# LACK OF DYNEIN ARMS IN IMMOTILE HUMAN SPERMATOZOA

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ABSTRACT

Spermatozoa from two brothers who are not twins were found to be straight and immotile. Examinations of the sperm showed that oxygen consumption and lactic acid production were normal; viability tests showed that the percentage of dead sperm was not increased.

The ultrastructural appearance of the sperm tail was normal except for a complete lack of dynein arms and some irregularities in the arrangement of the accessory fibers and the longitudinal columns of the fibrous sheath. The mitochondrial apparatus and the sperm head conform to the conventional model.

According to the sliding-filament hypothesis first proposed by Afzelius (1959. *J. Biophys. Biochem. Cytol.* **5**:269.), the arms are responsible for the bending movements of the tail. The simplest explanation for the simultaneous lack of arms and sperm motility appears to be that the two brothers have a genetic disorder involving production, assembly, or attachment of the dynein arms.

Spermatozoa swim by undulations of the sperm tail, which is a simple or a modified flagellum. The simple flagellum is prevalent in most lower animals and has two central microtubules surrounded by nine doublets, a 9 + 2 pattern indistinguishable from that of a cilium. Mammalian spermatozoa have a modified flagellum with an outer ring of accessory or coarse fibers, and thus a 9 + 9 + 2 pattern; this ring is further surrounded by the fibrous sheath and the cell membrane. The 9 + 2 or 9 + 9 + 2 filaments constitute the axoneme.

The detailed fine structure of the axoneme was first described in sea urchin sperm (1). It was shown that the nine doublets have projections which cross-bridge neighboring doublets. The projections were named "arms" and assigned a function in sperm movement. They were assumed to be capable of both holding the neighboring doublet in a steady grip and generating an active sliding of any one doublet relative to its neighbor.

Support for this sliding-filament hypothesis came from biochemical investigations by Gibbons and his co-workers (9, 10), who isolated the arms and showed them to be an ATPase. This is to be expected of a structure in which a transformation of chemical into mechanical energy takes place. Direct experimental evidence came from ultrastructural studies by Satir (20) who investigated the way by which the doublets terminate in straight and bent cilia, and from dark field microscope observations of activated fragments of detergent-treated sperm tails (11, 22).

The purpose of the present communication is to report on the ultrastructural alterations in the tails of the sperm of two brothers who have immotile spermatozoa although the percentage of dead spermatozoa is not increased. The reason for

sperm immotility seems to be a lack of "dynein" arms.

## MATERIALS AND METHODS

Patient A was born in 1938 and his brother (patient B) in 1943. Both brothers have chronic sinusitis and bronchitis and have been treated with various antibiotics for many years. Patient A also suffers from chronic otitis media, bronchiectasis and situs inversus totalis (Kartagener's syndrome). Careful clinical examination of the two brothers has not revealed any signs of hypogonadism, endocrine disorders, or any somatic disturbances other than those presented above. Their sister has no history of chronic infections and has children of her own.

Semen analyses were carried out as described in detail by Eliasson (4, 5) and included determinations of the number of spermatozoa, spermatozoan motility, morphology and viability (8), oxygen consumption (4), fructose consumption, and lactic acid formation. Fructose in the seminal plasma was determined according to Karvonen and Malm (14), and lactic acid according to Barker and Summerson (3). Zinc and magnesium were determined in seminal plasma and in the spermatozoa (6, 7, 15). The effect of caffein on sperm motility and metabolism was determined according to Johnsen et al. (13).

The spermatozoa were fixed according to the method of Pedersen (17, 18), by mixing the ejaculate with 2 vol of fixative consisting of 4% glutaraldehyde and 4% sucrose in 0.2 M collidine buffer (Sigma Chemical Co., St. Louis, Mo.), pH 7.4.

After a light centrifugation and a short rinse in sucrose-collidine, the samples were postfixed in 1.5% $OsO_4$ in a 0.2 M collidine buffer containing 6.6% sucrose. The material was dehydrated in a graded ethanol series and embedded in Epon 812. Ultrathin sections (silver to dark gray) were cut on an LKB Ultrotome ultramicrotome (LKB Producter, Stockholm, Sweden) and stained with uranyl acetate and lead citrate before being examined in a JEOL 100 C electron microscope. Control spermatozoa from healthy volunteers were treated the same way.

