obtained at biopsy of those patients and of all other patients with early acute glomerulonephritis (Fig. 1). Serums taken during the first few days after onset stained material from biopsy obtained early in the disease less intensely, compared to serums obtained 2 weeks later. Blocking tests with the patient's unlabeled γ-globulin eliminated the fluorescent staining. The labeled γ-globulin fractions from patients with acute glomerulonephritis did not stain normal renal tissue or that from patients with subacute glomerulonephritis, chronic glomerulonephritis, systemic lupus erythematosus, pure nephrosis, diabetic nephropathy, discoid lupus, and pyelonephritis.

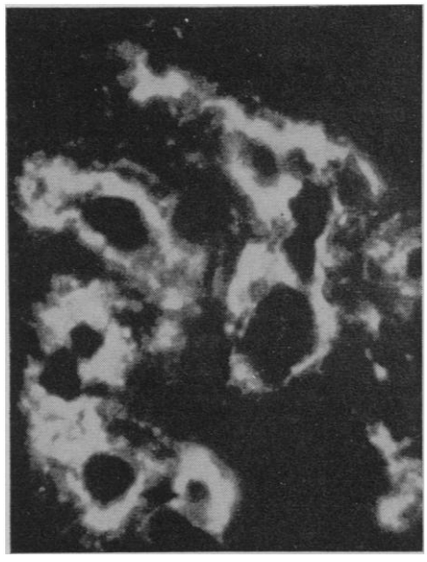

Fig. 1. Part of glomerulus from a patient with acute poststreptococcal glomerulonephritis stained with his own fluorescein-labeled IgG. Day 4 of clinical disease. Oil immersion (× 750).

Fig. 2. Part of glomerulus from the same patient as Fig. 1; stained with fluorescein-labeled antiserum to streptococcal plasma membrane. Oil immersion (× 750).

Tissues taken from two patients 3 months after onset of disease continued to stain with antiserums to human IgG and complement; however, they no longer stained with the patient's own labeled IgG or with any of the other serums from patients with acute nephritis. Serums obtained at time of this later biopsy, however, continued to stain renal tissues of patients in the early phase of the acute glomerulonephritis.

Renal tissue of patients in the early stage of acute glomerulonephritis was stained positively, but less intensely, by labeled IgG fractions from 11 out of 15 serums obtained from "normal" adults.

To investigate the nature of the factor combining with the IgG component —probably a circulating antibody—we used various bacteria for absorption of the labeled serums. When the labeled serums were absorbed with uniform amounts (5 mg) of sediments from repeatedly frozen and thawed *Streptococcus mitis, Streptococcus fecalis, Staphylococcus aureus, Staphylococcus epidermidis,* and *Escherichia coli,* their staining capacity was not abolished. However, when the serums were absorbed by three identically treated nephritogenic strains of β-hemolytic streptococci (Lancefield group A) the staining was markedly reduced or completely abolished.

The nephritogenic streptococci were fractionated into bacterial wall (*6*), M protein (*7*), and plasma membrane (*8*). The fluorescein-labeled serums of the nephritic patients and certain normal individuals first absorbed with the plasma membrane did not stain. Previous absorption of the serums with streptococcal cell wall and M protein reduced the staining capacity slightly, but all our preparations still contained some plasma membrane constituents. Antiserums against β-hemolytic streptococcal plasma membrane were produced in the rabbit and labeled with fluorescein isothiocyanate. These labeled serums stained the glomeruli of patients with early acute glomerulonephritis just as they stained the serums from patients with glomerulonephritis (Fig. 2).

Our results indicate that streptococcal components and, more specifically, plasma membrane constituents are present in the glomeruli of patients with acute poststreptococcal glomerulonephritis, but apparently these can be detected only during the early phase of the disease when not all antigenic sites are fully saturated and when only insufficient amounts of antibody are available. The possibility that antigen is removed after the initial insult appears improbable because antibody and complement that are bound in the original process can still be demonstrated after antigen can no longer be stained. The constituent in the IgG fraction is directed against a specific component of β-hemolytic streptococci, as the absorption studies suggest. All patients with acute glomerulonephritis examined so far seem to share the same antibody, and many normal individuals also have it. The presence of the antibody in "normal" individuals may be due to previous experience of these adults with the specific streptococcus resulting in a long-lasting antibody level.

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Cerumen Types in Choctaw Indians

Abstract. *Cerumen from 432 Choctaw Indians from Mississippi was classified as sticky or dry. The frequency of the autosomal recessive dry type was intermediate between that for Indians of western North America and that for Mayan groups of southern Mexico.*

Human ear cerumen, which occurs in two distinct forms, wet and dry, is under the control of a single gene pair in which the allele for the wet trait is dominant over that for the dry (*1*). Thus cerumen types are inherited as a simple Mendelian trait. Homozygous

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