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Electron Microscope Study of Sperm.*

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Originally the following study was undertaken with the hope of demonstrating degenerative changes occurring in the aged sperm which allow it to retain its fertilizing ability for a time but do not permit eggs fertilized by such sperm to develop into zygotes capable of hatching.[1]

Studies were begun with chicken sperm obtained by massage, and continued on bull sperm obtained by ejaculation into an artificial vagina. Attempts were also made to study human spermatozoa.

Human sperm, because of the presence of heavy secretions of the accessory glands which tend to coagulate and thus obscure the field of vision, were found in this preliminary study to be unsatisfactory.

All the bull sperm used came from fertile animals which are routinely used for artificial insemination. The bulls are permitted to ejaculate following a definite schedule and are not used to excess. This point is made in view of some of the findings reported below which do not agree with conclusions reached following sperm studies with the optical microscope.

The electron microscope preparations were made according to the directions given by Marton.[2] The bull and chicken semen were usually studied in a 1:100 dilution in distilled water. The suspension was applied to the collodion film with a bacteriological loop and allowed to dry at room temperature. Attempts were made to observe sperm in seminal fluid, however the density of the fluid obscured the field and made detailed study of the sperm impossible.

Since it is probable that drying of unfixed sperm may produce artifacts, part of the studies were undertaken on sperm fixed in alcohol, formalin, and mercuric chloride and some observations were made on unfixed sperm (hereafter referred to as "fresh sperm"). The stains used were: a silver preparation which is specific for lipoidal material and Harris' haematoxylin. In order to obtain stained sperm, semen was mixed with the water in a centrifuge tube, the mixture being centrifuged after each treatment and the residue containing the sperm cells being resuspended in the majority of cases in distilled water and occasionally in alcohol. The entire range of magnification of the electron microscope (1,000-100,000) was used in this study.

Results with Bull Sperm. The appearance of the head of bull sperm varied greatly de-