The micrographs in this paper are printed so that the tail profiles are observed in the head-to-tail direction. In such prints the A tubules lie in the clockwise direction relative to the B tubules.

## RESULTS

### *Biochemical and Clinical Data*

Patient A presented almost 20 semen samples between 1970 and 1974. During the 1st yr the volume was small ($<2$ ml) and the fructose was $<1.0$ mg/ml, indicating a vesiculitis. This was confirmed at clinical examination. This infection was successfully treated with antibacterial and

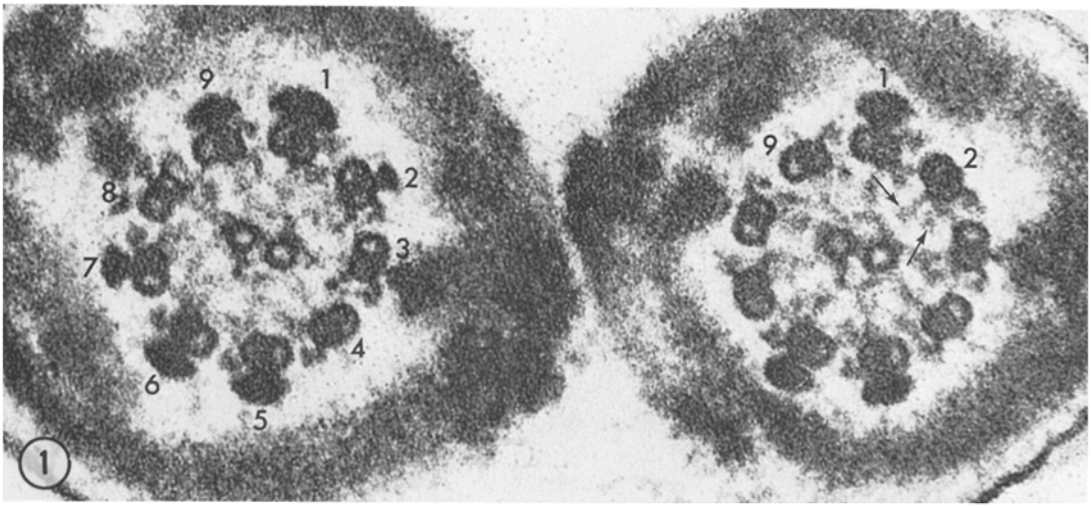
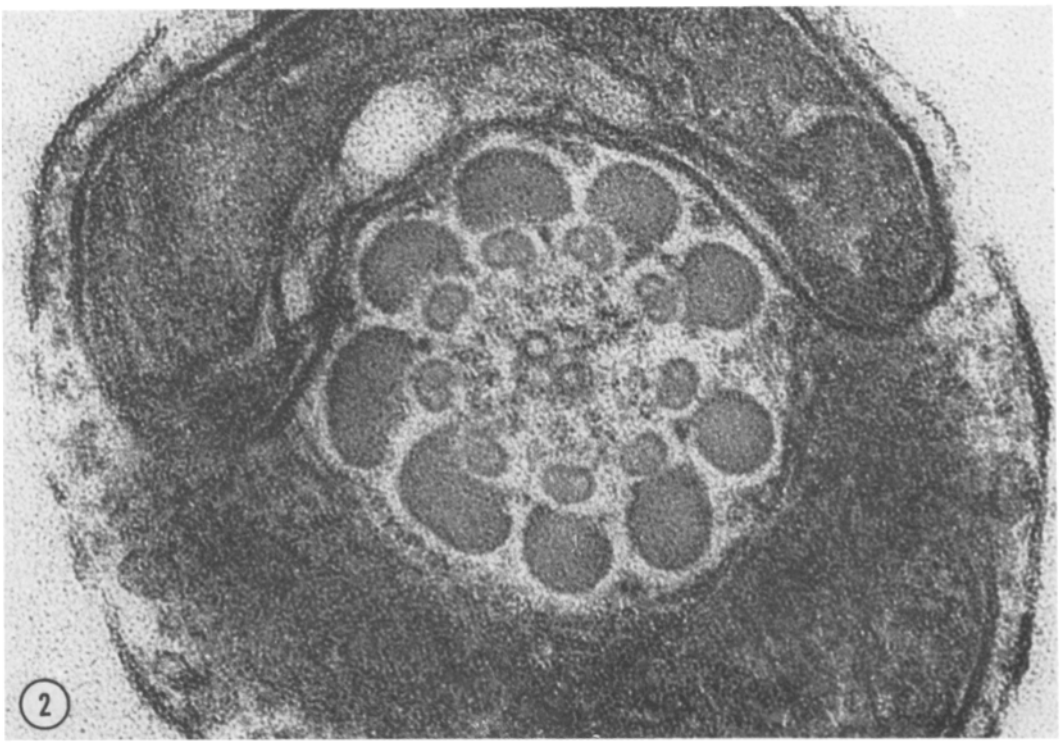



