

adult, whereas in the Brown Leghorn adult the differentiation of the feather-pigmenting melanophores is markedly affected by oestrogens. Although this argument was never substantiated by experiments, it was generally accepted, and the studies of HAMILTON were discarded⁵.

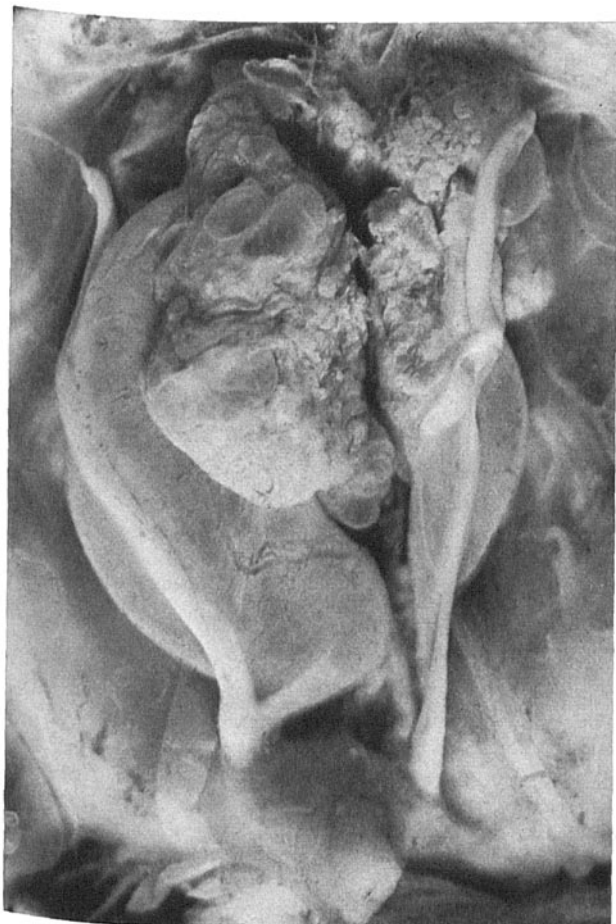


Fig. 6. Internal sex organs of case No. 4885. Minute remnant of right ovary. Right Md is fully preserved, the left Md is interrupted in its mid-portion due to mechanical damage. The Wolffian ducts are tremendously distended as a result of the action of the testosterone propionate; both mesonephroi show multiple cysts.

The experiments reported here offer the *in vivo* duplication of HAMILTON's *in vitro* experiments. Substitution of the ovarian hormone by oestrogens, androgens or embryonic testicular hormone restores the production of red pigment by the feather-pigmenting melanophores. The degree of restoration depends upon the dose of gonadal hormones administered. This suggests a local influence of the hormones on the melanophores. The latter do not distinguish between oestrogens and androgens. This explains why in the experiments of WILLIER and RAWLES⁶ skin ectoderm of 72-hour-old chick embryos of various crosses with a sex-linked down, transplanted into hosts of the same age, invariably developed the feather pigmentation of the genetic sex, independently of the sex of the host. In the case of the Rhode Island or New Hampshire breed, the implanted skin ectoderm always encountered a hormonal environment in the host, either male or female.

The melanophores of the genetic males of the cross New Hampshire ♂ × Light Sussex ♀, apparently lack the potency of developing red pigment due to their genetic constitution^{7,8}.

Zusammenfassung. Die rote Daunenpigmentierung der weiblichen F₁ Hybriden der Hühnerrassen Cross New Hampshire ♂ × Light Sussex ♀ entsteht nicht ohne Ovarialhormone, wie Kastrationsexperimente beweisen. Sie kommt aber zustande, wenn man ganz oder subtotal kastrierten Embryonen Östrogene oder Androgene zuführt oder embryonale Keimdrüsen implantiert.

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⁶ B. H. WILLIER, *Archs Anat. microsc. Morph. exp.* 39, 269 (1950).

⁷ B. H. WILLIER and MARY E. RAWLES, *Physiol. Zool.* 13, 177 (1940).

