THE RELATION BETWEEN CILIA AND MITOSES IN THE MOUSE ADENOHYPOPHYSIS

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INTRODUCTION

In 1961, Barnes (1) reported the presence of cilia in secretory cells of the mouse adenohypophysis. She noted that these cilia differ in several respects from "normal" cilia and flagella with an apparent motile function, but are similar to most cilia to which no motile function can be attributed. Generally speaking, "normal" cilia are located on free cell surfaces and possess a "9 + 2" fibrillar pattern and one basal body, whereas cilia of the second type are not necessarily located on free surfaces and possess a "9 + 0" (or "8 + 1," see reference 2) fibrillar pattern and a basal structure consisting of the pair of centrioles of the cell.

Since the paper by Barnes, the presence of 9 + 0 cilia has been reported in pituitary glands and in pituitary adenomas of a number of mammalian species (3–8) and in cells from many other mammalian organs (e.g., references 9, 10). Although a number of functions has been attributed to the 9 + 0 cilia (e.g., references 2, 9, 11, 12), their physiological role—if existing—is still unknown.

The present work reports on the frequency and distribution of 9 + 0 cilia in the adenohypophyses of mice in a number of physiological conditions, determined by means of large-scale electron microscopic counts. It appears that the distribution of the cilia in the different cell types can provide data concerning cell multiplication in the gland.

MATERIALS AND METHODS

The animals used were: in Experiments I, II, V, and VII, male (C57Bl × DBA)F1 mice; in Experiments III and IV, male C57Bl; in Experiment VI, (C57Bl × DBA)F1 fetuses of unknown sex on the 18th day of gravidity.

Inhibition of the thyroid gland in order to induce a TSH[1]-producing pituitary tumor was effected by a combination of radiothyroidectomy (200 $\mu c^{131}I$, i.p.) and a low-iodine diet containing 0.4% methyl thiouracil, plus distilled water.

Stimulation of the LTH-producing activity of the pituitary gland in order to induce an LTH-producing tumor was effected by 2 mg of estrone per ml of drinking water, in Experiment III supplemented by 0.4 mg of estrone, applied once a week subcutaneously in a cholesterol pellet, and in Experiment IV by castration of the animal.

In Experiment I, differential counts of cells and cilia were performed on the pituitary glands of nine animals in which the thyroid activity was inhibited for 12–19 days. The initial ages of the animals were 5, 8, and 10 wk. 11 animals of the same ages served as controls. The material in this experiment was the same as the material used in reference 13. Ciliary profiles observed on systematically taken photographs were counted, and the type of cell in which each was found was recorded. Extracellular profiles were not taken into consideration. The cells were classified into the following categories: *a*, GH cells; *b*, LTH cells; *c*, LH cells; *d*, FSH cells; *e*, TSH cells; *f*, LH/TSH cells, i.e. those cells which are obviously either LH or TSH cells, but cannot be classified in one of the categories with certainty; *g*, cells of a type provisionally indicated as "halo cells"; *h*, thyroidectomy cells, defined as all cells with an abnormally swollen endoplasmic reticulum; *i*, undifferentiated cells; *j*, "unknown cells," i.e. all cells which could not be classified with certainty in one of the other categories. Details of the electron microscopic technique, the cell-counting procedure, and the criteria used for the classification of the cells are given elsewhere (13).

[1] Abbreviations: TSH, thyroid-stimulating hormone; LTH, luteotrophic hormone; GH, growth hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

In Experiment II, counts were performed on micrographs of arbitrarily chosen blocks from two TSH-producing tumors, 11 months after the induction in a 12-month-old animal. The cells were classified as follows: *a*, thyroidectomy or TSH-tumor cells; *b*, unchanged cells of different types; *c*, cells which could not be classified in *a* or *b* with certainty. The latter category was probably mainly composed of undifferentiated cells and TSH-tumor cells with not very distinct characteristics.

In Experiment III, the pituitary glands of two animals, the LTH production of which was stimulated 16 days before sacrifice, were compared with those of two control animals. The initial age of the animals was 13 wk. The procedures of counting and classification of the cells were exactly as in Experiment I.

In Experiment IV, counts were performed on micrographs of arbitrarily chosen blocks from an LTH-producing tumor, 14 months after the induction in a 3-month-old animal. The cells were classified as follows: *a*, LTH cells; *b*, cells of other types; *c*, cells which could not be classified in *a* or *b* with certainty. The latter category was probably mainly composed of undifferentiated cells and LTH cells with not very distinct characteristics.

