NOTE ON THE PREPARATION OF SPECIMENS OF ORIENTED SPERM HEADS FOR X-RAY DIFFRACTION AND INFRARED ABSORPTION STUDIES AND ON SOME PSEUDO-MOLECULAR BEHAVIOUR OF SPERM

by

M. H. F. WILKINS

Medical Research Council Biophysics Research Unit, King's College, London (England)

AND

B. BATTAGLIA

Stazione Zoologica, Naples (Italy)

INTRODUCTION

Measurement of birefringence[1] and ultraviolet dichroism[2] has shown that the nucleic acid molecules in some sperm heads lie parallel to one another. This suggests that in these cells there is a specially simple molecular arrangement of nuclear material which is suitable for study by many physical methods[3]. While birefringence and ultraviolet observations may be made on individual sperm heads, X-ray diffraction and infrared absorption studies require specimens containing hundreds or thousands of sperm heads lying parallel. In many cephalopods mature sperm lie in the spermatophore immotile and in parallel arrangement, and such naturally-occurring macroscopic specimens of oriented sperm have been used in X-ray diffraction studies[4]. Spermatophore are, however, somewhat unsuitable objects owing to the fact that the walls of the spermatophore tend to obscure the effect due to the sperm in both infrared and X-ray investigations, and the thickness of the specimen is rather too great for infrared measurements. The purpose of this note is to describe methods of orienting sperm in convenient macroscopic specimens. We feel it worthwhile to give full details of these methods because sperm provide one of the few means of bridging the gap between studies of molecular structure in living cells and in crystals of purified biological substances.

EXPERIMENTAL METHOD

If fresh cephalopod spermatophore are placed in water they burst and the sperm are ejected *en masse* as a thread. In sea water the thread disperses and the sperm are motile; but in distilled water the thread remains intact and swells slowly. The arrangement of the sperm in the thread appears to be identical with that inside the spermatophore. This applies to *Loligo*, *Sepia*, etc., where the thread is straight in the spermatophore; but in some species, *e.g. Octopus vulgaris* and some giant squids, the thread is wound in a close helix (of length $\sim$ 1 cm) inside the spermatophore and straightens out after ejection into a thread about 10 cms long. The thread of sperm may be lifted easily with tweezers out of the water and hung to dry on a wire frame. While lifting the thread the lower end must not be pulled out of the water or surface tension causes the mass of sperm to coalesce into a round drop. If the thread is allowed to swell to more than about three times its initial diameter it becomes weak and breaks when stretched. The dried thread is several hundred μ in diameter and several cms long, depending on the size of the spermatophore (in cephalopods the weight

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of a spermatophore is often roughly proportional to body weight). Such fibres contain sperm lying parallel, and give X-ray diffraction photographs[5] showing good molecular orientation. Extinction between crossed nicols is very poor on account of the slight disorientation of the sperm heads relative to one other. It appears that the sperm in the thread while in water are held together by a dilute gel, but the larger part of the fibre consists of sperm. Use of distilled water enables much of the salts to be washed out of the sperm thread and hence, while drying, the sperm are not exposed for too long to the action of concentrated salt solution which might extract the nucleoprotein from the heads[6].

The technique may be developed in several ways. Fibres of greater diameter may be made by winding the wet thread on a wire frame and allowing the separate turns to coalesce. The length of the fibre, and possibly the degree of orientation, may be increased by stretching the thread when partly dried. To obtain threads of flat section the wet thread may be stretched and laid on a glass surface which has been made non-polar by the use of Teddol (trimethylchlorosilane). When thoroughly dry the sperm ribbon may be lifted off the glass with a razor blade. The sperm, which tend to lie parallel to the length of the thread, are also constrained by surface tension to lie parallel to the glass surface. Hence, when taking X-ray diffraction photographs, the X-ray beam may be passed in the plane of the ribbon at right-angles to its length and in this way the degree of orientation indicated by the photograph may be greater than that obtained with fibres of round section. If the thread is swollen to approximately 1 mm diameter, ribbons of about the same width are produced and the thickness of the ribbon can be made small enough for measurements of the fundamental frequencies of infrared absorption.