⁸ It is a pleasure to thank Prof. Dr. W. K. HIRSCHFELD, Chairman of the Department of Poultry Breeding and Husbandry of Utrecht University, for his kind cooperation in supplying eggs from the cross New Hampshire ♂ × Light Sussex ♀, used in these experiments.

⁹ The generous supply of Orchisterone by Frosst Company and of Dimenformon by Organon Ltd. is gratefully acknowledged.

Ciliated Biliary Epithelial Cells in the Livers of Non-Human Primates

Cilia¹ in epithelial cells of intrahepatic bile ducts (ductular cells) have been observed in normal livers of just one species (rat)². They have been found much more frequently in a variety of conditions of pathological nature affecting livers of not only rat^{3,4} but also man^{4,5}. Cilia in normal liver of man or any other primate have not been described as a regular feature of the biliary tree. This paper reports the constant occurrence of ciliated cells in livers of squirrel monkeys (*Saimiri sciureus*,

Voigt 1831) shortly after admission to the colony and before any treatment was instituted.

We have studied liver biopsies from 14 young male *S. sciureus* monkeys, which were obtained with the

¹ E. D. DE ROBERTIS, W. W. NOWINSKI, and F. A. SAEZ, *Cell Biology* (W. B. Saunders Co., Philadelphia 1965), p. 385.

² J. W. GRISHAM, *Proc. Soc. exp. Biol. Med.* 114, 318 (1963).

³ J. W. GRISHAM and E. A. PORTA, *Expl Cell Res.* 37, 190 (1963).

⁴ J. W. GRISHAM and E. A. PORTA, *Expl Molec. Path.* 3, 242 (1964).

⁵ I. STERNLIEB, *J. Microsc.* 4, 71 (1965).

Menghini needle under light sedation. Light microscopic studies were carried out in portions of the biopsies fixed in Baker's solution⁶. Frozen sections were stained with oil red O⁷, and paraffin sections with hematoxylin and eosin, periodic acid Schiff's method and Masson's trichromic stain for connective tissue. Other portions were immersed in Dalton's fixative⁸ and embedded in Epon 812⁹ for electron microscopy. Thick sections of plastic-embedded tissue (0.5–1.0 μ) were stained with toluidine blue¹⁰ and suitable fields containing bile ductules were selected under the light microscope. This technique enables precise identification of the lobular area to be examined under the electron microscope. Lead¹¹ and uranyl acetate¹² stains were used.

By light microscopy the hepatic architecture in all cases was normally preserved and no signs of cholestasis were found. In some livers (9 out of 13), mild to moderate degrees of fatty changes of imprecise lobular distribution consisted of droplets and small globules in the cytoplasm of hepatic parenchymal cells. Nuclear displacement by accumulated cytoplasmic fat has not occurred. However, these changes did not correlate with the presence of cilia (Table).

Biliary epithelial cells of monkeys do not differ in their ultrastructure from those of other species studied, including man^{13–17}. Ductular cell configuration, microvilli, attachment zones and cell membrane indentations all conformed to the previous descriptions cited.

An important additional feature however for the biliary epithelial cells of squirrel monkeys was the presence of cilia. They were observed within the lumina of most intrahepatic bile ductules and their number ranged from

1–3 in the plane of each section (Figure 1). In such a single plane, one cilium could be expected for every 3 or 4 cells in the section. Some cilia were found in biliary recesses (Figure 2). A basal body (kinetosome) was frequently found in the cytoplasmic portion of the ductular cells facing the lumen (Figure 3), usually in continuity with the ciliary shaft (Figure 4)). Cilia were consistently

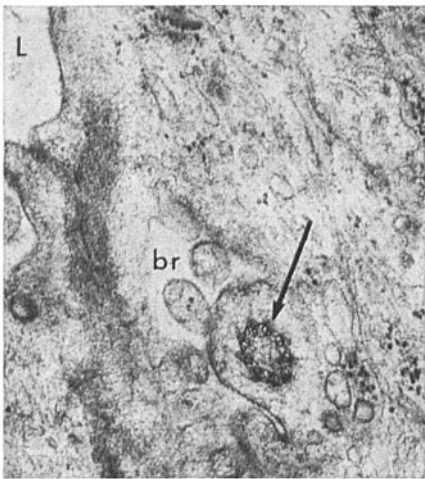


Fig. 2. Cross section of basal body (arrow) found in a biliary recess (br). The lumen of the duct is indicated by L (top left). Lead stain, $\times 45,400$.