In Experiment V, the ciliary profiles: nuclei ratio was determined on randomly taken micrographs of sections of the pituitary gland of a 10-day-old animal. The cells were classified as undifferentiated cells and cells of other types.

In Experiment VI, the overall ciliary profiles: nuclei ratio was determined on randomly taken micrographs of coronal sections of fetal pituitaries. Since most of the cells were small and only sparsely granulated, no attempt was made to distinguish between differentiated and undifferentiated cells.

In Experiment VII, all nuclei and ciliary profiles were counted on complete sections through the growing zone ("voile") of a tissue culture. The tissue had been taken 32 days before from a TSH-producing tumor explant which had been growing in the thigh of its host for 9 months. The medium consisted of 17% human serum and 83% Hanks' balanced salt solution which contained 0.5% lactalbumen hydrolysate; the pH was kept between 7.1 and 7.5.

RESULTS

The morphology of the cilia in the present experiments corresponds to the descriptions of others (e.g., references 1, 2, 11). Some micrographs are given in Figs. 1–3. The presence of two cilia in one cell was found only nine times (see references 1, 2, 9–11, 14–16).

The absolute cilia: nuclei ratio was determined on the basis of the data of Experiment I. For reasons which will follow, undifferentiated and thyroidectomy cells were excluded from the calculation. Ciliary profiles are often poorly recognizable (see reference 11), and the average length of the cilia is unknown, so that no estimate of the absolute number of the cilia can be made by counting ciliary profiles. Therefore, in this calculation only cilia with visible bases were taken into consideration, as ciliary bases are easily recognizable structures with known dimensions. The total number of bases recorded was 204, and the total number of nuclei was 7124 (two partly visible nuclei were considered as one nucleus; cf. the procedure used in a cell-counting chamber). Thus, the ratio in the sections is 204:7124 or 1:34.5.

It can be calculated that ciliary bases are, on the average, visible in 3.0–3.5 sections (of 750 A thickness), and nuclei (with an average diameter of 6 μ in the present material) in 80 sections. Thus, the probability of observing a ciliary base in a cell, divided by the probability of observing the nucleus of the same cell, is 3.0–3.5:80, or 1:23–27.

If each nucleus were accompanied by a cilium, a total of $\frac{7124}{23\text{–}27}$ or 265–310 ciliary bases should have been observed. The number actually observed, 204, is about 70% of this figure. This means that about 70% of the cells contain a cilium.

The distribution of the ciliary profiles in the different cell types does not show striking irregularities: the counts in Experiments I–V, given in Table I, indicate that cells of most types have ciliary profiles: nuclei ratio which lies between rather narrow limits, viz. 1:15.6 and 1:38.8. Exceptions to this rule are the undifferentiated cells which never contained cilia and the cell type which was stimulated in the experiment concerned (thyroidectomy or LTH cells) in which the frequency of the ciliary profiles was considerably lower. The different ages of the animals in Experiment I had no influence on the ciliary profiles: the over-all frequency (thyroidectomy and undifferentiated cells excluded) in the 5-wk-old animals was 1:28.9, in the 8-wk-old animals 1:24.8, and in the 10-wk-old animals 1:29.7. Therefore, the counts in the three age groups were pooled.

In the fetal material in Experiment VI (Table I), 827 nuclei and 11 ciliary profiles were recorded.

