wet earwax cannot be phenotypically distinguished from the heterozygous type. Dry cerumen occurs with low frequency in Caucasians and Negroes whereas it is preponderant in Mongoloids.

We have examined the cerumen from 432 Indians of the Mississippi Choctaw tribe. Our sample was drawn from patients, outpatients, and ward visitors at the Public Health Service hospital in Philadelphia, Mississippi, as well as from Indians seen in their homes, at field clinics, and at the annual Choctaw Indian fair. They were randomly selected from all age groups; individuals with ear infections were excluded.

Bilateral otoscopic examination was performed. Wet cerumen was light tan to dark brown in color, thick, and always sticky. The dry type was flaky or granulated, ranged in color from black through tan to light grey, and did not adhere to the instruments or the fingers. Stickiness was the ultimate criterion in cases of doubt on inspection, requiring curettage and extraction of cerumen.

Ninety individuals had dry cerumen, 333 had wet cerumen, and 9 could not be classified. This represents a phenotype frequency of 21 percent dry and 79 percent wet, excluding indeterminates. Gene frequencies were calculated by the Hardy-Weinberg law. The estimated gene frequency for the recessive dry allele (q) is 0.46, and the overall genotype frequencies are 0.212 homozygous dry, 0.291 homozygous wet, and 0.497 heterozygous wet.

Petrakis *et al.* (*2*) found an overall incidence of 51.3 percent of dry cerumen in a miscellaneous group of 482 Indians, predominantly Sioux and Navaho. They found that the greater the proven admixture of Caucasian with Indian, the lower the frequency of dry earwax. They suggested that Caucasian admixture was the major cause of the lower frequency of dry cerumen among American Indians as compared to their presumably Mongolian ancestors, who have frequencies of dry cerumen as high as 90 percent or greater (*1*).

The frequencies of dry cerumen (ranging from 3 to 8 percent) among Mayan Indian groups in southern Mexico (*3*) are extremely low in comparison to those for North American Indians, in spite of almost complete isolation of the Mayans from their Spanish conquerors. The frequency of only 21 percent of dry cerumen among Choctaw Indians is intermediate between that of the Mayan and western North American Indians. The Choctaws claim to be full-blooded and have distinctly Indian physiognomy. Hospital patients were routinely typed as blood group O, although the exact frequency of ABO blood groups has not been determined. With this evidence of decreasing frequency of dry cerumen with increasing distance from the Bering Strait, the possibilities of early genetic drift, or mixture with prehistoric non-Mongoloid immigrants, deserve further consideration as additional explanations for variation in cerumen types. It would be interesting to compare the frequency of cerumen types among Mississippi Choctaws with that of Oklahoma Choctaws, who were removed from Mississippi by treaty in 1830.

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Microtubules in Spermatozoa of Childia (Turbellaria, Acoela) Revealed by Negative Staining

Abstract. *Many intact axial units, with attached basal plates, are found in spermatozoa of* Childia groenlandica *negatively stained with phosphotungstic acid. Electron micrographs show a total of nine doublet microtubules, confirming observations on sectioned material, where nine peripheral doublets, but no single central ones, occurred. Living spermatozoa move by waves progressing along a double undulating membrane.*

It has been thought that nonmotile or sensory flagella and cilia are characterized by the presence, in their axial units, of nine doublet microtubules and by the absence of the two central microtubules. Conversely, motile and nonsensory flagella and cilia have the usual 9 + 2 arrangement (*1*) or the so-called 9 + 1 arrangement found in several platyhelminth spermatozoa (*2*). The spermatozoa of the acoel turbellarian *Childia groenlandica* are an exception, however. They are motile (*3*), but both sectioned material and negatively stained preparations reveal the presence of only nine peripheral doublets, with no evidence whatsoever of the central two. (This will be referred to hereafter as the 9 + 0 pattern.)

For electron microscopy, entire animals were fixed in 6 percent glutaraldehyde in cacodylate buffer for 2 hours, treated with 1 percent osmium tetroxide, embedded in Araldite, and sections were stained with uranyl acetate. Thin sections were examined with the Philips 100 or Zeiss electron microscopes. Negatively stained material was prepared by cutting up the intact animals with fine needles, and digesting them in 1 percent phosphotungstic acid (PTA) (*4*); the digests were then transferred to 200-mesh copper grids coated with Formvar and carbon, and examined with the Zeiss electron microscope.

During spermiogenesis in *Childia,* two free flagella are given off by the young spermatid. As seen in sections, these have a 9 + 0 pattern of doublet microtubules (Fig. 1), but are quite motile in the living condition. By a complex series of events (*5*), these free flagella are then incorporated into a cytoplasmic sheath which appears rather suddenly and which undergoes further changes to become the two undulating membranes of the mature spermatozoon's tail (*3*). Movement of the spermatozoon occurs by waves which arise independently. These pass along the edges of the undulating membranes, from the tip of the tail toward the head; the motion is similar to that of trypanosomes.

In thin sections (Fig. 1), the two axial units (each about 0.18 μ in diameter) of the incorporated flagella are completely surrounded by the cytoplasm of the undulating membranes, and lack the two central microtubules. In contrast, the cilia on the body surface have the usual 9 + 2 pattern.