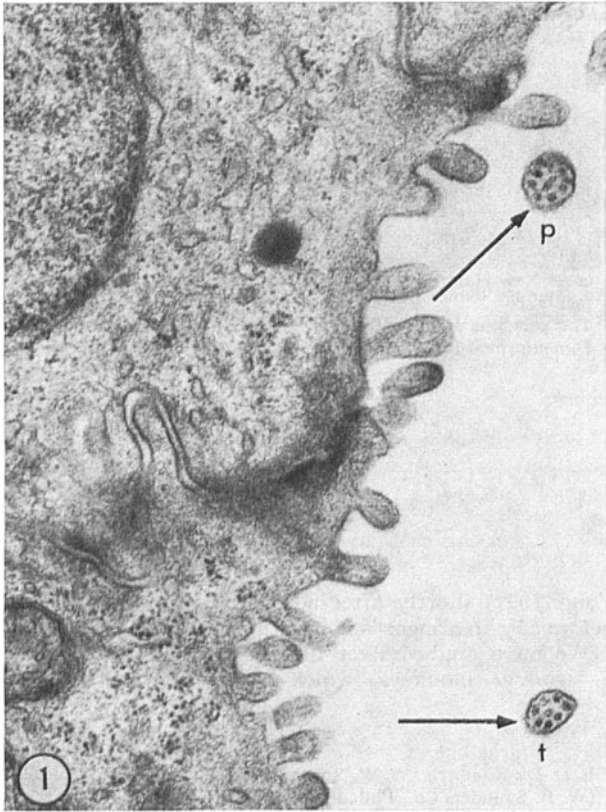


Fig. 1. Cross section of cilia (arrows), in the lumen of a bile duct. 2 different patterns of the disposition of ciliary filaments are observed: proximal (p) and distal (t). Lead stain, $\times 32,500$.

Correlation between fatty changes in hepatic parenchymal liver cells and the presence of cilia in biliary epithelial cells of monkeys^a

Degree of fatty changes	No. of animals	Presence of cilia ^b
–	4	present
+ or ++	5	present
+++	4	present
++++	0	–

^a The criteria employed were as follows: + = small droplets equal to the nucleolus in size, sparsely distributed in few periportal parenchymal cells; ++ = almost every cell in periportal regions and eventual globules; +++ = diffuse distribution of droplets and globules through the lobule; ++++ = formation of large vacuoles and nuclear displacement. ^b Cilia found in a frequency of 1 for every 3–5 ductular cells.

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¹³ J. W. GRISHAM and W. S. HARTROFT, *Lab. Invest.* 10, 317 (1961).
¹⁴ J. E. MOSQUERA, F. A. DE LA IGLESIA, and E. A. PORTA, *Revta Fac. Cienc. med. Univ. nac. Cordoba* 27, 7 (1963).
¹⁵ E. A. PORTA, Thesis, Univ. of Buenos Aires, 1961.
¹⁶ J. W. STEINER and J. S. CARRUTHERS, *Am. J. Path.* 38, 639 (1961).
¹⁷ J. W. STEINER, J. S. CARRUTHERS, and S. R. KALIFAT, *Expl Molec. Path.* 1, 162 (1962).

found in all cholangioles observed. In cross sections of cilia, a single membrane could be traced surrounding them. The patterns displayed by the matrical filaments were variable ($9 + 2$, $8 + 2$ and $7 + 2$), whether the sections passed through the proximal or distal part of the ciliary shaft (Figure 1). The total length of the basal body fell on the range of 450–470 nm and the diameter 220–250 nm. Diameters of cilia in different cross sections ranged from 225–250 nm and the longest shaft measured in the photograph about 1.2μ .