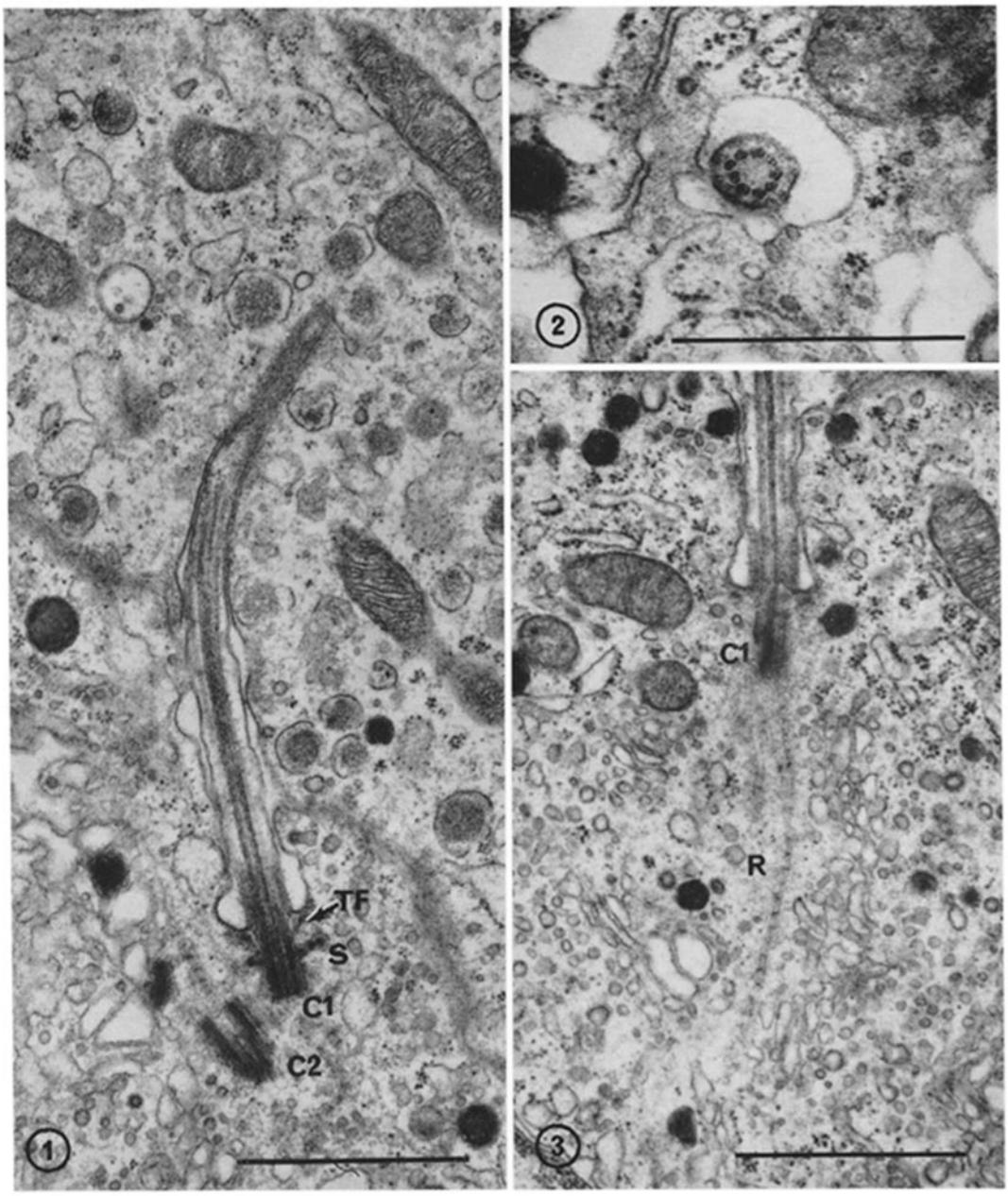

FIGURE 1 Survey micrograph of a 9 + 0 cilium. *TF*, transitional fibers; *S*, satellites; *C1*, distal centriole or basal body; *C2*, proximal centriole. × 32,000.

FIGURE 2 Cross-section of a 9 + 0 cilium. × 41,000.

FIGURE 3 9 + 0 cilium with a long rootlet (*R*) attached to the distal centriole. × 32,000.

TABLE I

Distribution of Ciliary Profiles in Adenohypophyseal Cell Types under Different Conditions

			GH Cells	LTH Cells	LH Cells	Unknown Cells	FSH Cells	TSH + LH/TSH + Halo Cells	Thyrect. Cells	Undifferentiated Cells	Total of Cells*
Experiment I (Effect of thyroid inhibition)	Control	Nuclei	2705	606	473	777	374	266	13	119	5201
		Ciliary profiles	80	21	22	30	16	9	0	0	178
		Cil. prof./Nuclei	1:33.9	1:28.8	1:21.5	1:25.9	1:23.4	1:29.5	—	—	1:29.3
	Exptl.	Nuclei	2098	364	364	707	328	132	409	95	3993
		Ciliary profiles	73	13	19	20	18	8	6	0	151
		Cil. prof./Nuclei	1:28.7	1:28.0	1:19.2	1:35.4	1:28.2	1:16.5	1:68.2	—	1:26.4

			GH Cells	LTH Cells	LH Cells	Unknown Cells	FSH + TSH + LH/TSH + Halo + Thyrect. Cells	Undifferentiated Cells	Total of Cells‡
Experiment II (Effect of estrone treatment)	Control	Nuclei	698	144	85	243	156	27	1326
		Ciliary profiles	18	7	4	9	10	0	48
		Cil. prof./Nuclei	1:38.8	1:20.6	1:21.2	1:27.0	1:15.6	—	1:27.6
	Exptl.	Nuclei	817	335	151	250	88	25	1306
		Ciliary profiles	22	6	9	7	4	0	42
		Cil. prof./Nuclei	1:37.1	1:55.9	1:16.8	1:35.7	1:22.0	—	1:31.1