FIGURE 1 Cross section through two sperm tails from a man with normal sperm motility. The ciliary doublets are numbered according to the system of Afzelius (1). Each doublet has a denser A tubule and an empty-looking B tubule, and carries two dynein arms on its A tubule. There are spokes (upper arrow) extending from the doublets to the center and also a thin strand with a position between the spoke and the inner arms (lower arrow). Doublets numbers 3 and 8 are joined to the longitudinal columns of the fibrous sheath. Most of the other doublets have a neighboring accessory fiber. The thickest accessory fibers are those flanking doublets number 1, 5, and 6 (left cross section) and these fibers also are the longest ones, that is, they can be seen also at the more distal sperm tail level seen in the right cross section. Magnification, × 150,000.



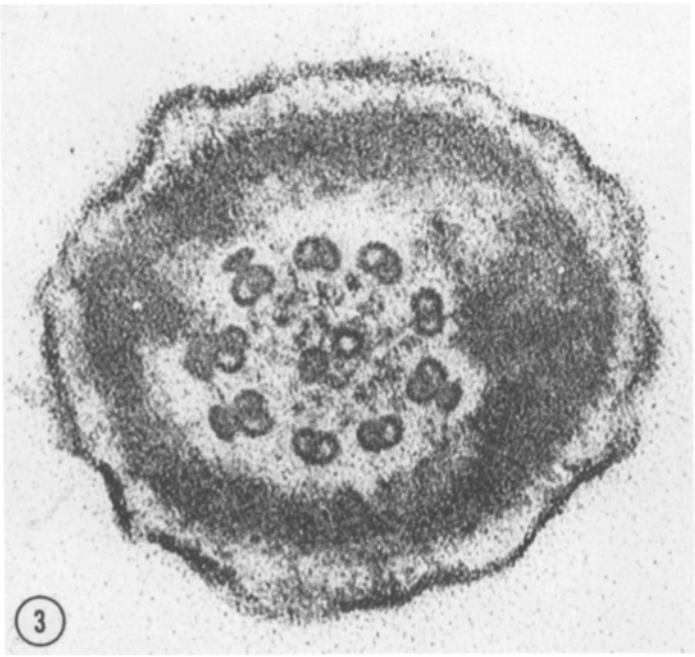
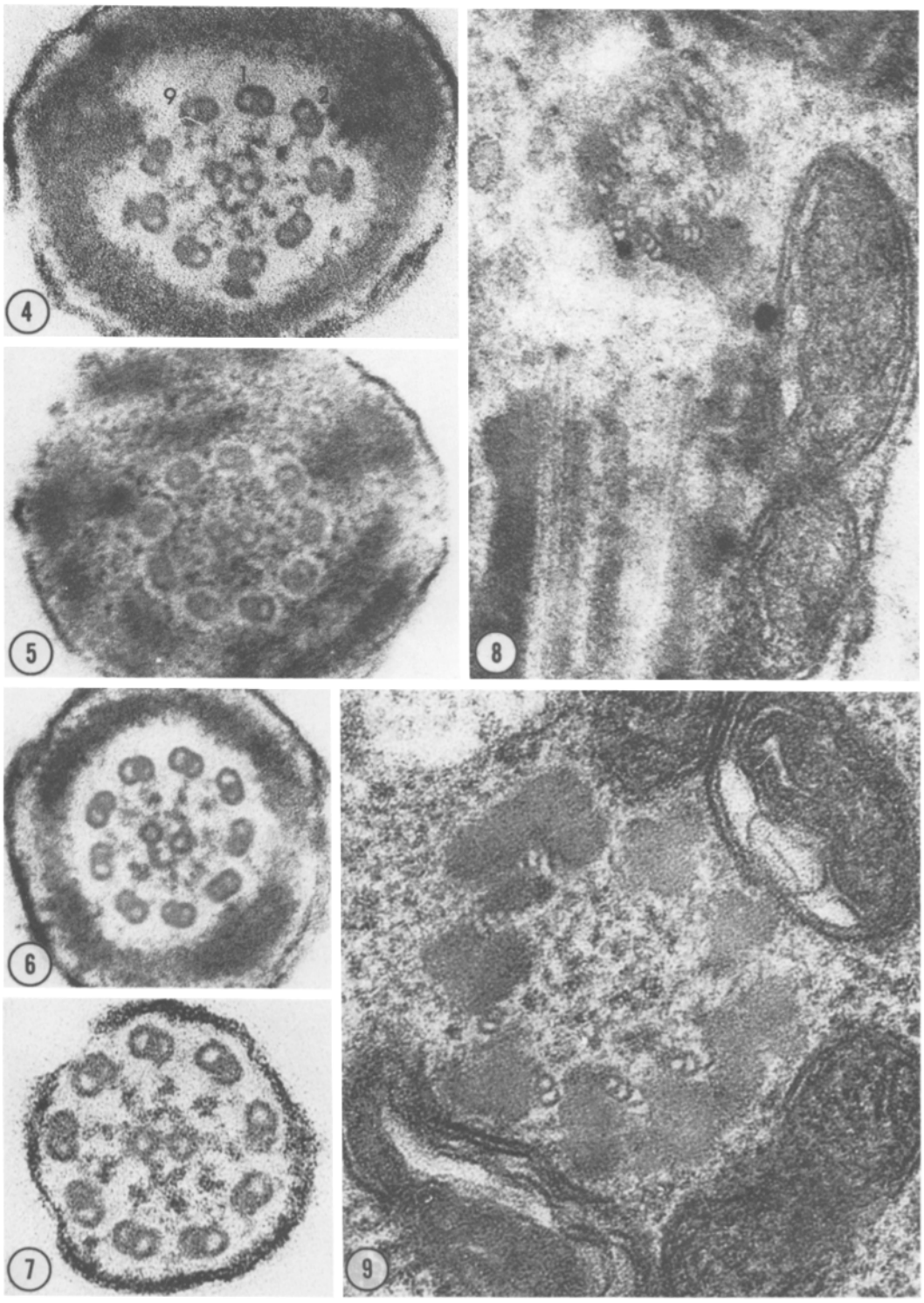




FIGURE 2 Cross section through a sperm middle piece from patient A. The axonemal 9 + 9 + 2 pattern is normal except for the lack of the dynein arms and the somewhat irregular size of the accessory fibers. Note also that each doublet is surrounded by a 50-Å wide empty space. The mitochondria have the common appearance. Magnification, × 150,000.

FIGURE 3 Cross section through the principal piece of the sperm tail from patient A. There is a fair amount of formed components between the central two tubules and the outer nine ones, but no arms. A numbering of the ciliary doublets cannot be performed with any degree of confidence as the longitudinal columns of the fibrous sheath do not lie in the same plane as the central tubules, and the localization of the accessory fibers likewise is abnormal. Magnification, × 140,000.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antiphlogistic drugs. Semen volume and fructose levels then returned to normal. Spermatozoan density varied between 57 and 149 $\times$ $10^6$/ml ($\bar{x}$ = 101.8). The viability test showed between 23% and 45% dead spermatozoa ($\bar{x}$ = 33.9). In none of the semen samples did any spermatozoa show motility.

Oxygen consumption and lactic acid production were normal. Addition of caffein (two experiments) increased lactic acid production by 70% but had no effect on sperm motility.

Patient B presented four semen samples during 1974. As in patient A, all parameters were normal except sperm motility; all cells were immotile.

The seminal plasma from patient A had no negative effect on the motility pattern or maintenance of motility of washed normal homologous spermatozoa in comparison with control seminal plasma.

