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11 April 1975; revised 10 May 1976

A Human Syndrome Caused by Immotile Cilia

Abstract. *Four subjects who produced immotile sperm were studied. In three of the subjects, who had frequent bronchitis and sinusitis, there was no mucociliary transport, as measured by tracheobronchial clearance. Electron microscopy indicated that cilia from cells of these patients lack dynein arms.*

Evidence has been found for the existence of an inborn disease that is due to an immotility of the cilia. This disease has been studied in four men, but the condition may not be exceedingly rare. A study of this disease may contribute to a better understanding of the role of ciliary movement.

1) The four subjects produced spermatozoa that were living but immotile. The sperm tail appeared as in rigor—that is, straight, stiff, and with no motility. Electron microscopy indicates the spermatozoa to be relatively normal except that so-called dynein arms were not present (*1*). Dynein arms are structures that in normal cilia, flagella, and sperm tails form temporary cross bridges between adjacent ciliary filaments and that are believed to be responsible for generating the movements of cilia or sperm tails (*2*).

2) Three subjects have had, since childhood, chronic sinusitis and bronchitis, and frequent pneumonias, common colds, and ear infections.

3) Study of the tracheobronchial clearance in the three subjects reveals that there is no measurable mucociliary transport (*3*). The investigation was performed by having the subjects inhale, by mouth, a test aerosol of 6-μm particles labeled with technetium-99, and making external measurements of the radioactivity in the chest for 2 hours. The subjects were asked not to cough during this time span. Ordinary tracheobronchial clearance was nearly or totally absent, when no coughing occurred, but a reasonably good substitute for this clearance could be obtained by permitting the subjects to cough.

4) No ciliary motion could be seen in biopsy material obtained from the bronchial mucosa of one subject. In a portion of the biopsy sample that was processed for electron microscopy, the appearance of the cilia was abnormal. There was dense matrix material between the ciliary filaments. Very few dynein arms were seen, and these seemed shorter than in normal cilia.

5) Three of the subjects have situs inversus totalis; the fourth subject is brother to one of the other three affected men.

From the above five findings two tentative conclusions are drawn. The cells of the four men cannot synthesize normal dynein arms, or, if such dynein arms are formed, they cannot attach to the ciliary filaments; this causes immotility of spermatozoa and cilia. Visceral asymmetry is determined through the movements of cilia of some embryonic epithelial tissues.

There is some support for the first conclusion. The human sperm tail is a modified cilium. Ordinary cilia in ciliated epithelia are present at the following sites in the human body: the respiratory tracts, the paranasal sinuses, the Eustachian tube, the ependyma lining brain ventricles and spinal cord, and, in women, the oviducts.

In the spermatozoa of the four subjects, tail paralysis and a lack of dynein arms can be directly observed (Fig. 1). Similarly, the bronchial cilia seem to be immotile and to have an abnormal ultrastructure (Fig. 2). An evaluation of the detailed pattern of the ciliary machinery is difficult as a dense matrix fills most of the space within the cilium. There is, however, a clear space around the nine ciliary filaments in distal regions of the cilia, and the dynein arms are usually absent. Occasionally, a short dynein arm is present, particularly in the basal region, but the appearance is atypical. The morphology of these cilia differs from that of normal cilia in the human respiratory tract (*4*).

The respiratory tract, sinuses, and auditory ducts are sites of repeated infections in these men, as might be expected in a person with no mucociliary transport mechanism. Chronic bronchitis and sinusitis may cause, as a secondary phenomenon, a particular anatomical lesion in the bronchi which is called bronchiectasis. The condition has been found in those two of the present subjects who have been examined in this respect.

