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Absence of Arms in the Axoneme of Immobile Human Spermatozoa [1]

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The spermiograms of an infertile man were unique in showing normal parameters except for the presence of total immotility in 100 per cent of the spermatozoa. In 100 per cent of the axonemes of such spermatozoa there was a consistent lack of arms on the doublets of the axoneme. The same feature was found in all stages of spermatid formation. Other less consistent abnormalities were the occasional appearance of extra coarse fibers and axonemal microtubules, and an abnormal relationship between the longitudinal columns of the fibrous sheath and the doublets of the axoneme. All other ultrastructural details compared well with normal spermatozoa.

Detailed descriptions of the substructural organization of axonemes from a wide variety of sources have to some extent helped to clarify our understanding of ciliary and flagellar motility. However, at the present time significant information at the molecular level is needed before a functional model comparable to the one known for muscle contraction can be achieved. The presence of a rather unique axonemal defect in the spermatozoa of a sterile man may help to throw some light on these problems. In this report the history of such a patient and the ultrastructural findings in his spermatozoa will be described.

MATERIALS AND METHODS

Our patient was born in 1941. On admission in 1971, his marriage had been childless for two years. He was healthy, and except for situs inversus no significant abnormalities were detected. He had no sexual problems other than the infertility. As shown in Table 1 his spermiograms were roughly normal except for the consistent finding of totally immobile spermatozoa. About 60 per cent of the spermatozoa were alive as indicated by eosin staining. The fructose and acid phosphatase content of the semen was normal. A testicular biopsy showed no quantitative or qualitative changes. After these studies his wife was inseminated with donor semen, and she conceived in the second series.

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Electron Microscopy

Liquefied semen samples were fixed in collidine buffered glutaraldehyde and osmium tetroxide, dehydrated in graded concentrations of ethanol and embedded in Epon 812 (Luft, 1961). Thin sections were stained with uranyl acetate and lead citrate (Venable and Coggeshall, 1965), and examined in a Philips 301 electron microscope. The technique has been described in detail previously (Pedersen, 1974). The same procedure was used for the testicular biopsy.

RESULTS

No significant abnormalities could be detected in the sperm heads compared to spermatozoa from normal ejaculates processed for electron microscopy as described above. The arrangement and internal structure of the mitochondria did not differ from normal spermatozoa.

TABLE 1

SPERMIOGRAMS

	Oct. 1971	Jan. 1972	Apr. 1973
Volume (ml)	5.4	5.1	5.2
Immobile sperm 30-45 min after ejaculation	100	100	100
Number of spermatozoa (mill/ml)	97	92	92
Abnormal sperm heads (%)	24	21	40

Axoneme

The most obvious defect was found in the axoneme, and the defect was seen in 100 per cent of the cells. There was a complete absence of arms on the outer doublets (Figs. 1–4), whereas the rest of the axoneme appeared to be completely normal with well preserved substructure of the axonemal matrix (for normal substructure, see Fig. 5). Occasionally single extra tubules could be seen (Fig. 1), a feature also found sometimes in normal ejaculates. In the testicular biopsy all spermatid flagella showed the same lack of arms on the doublets.