The zinc content of the spermatozoa from patient A increased from 3.9 to 8.2 $\mu g/10^8$ cells during 4 h of incubation. This is slightly in excess of that regarded as normal (up to 6 $\mu g/10^8$ cells). During the same incubation period the magnesium content increased from 5 to 9.5 $\mu g/10^8$ cells, which also is slightly in excess of that observed in normal semen samples.

### *Light Microscope Observations*

Sperm morphology was completely within normal limits in all samples from the two brothers. In unfixed samples the sperm tail appeared straight and stiff in contrast to the slightly undulated sperm tail of ordinary, dead spermatozoa.

### *Electron Microscope Observations*

The axoneme from a subject with normal sperm motility is presented for comparison in Fig. 1. The general fine structure does not differ from that of axonemes of sperm of most other mammals. Each doublet has an A tubule with two dynein arms and a B tubule which appears empty and has no projections. The outer arm is described as being sharply bent centrally, but an alternative interpretation of the micrographs seems equally valid: a straight outer arm and a dot on the mesial side of the arm. This dot might represent the cross section of a thin longitudinal strand (18). The "spokes" extend from the central region of the A tubule to the so-called central sheath. Thin strands named nexin fibers connect the A tubules on their inner aspect. There is a further projection towards the interior from the A tubule, namely a thin, short strand originating at the base of the inner arm. This projection will be called "inner arm companion." It seems not to have been described previously.

The accessory fibers are of unequal length. Those that flank doublets 3 and 8 (according to the terminology of Afzelius, reference 1) are the shortest ones. They are not present at the levels

FIGURE 4 Cross section of the sperm tail at the level of the distal ends of accessory fibers. The abnormal localization of the longitudinal columns of the fibrous sheath and of the accessory fibers make a numbering of the doublets uncertain. Between the doublets called numbers 1 and 2 a thin nexin strand can be seen. An "inner arm companion" can be seen on doublet number 2. Patient A. Magnification, $\times$ 140,000.

FIGURE 5 A cross section of the sperm tail at the same level as Fig. 4. Here the entire space around the axoneme is occupied by a dense material, except for a thin zone around each doublet. Patient A. Magnification, $\times$ 110,000.

FIGURE 6 The sperm tail at an even more distal level. The spokes and the central sheath are prominent but the arms are lacking. Patient A. Magnification, $\times$ 110,000.

FIGURE 7 Near the tip the appearance of the sperm tail is that of a simple cilium. As at other levels of the tail, there are no arms. Patient A. Magnification, $\times$ 175,000.

FIGURE 8 A longitudinal section through the neck region of a spermatozoon from patient B. The proximal centriole is crosscut and has a normal appearance. The mitochondrial spiral is also of a normal appearance. Magnification, $\times$ 85,000.

FIGURE 9 Cross section through the spermatozoon at the level of the distal centriole. The mitochondria and the centriole have the conventional appearance. Patient A. Magnification, $\times$ 110,000.

shown in the two axonemes in Fig. 1. The widest and longest accessory filaments are those that flank doublets 1, 5, and 6. At an even more posterior level, the axoneme has a 9 + 2 pattern and resembles an ordinary cilium or flagellum except for the presence of the fibrous sheath.

Spermatozoa from the two brothers examined appeared identical and will be described together. The most striking finding is the lack of dynein arms (Figs. 2–7). Hence, the contour of the A tubule is smooth. It is noticeable that each doublet is surrounded by a 50-Å wide zone that appears empty. The space between the inner and outer ciliary tubules contains formed components among which the nexin fibers, spokes, and "inner arm companions" can sometimes be recognized (Fig. 4). The amount of formed components may be increased over that normally seen, occasionally to the extent that the cross section of the tail is completely filled by a dense material in which the nine doublets can barely be discerned (Fig. 5).

In the middle piece, the nine accessory fibers have a normal appearance and distribution (Fig. 2). In the principal piece of the tail, the arrangement of the accessory fibers is irregular and abnormal. Whereas fibers 1, 5, and 6 invariably are the longest ones in normal spermatozoa, there seems to be no regularity in this respect in the spermatozoa of the two patients (Figs. 3 and 4). The longitudinal columns of the fibrous sheath likewise are not so regular as they are in normal spermatozoa.

