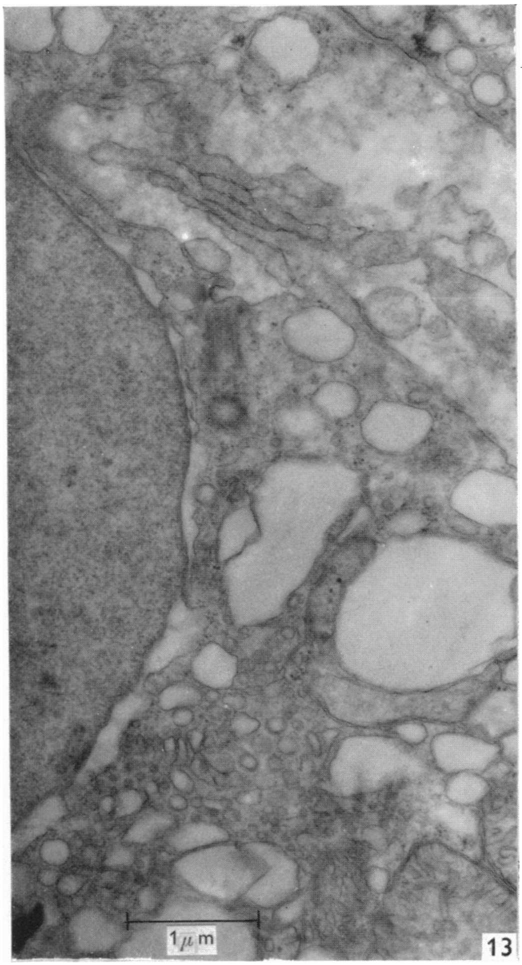
The increasing number of reports of cilia of a 9+0, diplosome associated, solitary nature suggest that these structures are more ubiquitous than was previously realized. It is possible that cilia are produced at some time or other in most tissues.



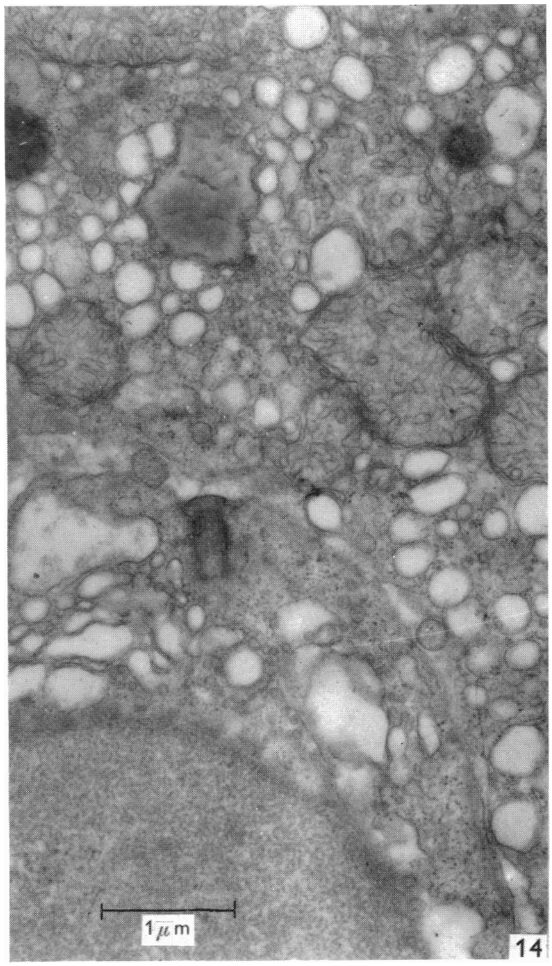




Fig. 13. Zona reticularis cell of a normal rat showing a diplosome. One centriole appears to be attached by a process to the cell membrane. No evidence of a cilium is found. $OsO_4$, ×18000.

Fig. 14. Zona reticularis cells with a single centriole seen in longitudinal section. A thickened rim of membrane occurs at its distal end but no evidence of a ciliary process is seen. $OsO_4$, ×18000.

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Fig. 10. Relatively oblique section of a cilium (near origin) in a zona glomerulosa cell; note the presence centrally of a vacuole (*A*). $OsO_4$, ×43500.

Fig. 11. Transverse section in the proximal region of a cilium showing 9+0 fibre pattern. $OsO_4$, ×43500.

Fig. 12. A transverse section towards the distal part of a cilium showing nine paired fibres. Two pairs of fibres appear to be displaced from the circle formed by the others. Distended vesicles of the Golgi membranes (*G*) surround the cilium. $OsO_4$, ×43500.

On the other hand, if only certain tissues possess cilia, it is of importance to determine why this distinction exists. This could give further indications as to their function.

SUMMARY

Cilia which are associated with diplosomes (paired centrioles) have been found in the zona glomerulosa of the rat adrenal cortex. The fine structure of the cilium-centriole relationship is described. Cilia are basically of the 9+0 fibre arrangement when seen in transverse section but, due to their tapering, rearrangement of fibres occurs in their distal regions. Cilia are often ensheathed in deep invaginations of the cell membrane but can also be found in a superficial position in a cell. After rats have been treated with 9,10-dimethyl-1,2-benzanthracene cilia of the zona glomerulosa often protrude into vacuoles of extracellular fluid lying almost completely within the cells. Although cilia are frequently seen in zona glomerulosa cells no evidence of their presence in the cells of the inner cortical zones has yet been found.

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