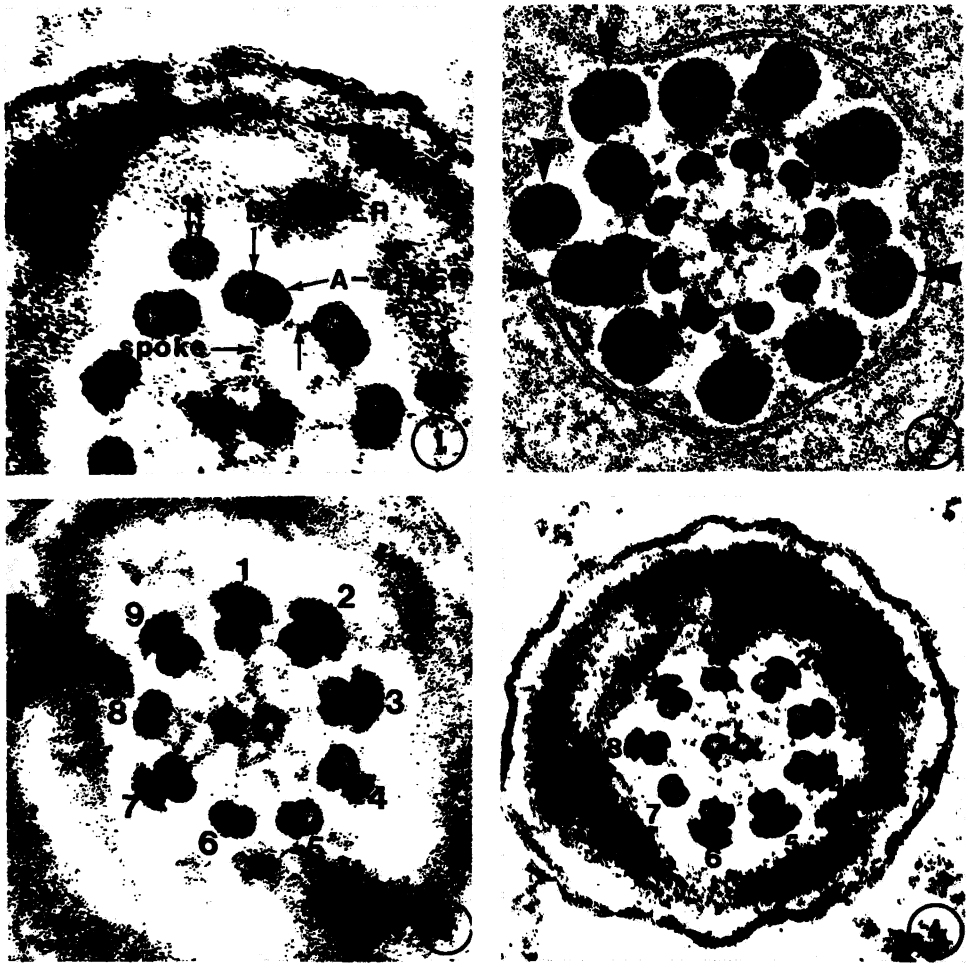

FIG. 1. The A-fibers are identified by their more dense appearance and by their relationship with the spokes. The absence of arms on the A-fibers is evident. There is only a thin strand interconnecting the A-fiber with its neighbouring B-fiber (single arrow), which is found in normal axonemes. Occasionally extra fibers are present (double arrow). ×225,000

FIG. 2. Supernummary or double coarse fibers are often seen (arrowheads), but this phenomenon is also often seen in normal ejaculates. The lack of arms on the doublets is evident at all levels of the flagellum. ×132,000

FIG. 3. The longitudinal columns of the fibrous sheath are usually abnormally situated (arrowheads). In this micrograph there is a normal relation to doublet number eight, but the other longitudinal column is abnormally related to number five instead of number three. ×163,000

FIG. 4. In this micrograph only one longitudinal column is seen, and it is abnormally related to doublet number one. ×119,000

Coarse Fibers

The coarse fibers showed the fine cross striation as previously described in normal spermatozoa (Pedersen, 1972), but the order of the distal termination of these fibers was abnormal and there was considerable variation between cells (Pedersen, 1974). In addition extra coarse fibers were occasionally present, usually in the anterior part of the tail (Fig. 2).

Fibrous Sheath

In normal spermatozoa the longitudinal columns of the fibrous sheath are closely related to doublets number three and eight of the axoneme. As illustrated in Figs. 3 and 4 this normal relationship was found to be disturbed in the spermatozoa of the patient, and several other relations were seen.

DISCUSSION

At the light microscopical level, the findings in our patient were unusual in that the characteristics of his spermiograms were all normal except for a total lack of motility. On rare occasions in an infertility practice severe asthenospermia is seen, but usually in such cases at least a few cells are motile, and as a rule, other details of the spermiograms are abnormal (Pedersen et al., 1971). Initially absence of such detectable abnormalities led us to believe that there were only biochemical or substrate defects involved in this patient, and it was hoped that correction of these might restore his fertility. However, this possibility was excluded by the electron microscopic demonstration of a complete lack of arms on the axonemal doublets. This defect was apparently inborn in the spermatozoan development inasmuch as the same defect could be demonstrated in all stages of spermatid formation—thus excluding the possibility that the defect could have developed secondarily during epididymal passage of the spermatozoa. Normal appearance of the spermatozoa of a normal ejaculate processed by the same technique at the same time ruled out the possibility that the findings could be preservation artefacts.

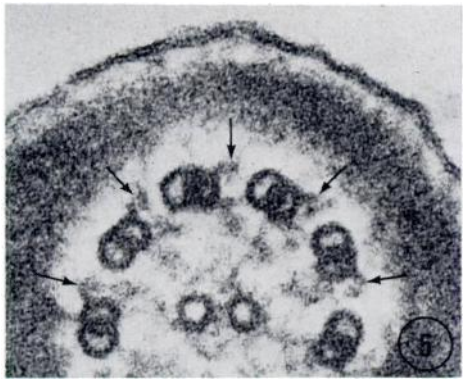

FIG. 5. For comparison findings in normal axonemes are illustrated here. The arms on the A-fibers are indicated by arrows. ×173,000

The functional significance of the present findings is not immediately obvious at a time when the mechanism of flagellar motility is still somewhat controversial (Bishop, 1962; Fawcett, 1961; Gibbons, 1968; Holwill, 1967; Sleigh, 1969). Biochemical evidence points to the doublets and arms as active agents in the propagated wave of bending, and to the arms as the sites of ATP-ase activity, but the subject is still controversial (Gibbons, 1963, 1965, 1968; Gibbons and Rowe, 1965; Gordon and Barnett, 1967; Nagano, 1965; Nelson, 1958, 1962; Satir, 1968). However, in the light of such work it appears that the arms may have a central role in flagellar motility. Thus it is not surprising that the total lack of axonemal arms is accompanied by a complete lack of motility of such flagella. It must be kept in mind, though, that the other abnormalities described here might also play a role in sperm immobility—reflecting profound biochemical changes that may not be detectable at the morphological level.

It appears that a more extensive use of the electron microscope in the evaluation of human infertility might provide a sound basis for a better understanding of the pathophysiology involved, and thus for a more effective treatment of our patients. At the present time several conditions have been clarified by this tool (Pedersen and Rebbe, 1974; Pedersen et al., 1971; Ross et al., 1971; Schirren et al., 1971), and further studies along such lines

will hopefully improve our understanding of normal human reproduction and the basis of many cases of infertility.

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