The endpiece of the sperm tail appears as a simple 9 + 2 axoneme surrounded by a membrane (Fig. 7). In the terminal part, the axoneme is broken up into a bundle of 20 separate simple tubules as described for normal spermatozoa by Pedersen (18).

Spermatozoa from the two brothers show no other structural peculiarities. The general shape of the sperm head including acrosome, of the neck region and of the tail is normal. The proximal centriole (Fig. 8) and the distal one (Fig. 9) also conform to the normal appearance of nine triplets arranged in a skew fashion. It is of interest that the mitochondrial spiral is normal and made up of 11–16 turns.

## DISCUSSION

Analysis of human semen samples has shown that the rate of fructolysis is usually positively correlated with the concentration of motile spermatozoa (16). Sperm motility and sperm fructolysis may even be coupled. It is believed that a stimulus on the sperm surface (for instance, that provided by caffein) starts a series of connected events: an elevation of the cyclic AMP level, stimulation of motility, a lowering of the ATP to ADP ratio and increased fructolysis (12). The finding of spermatozoa which, by several criteria, are living yet are immobile prompted a further investigation.

All biochemical tests showed the abnormal spermatozoa to behave like normal motile ones; even the rates of oxygen consumption and lactic acid production were normal. Evidently the metabolic events are uncoupled from the mechanochemical machinery in these spermatozoa.

Ultrastructural investigations revealed that the sperm tails were devoid of dynein arms. According to current concepts these arms are responsible for the bending movements of the sperm tail, and a lack of arms would thus lead to sperm immobility. Other components of the spermatozoon, including mitochondria and centrioles, appeared intact.

There are many possible explanations for the simultaneous loss of arms and sperm motility, but the simplest would be that the two brothers have a genetic disorder in which synthesis of the dynein proteins, or their assembly into dynein arms, is defective. Alternatively, the defect lies in the tubulin molecules of the microtubular doublets, preventing a normal association between tubulin and dynein, or else in the transport of the dynein to its functional site. It is meaningless to guess between these and other alternatives. It is unlikely that medical treatment of the two brothers caused the loss of dynein arms, as the antibacterial drugs that they received are commonly used and have no known effect on sperm motility.

A lack of the dynein arm has been found also in a Danish patient, whose sperm also is immotile.[1] At the Reproductive Physiology Unit of the Karolinska Institutet many thousands of patients with infertility problems have been examined, but only the two brothers described here and one other patient were found to have totally immotile, stiff spermatozoa with a normal percentage of dead spermatozoa. The disorder thus seems to be rare although not extremely rare.

A survey of spermatozoa from different animal species and of cilia from other cell types has shown

[1] H. Pedersen, personal communication.

that motile spermatozoa and cilia always have dynein arms (2). It is possible to produce mutants of micro-organisms in which the mutation affects the motility of the flagella. Hundreds of flagellar mutants of *Chlamydomonas reinhardtii* have been screened in such experiments, and the ultrastructure of the flagella has been examined (19, 23). Surprisingly enough, no mutant seemed to involve a loss of the dynein arms; the central two microtubules often were affected.

The finding of a cell material in which the axonemal structure and biochemistry seem normal except for the loss of the dynein arms provides a means of examination of the role of these arms in motility and ciliogenesis. Hence it is of interest that the 9 + 9 + 2 filaments keep together in a normal arrangement. Evidently the spokes and the nexin fibers (21) are responsible for maintaining the axonemal pattern. The irregular length of the accessory fibers and the faulty arrangement of the longitudinal columns may be a consequence of the lack of arms, although the mechanisms are not understood. The accessory fibers are formed at a later stage in spermatogenesis than the arms.

The fact that the two brothers examined suffer from chronic sinusitis and bronchitis is of interest since the upper respiratory tract is ciliated and since ciliated epithelia normally function by removing bacteria and other particulate matter. We plan to investigate these epithelia to determine whether the cilia are defective. If these and other cilia also lack the dynein arms, then subjects with this disorder provide a unique insight into the role of motile cilia in the human body, for the respiratory tract, and for the ependyma in the central nervous system.

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