It is possible, however, to orient many sperm (*e.g. Sepia, Scyllium*, and many insects with long straight sperm heads) without making use of the naturally-occurring orientation in the spermatophore, and in many species there is no alternative as spermatophore do not exist. The thick suspension of motile sperm in the vas deferens is squeezed on to a glass slide (or for infrared study on to a silver chloride strip). The suspension is then smeared with a single stroke (a razor blade is convenient). On drying, an oriented sheet of sperm results, the length of the sperm being parallel to the direction of shear. This kind of technique is a standard one for orienting rod-shaped macro-molecules such as tobacco mosaic virus, desoxyribose nucleic acid, etc. Such preparations obtained from the vas deferens include the dried material from the fluid in which the sperm were suspended, and the proportion of material in the specimen which consists of sperm is less than in specimens prepared from spermatophore; sometimes the extraneous material can be removed by washing the sperm before smearing. The dried sheets of oriented sperm may be stripped off the glass. Fibres also may be made by drawing out the sperm suspension in a sticky condition as it dries.

DISCUSSION

The purpose of preparing these specimens has been to study the submicroscopic and molecular structure of the nucleoprotein in the sperm head, and the question arises as to what extent the treatment has produced artefact. A good approach is to compare the X-ray diffraction etc. produced by the dried fibres with that due to sperm contained in a spermatophore and living at the beginning of the experiment. Preliminary results suggest that the drying has produced little change, and this is rather to be expected as the water content of the heads is low and ionic concentration has been kept to a minimum during drying. The general microscopic appearance of many sperm heads is unaltered during drying in distilled water. A more careful examination was made in the case of *Sepia*. When viewed with 1.3 numerical aperture and crossed nicols the sperm heads, whether dried or in distilled water, appeared identical with those in life; the general direction of the optic axis followed the characteristic bent shape of the head[1], but when examined more closely the head was seen to consist of regions about 1 μ square each extinguishing in a direction which varied from one region to the next to the extent of about one degree. Phase-contrast examination (1.3 numerical aperture) showed that live sperm have a small transparent extension at the tip of the head and this disappears in distilled water. A few percent. of *Sepia* sperm heads sometimes swelled laterally in distilled water to form a refractile birefringent mass of fibres. Cephalopod sperm differ from those of many species, *e.g.* sea urchin, in that they do not in general swell in hypotonic solution[6]. If, however, the sperm are dried at room temperature

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($\sim 20°$ C) from sea water the heads frequently flatten, the nucleoprotein being disaggregated by the concentration of salts. The above observations gave no suggestion that nucleoprotein was dissolved by water[10] from the intact sperm heads.

It is desirable that the specimens contain a large proportion of sperm head nucleoprotein. In the case of dry sperm of *Sepia* and *Loligo*, the head is $\sim 7\ \mu$ long and $\sim 1.2\ \mu$ diameter, and the tail $\sim 0.3\ \mu$ diameter and $\sim 100\ \mu$ long (electron microscope observation by Dr. S. T. BAYLEY). The head and tail therefore contain approximately the same mass of material. Thus it is not true, as has been previously stated[4], that the mass of the tail is small compared with that of the head. However, in many experiments the effects due to the tail material fortunately do not obscure those due to the nucleoprotein. The slightly bent shape of the head appears to be responsible for much of the disorientation observed in X-ray photographs[5], and a search was therefore made to find suitable straight sperm heads. *Octopus vulgaris* has sperm with heads which are exactly straight, but unfortunately the proportion of the sperm fibres that consist of nucleoprotein is small, and poor X-ray diffraction results are obtained.

Some pseudo-molecular behaviour of sperm

There is a certain degree of usefulness in indicating, with suitable reservations, the similarity of properties between macromolecules and some microscopic biological bodies of simple form and uniform size (*e.g.*[7]). The sperm referred to above are very uniform in size and approximately rod-shaped; it is therefore not surprising that the sperm may