Many intact axial units (nine doublets with at least the basal plate of the basal body still attached) were found in negatively stained preparations (Fig. 2). No cases have been observed where more than nine doublets were present. The very few cases where less than nine could be counted presumably would be a consequence of the breaking away of one or more of the tubules. The fact that no more of the basal body was present in any of the observed axial units than is shown in Fig. 2 may indicate that an inherent weakness in the organelle causes the basal plate to split away from the remainder

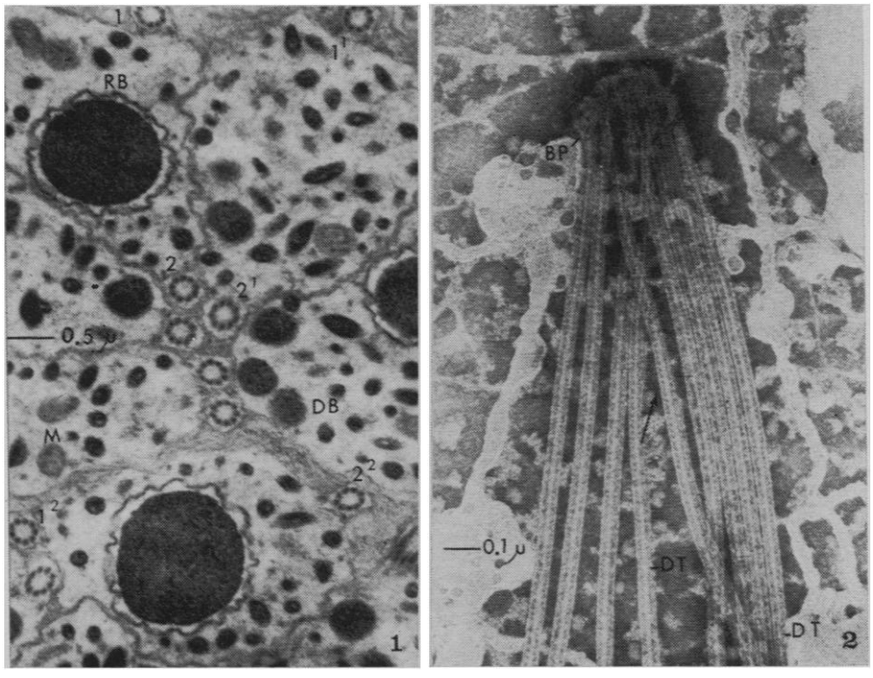

Fig. 1 (left). Transverse section of spermatozoa in testis of *Childia groenlandica*, through tail region. Paired axial units of three spermatozoa are labeled (*1* and *2*; *1'* and *2'*; *1²* and *2²*, respectively), each with nine peripheral doublet microtubules but no central ones. *DB*, dense bodies; *M*, mitochondria; *RB*, refractile body (× 15,000). Fig. 2 (right). Isolated negatively stained intact axial unit from tail of *Childia* sperm, with attached basal plate. Nine doublet microtubules are present; arrow indicates region showing periodicity. *BP*, basal plate; *DT*, doublet microtubules (× 50,000).

of the structure, or this may be an unusually flat, but entire, basal body. Evidence from sectioned material favors the second interpretation, but the question is not yet definitely settled.

The structure of the microtubules has well-defined periodicity (Fig. 2) with a repeating period for the striations of approximately 150 Å; the diameter of each doublet is about 400 Å. In micrographs made at greater magnifications, longitudinal protofibrils can be seen in the walls of the microtubules. Studies with higher-resolution microscopy will be necessary to establish the number and morphology of these filaments and the precise dimensions of the subunits.

Isolated microtubule doublets were often very sharply bent. This suggests that the microtubules, rather than being solid rods, have an outer wall differing greatly in density from the interior (*6*). The brittle quality of the outer walls, under the preparatory conditions employed, is also indicated. Similar sharp bends occur in microtubules of other platyhelminth spermatozoa (*7*).

In contrast to the findings of Pease (*6*), we observed no cases where microtubules were joined together at their distal ends by the amorphous cloud of material which he illustrated. Indeed, there was a definite tendency in our preparations for the doublet microtubules to separate from one another at their distal ends. The macerating action of PTA on spermatozoa of *Childia* appears to begin at the distal ends of the axial units, and to progress proximally quite rapidly.

We have found that the 9 + 0 pattern of microtubules occurs in spermatozoa of the acoel *Polychoerus carmelensis* which, under certain specific circumstances (*8*), are motile (*3*). There are only two other reports of such a 9 + 0 configuration in motile male gametes, that of Desportes (*9*) for the eugregarine *Stylocephalus*, and possibly that of Afzelius (*10*), for *Myzostomum*. The motility of male gametes of *Stylocephalus* was established by Léger (*11*). The acoels *Childia* and *Polychoerus* are also the first cases known to us where spermatozoa of platyhelminths have a 9 + 0 pattern. Several workers have described a configuration in turbellarian, trematode, and cestode spermatozoa (*2*), in which the central portion of the axial unit is occupied by a single electron-dense core unit, frequently connected to the nine peripheral doublets by amorphous strands to give a wagon-wheel effect. A system of cytoplasmic microtubules (*12*) just under the plasma membranes of the cells also occurred frequently in those spermatozoa. We found no evidence of such cytoplasmic microtubules in sectioned *Childia*, although at certain stages in spermiogenesis there may be a manchette of microtubules temporarily ensheathing the nucleus.

Grimstone and Klug (*13*) found intact axial units in the negatively stained flagella of *Trichonympha* and other flagellates, but they published no illustrations of the basal body. Anderson *et al.* (*14*) also isolated intact axial complexes of microtubules from cilia and Burton has an illustration (*2*, Fig. 21) of the entire complement of microtubules at one level of the spermatozoon of the lung fluke.

Silveira and Porter, Hendelberg, and Tulloch and Hershenov (*2*) have all suggested that the so-called 9 + 1 pattern may be the normal one for spermatozoa of Platyhelminthes. Our results contradict this generalization, and in addition provide a case where spermatozoa and cilia of the same animal have a 9 + 0 and a 9 + 2 pattern, respectively, both being motile.

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