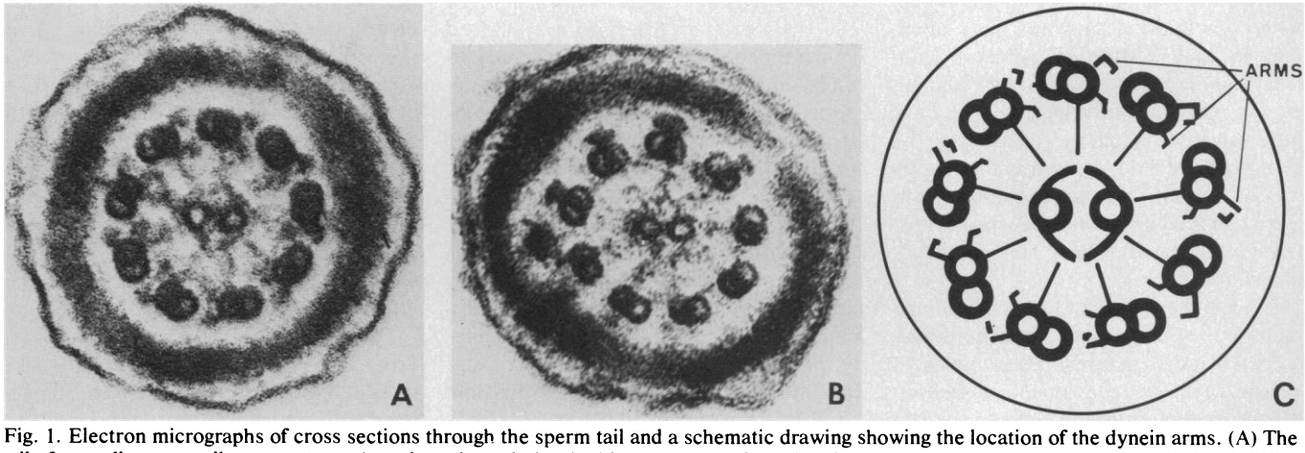

Fig. 1. Electron micrographs of cross sections through the sperm tail and a schematic drawing showing the location of the dynein arms. (A) The tail of an ordinary, motile spermatozoa has nine microtubular doublets; on each of the doublets there are two dynein arms (× 128,000). (B) The sperm tail of patient L.P. is devoid of the dynein arms. The pattern is indistinguishable from that of three patients previously described (*1*) (× 128,000). (C) Drawing of the sperm tail or ciliary cross section.

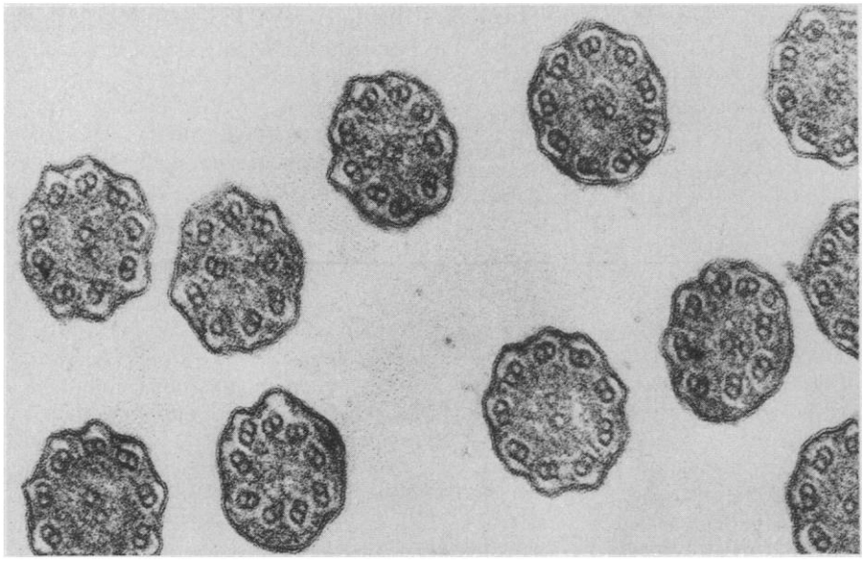

Fig. 2. Electron micrograph of cross-sectioned cilia from the respiratory epithelium of patient L.P. The ciliary ultrastructure is abnormal in two respects: (i) There is a dense matrix occupying most of the cilium; and (ii) the vast majority of the microtubular doublets are devoid of dynein arms and may be surrounded by a clear space. In some cases an arm may be seen, but it does not have the normal length and appearance (× 84,000).

When excised sinus or bronchial mucosa from other persons suffering from chronic sinusitis or bronchiectasis (or both) have been examined in the living state and the ciliary beat rate measured, it has been found not to differ from that of cilia from normal mucosa (*5*). Chronic sinusitis and bronchitis will thus not affect the ciliary beat as such, or the mucociliary transport (*6*); the cilia from "ordinary" patients with chronic bronchitis have also been shown to possess normal dynein arms (*7*).

The ductuli efferentes of the testis have an epithelium that contains several cell types, some of which are ciliated (*8*). It is presumed that the beat of these cilia in healthy men is directed toward the epididymis. This epithelium has not been examined in any one of the four subjects.The only remaining ciliated epithelium in male humans is the ependyma, where ciliated cells are not very abundant in adults. Many investigators consider the ependymal cilia to be rudimentary in man and the ciliary beat to have little influence on the circulation of the cerebrospinal fluid (*4*). In this connection it is of interest that two of the four subjects complain of chronic headaches; two of them have or have had mental depressions.