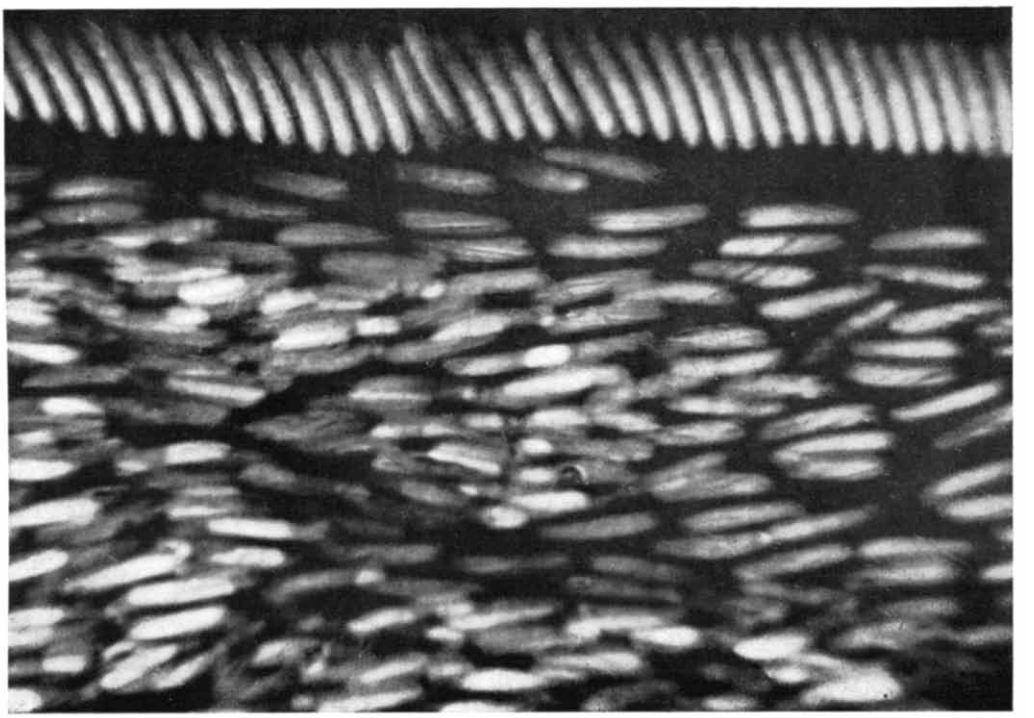

Fig. 1. Part of liquid-air interface at edge of drop of *Sepia* sperm suspension. The photograph is taken after drying, the arrangement of the sperm being preserved. Crossed nicols × 1000.

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be oriented in the same manner as macromolecules (see also[8]). During the above experiments some effects were observed where the sperm simulated the behaviour of polar molecules. A suspension of motile sperm from the vas deferens of *Sepia* was observed between coverslip and slide separated by a spacer ($\sim$ 0.2 mm). Near the air-liquid interface the sperm swam rapidly parallel and in groups, and often parallel to the surface, thus lying in the direction normally observed for rod-shaped macromolecules. At the actual interface, however, the sperm often behaved like polar molecules; all the tails pointed into the liquid and the heads lay perpendicular to the interface (see Fig. 1). This monolayer of sperm was relatively stable, but occasionally one sperm was seen to slip out of the monolayer and to be replaced by another. Spontaneous birefringence and flow birefringence of the sperm suspension were readily observed, both when the sperm were motile or immotile. A two-phase system corresponding to that found in solutions of purified tobacco mosaic virus[9] does not appear to exist. These sperm and macromolecule analogies must not, of course, be taken too literally.

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SUMMARY

Some sperm heads (e.g. those of *Sepia*) contain nucleo-protein molecules lying parallel to the length of the head. Fibre or sheet specimens consisting of an aggregate of intact sperm lying parallel may be made from spermatophore or from fluid from the vas deferens. Such specimens are used for infra-red and X-ray diffraction studies of the molecular structure of nucleo-protein in cells.

RÉSUMÉ

La tête de certains spermatozoïdes (p. ex. ceux de *Sepia*) contient des molécules de nucléoprotéines orientées parallèlement à son grand axe. On peut obtenir des échantillons en fibres ou en lamelles constitués par des aggrégats de sperme intact disposés parallèlement à partir des spermatophores ou du liquide du vas deferens. De tels échantillons ont été employés à l'étude, par les rayons infra-rouges ou la diffraction des rayons X, de la structure moléculaire des nucléoprotéines dans les cellules.

ZUSAMMENFASSUNG

Einige Samenfädenköpfe (z.B. die von *Sepia*) enthalten Nucleoproteinmoleküle, die parallel zur Längsrichtung der Köpfe liegen. Faserige oder blättrige Proben, die aus einer Anhäufung parallel liegender intakter Samenfäden bestehen, können aus Spermatophoren oder aus der Samenleiterflüssigkeit isoliert werden. Derartige Proben werden zu Infrarot- oder Röntgenstrahlenstreuungsuntersuchungen der Molekülstruktur des Nucleoproteins in Zellen benützt.

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