Cholangiocilia have been found after much search in livers of normal rats². But they have been observed in abundance in intrahepatic epithelium of rats and in man under abnormal conditions^{3,4}. They have been reported in cholangiolar proliferation in livers of rats fed choline-deficient diets^{3,4}, ethionine feeding^{3,4}, α -naphthylisothiocyanate^{3,4,18}, extrahepatic biliary obstruction^{3,4,17} and subcutaneous transplantation of the liver^{3,4}. In man, cholangiolar cilia have been reported in only 2 conditions: in cirrhotic livers associated with Wilson's disease⁵ and extrahepatic cholestasis³. Their occurrence in epithelial

cells of intrahepatic bile ductules in one species of non-human primate can now be added to the above. Lack of correlation between stainable fat and the presence of cilia led to the conclusion that the presence of fatty changes had no obvious bearing on the frequency of cilia.

GRISHAM² and GRISHAM and PORTA^{3,4} made no attempt to resolve the fine structure of the cilia they found in rats and man, but there is no evidence that the type encountered in their photographs differs significantly from what we are describing.

The different patterns in the arrangement of filaments found in cilia of monkeys could undoubtedly be attributed to the level of sectioning. The classic studies of SATIR¹⁹, using the gill of *Elliptio complanatus*, showed that they depend on the level in which cilia are sectioned.

In other situations, cilia have also been found in Schwann cells²⁰, fibroblasts²¹, rod cell retina²², chromaffin cells^{1,23}, neurones^{14,23,24}, tumor cells²⁵, immature β -cells of the pancreas²⁶, smooth muscle cells²¹, ciliated epithelia²⁷ and cells of the kidney²⁸ and pituitary²⁹. Different interpretations regarding their possible significance and function have been advanced in these varying contexts, and particularly their presence in the biliary epithelium of some species is still unknown. Their ubiquitous filogenetic character would suggest that they are possibly a constant feature of the normal biliary epithelium of several species, exaggerated in the pathologic conditions in which these cells proliferate³⁰.

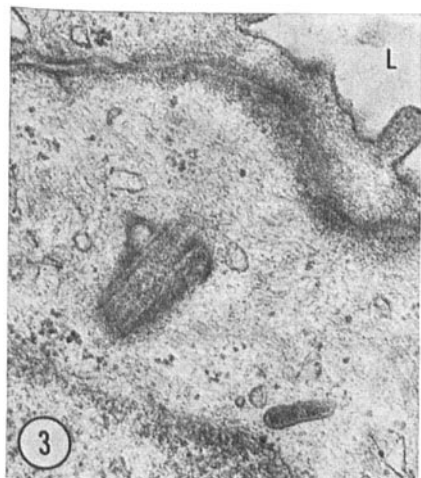


Fig. 3. A basal body (center) found in the cytoplasm of a biliary epithelial cell facing the lumen of the duct (L). Lead stain, $\times 37,400$.



Fig. 4. Ciliary shaft emerging from the cytoplasm and extending into the lumen (L). The cell membrane infolds and covers the ciliary elements (arrows). Lead stain, $\times 40,000$.

Zusammenfassung. Das Vorkommen von Kinocilien im Epithel der intrahepatischen Gallenwege bei einer Affenart (*Saimiri sciureus*) wird beschrieben. Diese Cholangiocilien finden sich relativ häufig in vollständig normalen Lebern bei dieser Affenspezies. Die Ultrastruktur dieser Cilien variiert entsprechend der Schnitthöhe und stimmt mit dem typischen Bau der Cilien anderer Zellen überein.

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²¹ S. SOROKIN, J. Cell Biol. 15, 363 (1962).

²² E. D. DE ROBERTIS, J. biophys. biochem. Cytol. 2, 319 (1956).

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²⁵ K. MANNWEILER and W. BERNHARD, J. Ultrastruct. Res. 1, 158 (1957).

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³⁰ Acknowledgment: The kind criticism and suggestions of Prof. Dr. W. S. HARTROFT are greatly acknowledged. This work was supported by a grant from the Medical Research Council of Canada (Fund No. MT-1904).

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