Even if there are no more ciliated epithelia in the human body, other cells may carry ciliary structures. Many types of sensory cells have a so-called sensory hair protruding from the cell surface into the extracellular space. The sensory hair usually is a cilium. It may be modified in its structure; most often it is devoid of dynein arms and is also immotile. The sensory hairs of the olfactory cells (*9*) and of the vestibular apparatus (*10*) do, however, carry dynein arms and are capable of some motility (*9, 11*). Three subjects said that their sense of smell is very poor, which is common in people suffering from chronic sinusitis and bronchitis. The sense of balance of the affected men has not been tested. One subject, aged 37, has a progressive decrease in hearing ability.

A further category of cells which carry cilia is the differentiating cells of vertebrate embryos (*12*). Early human embryos have not been investigated in this respect; there are no reasons to believe that they differ from other vertebrates. It has been suggested that the blockage of mitosis is associated with the formation of a cilium, which then stores the proteins of the mitotic spindle outside the cell body proper (*12*). This may indeed be the function of cilia on embryonic epithelia, although a more active role is suggested for some, namely, that they are instrumental in establishing the chirality or enantiomorphism of the visceral situs. This is the second of the tentative conclusions mentioned above.

It is conceivable that situs inversus is caused by a factor that is genetically linked to an inability to synthesize dynein, although it would appear more likely that there is one primary defect causing the many deviations. A possible mechanism is presented below.

I postulate that cilia on the embryonic epithelia have a certain position and a fixed beat direction (in normal embryos), and that their beating somehow is instrumental in determining the visceral situs. In the normal course of development, dextral rotation of the viscera takes place, so that bilateral symmetry gives rise to spiral symmetry (*13*). Situs inversus consists of the formation of a sinistral instead of a dextral spiral. It is not unreasonable to assume that a malrotation may occur when the ciliary movements causing rotation are lacking. Experimental support for this assumption can be sought in the development of lower animals. An increased proportion of starfish larvae with situs inversus was obtained by Newman by chilling the unhatched blastulae for 1 or 2 hours (*14*). This treatment stops their ciliary beating. Pressler (*15*) reported that a 180° rotation of the archenteron roof in the amphibian larva, which corresponds to the developed duodenum, will bring about situs inversus. Again, a more specific interference would be welcome.

An association of situs inversus with chronic sinusitis and bronchiectasis has been known for more than 40 years, and is called Kartagener's triad (*16*). This syndrome affects about one person in 68,000 in Europe (*17*) and the United States (*18*). An analysis of 215 cases of bronchiectasis showed that three patients or 1.4 percent had Kartagener's triad (*19*). Of all persons with situs inversus, about 15 percent also have the complete Kartagener's triad.

Although speculations on the association between situs inversus and chronic sinusitis and bronchitis have been set forth (*16, 20, 21*), none is convincing. According to Churchill bronchiectasis in Kartagener's triad is a secondary manifestation, and the basic abnormality may be an altered secretory activity of the respiratory tract epithelium (*22*). This explanation is more valid for cystic fibrosis, where it is believed that a dysfunction in the mucus production causes obstructions sometimes with dilations in the bronchi (*23*). No pathognomonic changes have been found in the mucosa of the respiratory tract in patients with Kartagener's syndrome (*21, 24*). But such negative findings are uninformative because the functioning of a ciliated epithelium cannot be evaluated on fixed and dead specimens. My data presented show that the cilia are nonmotile, and the ultrastructure is altered.

Familial studies of Kartagener's syndrome have shown that among 104 siblings in many families there were 30 persons with a partial (no situs inversus) or total Kartagener's syndrome (*21*). Family data show that the total and the partial syndrome are equally common (*25*).

The values are consistent with the opinion that the syndrome is inherited as an autosomal recessive mutant.

Whereas it has previously been assumed that Kartagener's syndrome is due to some gene with an incomplete penetrance (*26*), I suggest that the gene may be the one responsible for synthesis of the dynein protein or of a protein which binds dynein to the microtubules, and that the gene has an all-or-none action with respect to manufacture or attachment of normal dynein arms. It is further assumed that chance alone will determine whether the viscera will take up the normal or the reversed position during embryogenesis, when normal dynein arms are missing.

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27. I thank Dr. R. Eliasson who has given me information on three of these patients and Drs. H. Pedersen and H. Rebbe who have informed me on one patient. Supported by the Swedish Natural Science Research Council.