		TSH-tumor cells	Unknown cells	Total of cells of other types
Experiment III (TSH-producing tumor)	Nuclei	387	55	111
	Ciliary profiles	3	0	5
	Cil. prof./Nuclei	1:129.0	—	1:22.2

		LTH Cells	Unknown Cells	Total of cells of other types
Experiment IV (LTH-producing tumor)	Nuclei	609	103	174
	Ciliary profiles	5	0	9
	Cil. prof./Nuclei	1:121.8	—	1:19.3

		Undifferentiated Cells	Total of cells of other types
Experiment V (10-day-old animal)	Nuclei	30	781
	Ciliary profiles	0	29
	Cil. prof./Nuclei	—	1:26.9

		Total of cells§
Experiment VI (Fetuses)	Nuclei	827
	Ciliary profiles	11
	Cil. prof./Nuclei	1:75.2

		LTH Cells
Experiment VII (Tissue in vitro)	Nuclei	571
	Ciliary profiles	14
	Cil. prof./Nuclei	1:40.8

* In the experimental group, the thyroidectomy cells and the ciliary profiles therein have been excluded from the totals. In both the control and the experimental groups, the undifferentiated cells have been excluded from the total.

‡ In the experimental group, the LTH cells and the ciliary profiles therein have been excluded from the totals. In both the control and the experimental groups, the undifferentiated cells have been excluded from the total.

§ Differentiated plus undifferentiated cells.

Thus, the over-all ciliary profiles:nuclei ratio was low (1:75.2).

In Experiment VII, practically all of the nuclei of epithelial cells in proliferating pituitary material in vitro had the characteristics of those of LTH cells (see reference 17). There were 571 nuclei of LTH cells, accompanied by 14 ciliary profiles, which gives a ciliary profiles:nuclei ratio of 1:40.8. This value is low in comparison with the values for LTH cells in Experiments I and II (Table I). There was some indication that especially in the border of the voile the frequency of the ciliary profiles was low, but the number of profiles was too small to establish this point with certainty.

DISCUSSION

It is well known that fully differentiated cells in the pars distalis of the pituitary gland have the capacity to undergo mitosis (e.g. references 18–21). In the normal, postnatal pituitary gland this capacity is seldom manifested, but different treatments can restimulate cell multiplication. The present work shows that most of the cells contain a cilium, but that cells which can be considered to have a high mitotic activity contain one less frequently. This holds true: *a*, in the fetus, in which the over-all frequency of the cilia is low; *b*, in glands to which a tumor-inducing treatment was applied which stimulates one of the cell types excessively, and where the cells of the type concerned lose their cilia as a reaction to the treatment; *c*, in pituitary material in vitro consisting of multiplying LTH cells which have also relatively few cilia. Considering that centrioles cannot simultaneously be involved in mitosis and constitute a part of the basal structure of a cilium, the reverse relationship between the presence of 9 + 0 cilia and the occurrence of mitoses is not surprising.

The significance of the absence of cilia in undifferentiated cells in the postnatal pituitary will be discussed in a separate paper.

The frequency of mitoses in the normal as well as in the stimulated pituitary gland is so low (13, 22) that it is extremely difficult to determine which cells are multiplying at any given moment. However, since the period during which the cilium is absent is apparently much longer than the visible phase of the mitosis, the multiplying cells can be detected by determining the frequency of the ciliary profiles in the different cell types.

The author wishes to thank Drs. Constance A. Feltkamp, Peggy Nakayama, H. G. Kwa, G. Zeilmaker, and C. Homburg for their help during the experiments and during the preparation of the manuscript.

Received for publication 16 January 1969, and in revised form 2 June 1969.

Note added in poof: After completion of the manuscript, the selective enhancement of mitotic activity in thyroidectomy cells soon after thyroid inhibition was confirmed by the results of a preliminary radioautographic experiment. In this experiment a male mouse, made thyroprivic 16 days before, was injected with 5 mc thymidine-^{3}H, spread over a period of 10 hr. In electron microscopic radioautographs, 42 labeled nuclei of epithelial cells were detected; 29 (or about 70%) of these belonged to thyroidectomy cells, whereas the thyroidectomy cells constituted only about 10% of the population.

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