20 October 1975; revised 1 March 1976

Lymphocyte-Differentiating Hormone of Bursa of Fabricius

Abstract. *Induction of early lymphocyte differentiation was studied in vitro in fractionated bone marrow cells of newly hatched chickens, with alloantiserums to identify newly differentiated B cells (Bu-1$^+$) and T cells (Th-1$^+$). Thymus extract induced selective T cell differentiation; the activity of the extract corresponds to that of thymopoietin. Bursal extract induced both B cell and T cell differentiation, but at lower concentrations B cell differentiation was always greater. This activity is ascribed to a lymphocyte-differentiating hormone of the bursa of Fabricius, for which the name bursopoietin is suggested.*

Two main classes of lymphocytes occur in the immune systems of vertebrates: (i) T cells, which differentiate in the thymus, and (ii) B cells, which differentiate in the bursa of Fabricius of birds and presumably in some homologous organ in vertebrates that have no bursa. The immediate precursors of T cells and B cells are found in the bone marrow. The T cell precursor (prothymocyte) is induced by the polypeptide hormone or "inducer" thymopoietin to differentiate into a thymic lymphocyte (thymocyte) which is identified serologically in the mouse by expression of distinctive surface components TL, Thy-1, Ly-1, Ly-2/3, and Ly-5 (*1*). This prothymocyte-to-thymocyte transition occurs without cell division (*2*), and it can be assayed in vitro. For this purpose a subpopulation rich in prothymocytes is prepared from bone marrow (or spleen in mice) and is incubated for 2 hours with thymopoietin. Complement-dependent cytotoxicity assay then reveals that 20 to 30 percent of the cells have acquired TL, Thy-1, and Ly antigens.

Evidently thymopoietin induces thymocyte differentiation via an adenosine 3′,5′-monophosphate (cyclic AMP) "second message" (*3*), which would account for induction by various agents that have no special relevance to the thymus (*4*) but elevate intracellular cyclic AMP. In this category of nonspecific inducers ubiquitin is of particular interest because this polypeptide is highly conserved in evolution and has been found in all tissues and all species tested (*5, 6*). In the in vitro assay ubiquitin is probably responsible for thymocyte induction by crude extracts of tissues other than thymus (*4*).

Steps in B cell differentiation also appear to be mediated by cyclic AMP (*3, 5, 7*), and this permits distinction between specific and nonspecific induction in a dual assay. Thus thymopoietin induces only T cell differentiation while all other inducing agents so far tested trigger both T cell and B cell differentiation (*3, 5*).

Both birds and mammals have a thymus but birds also have a discrete organ, the bursa of Fabricius, in which B cells differentiate. Early removal of the bursa, which is a dorsal diverticulum of the cloaca, results in failure of B cell development and agammaglobulinemia (*8*). Thus birds are especially suitable for studies of a possible B cell inducer (*9, 10*) that would correspond to the T cell inducer thymopoietin and would be ex-

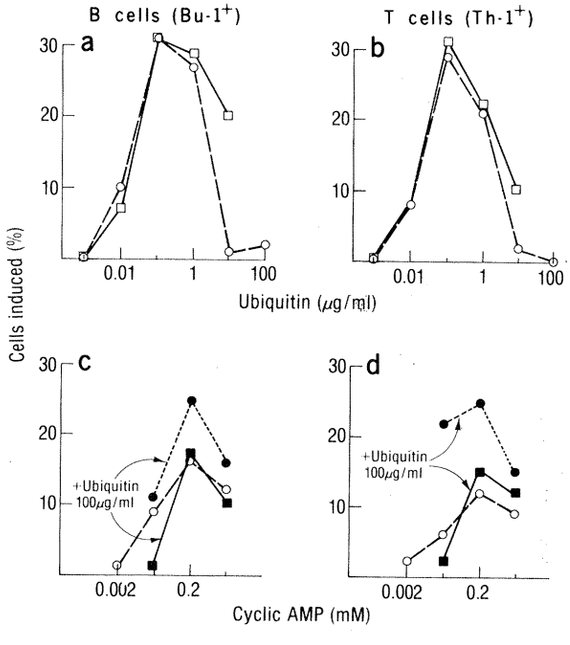

Fig. 1. The relation between concentration of ubiquitin or cyclic AMP (*15*) and induction of Bu-1$^+$ or Th-1$^+$ cells in fractionated bone marrow from newborn chickens. Ubiquitin produced maximal induction at a concentration of 0.1 μg/ml with complete inhibition at 100 μg/ml (a and b). Cyclic AMP produced maximal induction at 0.2 m*M*, and this was not affected by the presence of ubiquitin (100 μg/ml) (c and d). Each symbol represents a separate experiment; each point is the mean of duplicate or triplicate tubes.