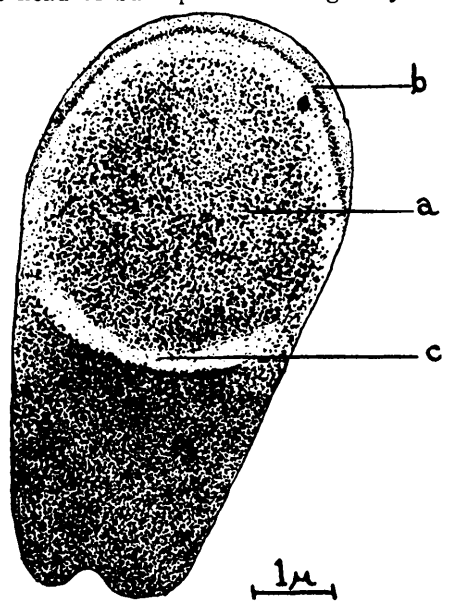

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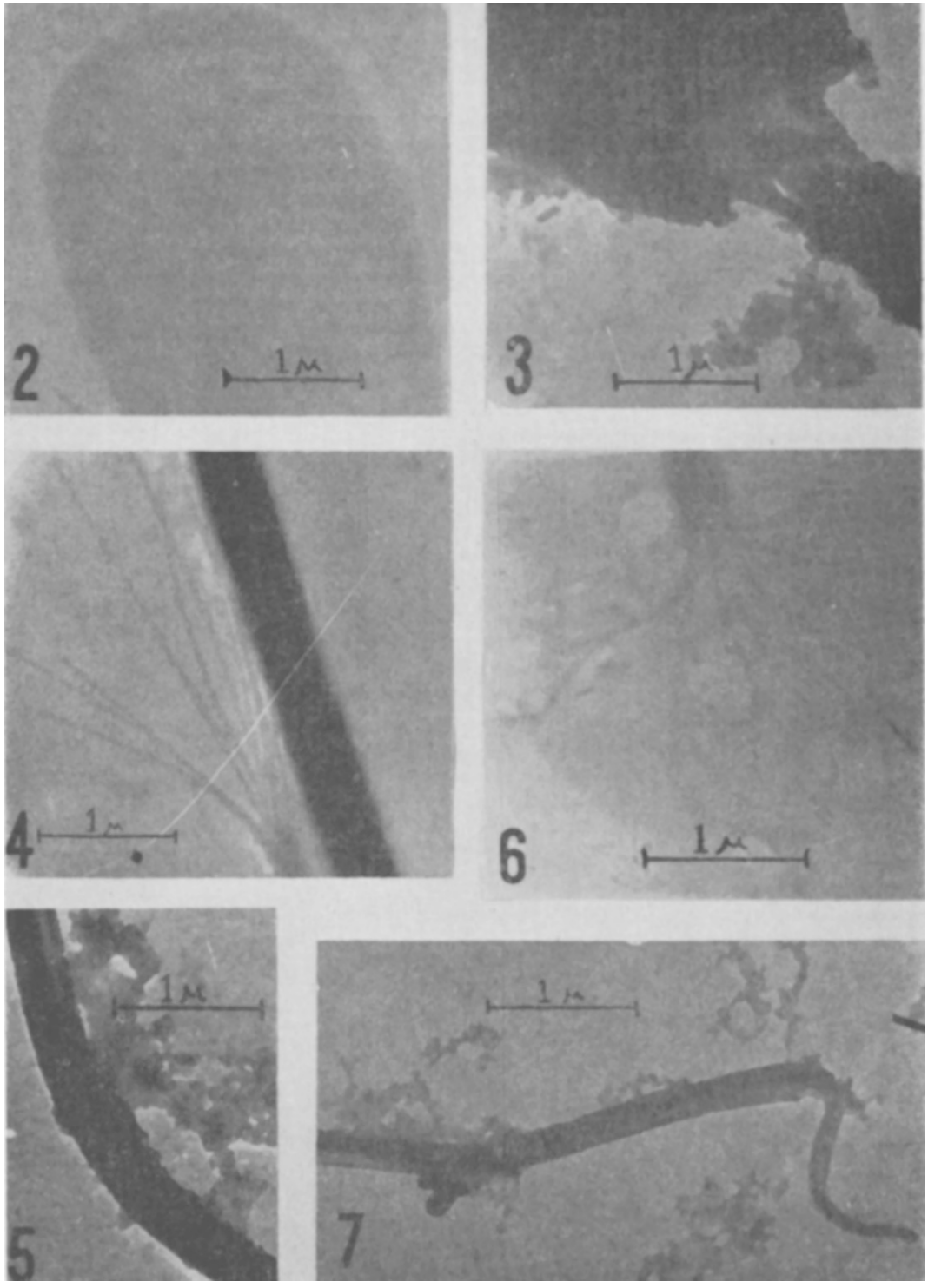
Fig. 1.

* We are indebted to Dr. Kruger, Veterinarian in charge of the Eastern Illinois Live Stock Improvement Co-operative, for supplying us with samples of bull semen and breeding records of these bulls.

[1] Nalbandov, A., and Card, L. E., *Poultry Sci.*, 1943, **22**, 218.

[2] Marton, *J. Bact.*, 1941, **41**, 397.


2
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pending on whether the sperm was studied fresh, fixed, or stained. In all cases where no fixatives or stains were used, the head was enclosed in a protoplasmic cap which was always present but which varied in size depending on the individual sperm. (Fig. 2). This condition has been previously observed under the light microscope but has been described as a sign of immaturity or abnormality of the sperm.[3] In our study in which sperm samples from many bulls were used the protoplasmic cap appeared in all fresh specimens indicating that it is probably a normal part of the sperm. Presumably this protoplasmic cap is frequently dissolved when fixatives or stains containing solvents are used in preparing the sperm for examination under the optical microscope. We have found that when either stains or fixatives were employed, the cap was absent, or if present, was greatly shrunken and distorted.

In stained preparations the head appears to have 2 distinct zones of density. (Fig. 1.) These zones have been seen under the light microscope, but the electron microscope has revealed additional details. In some preparations the head appears to consist of 2 distinct and well-defined regions, one of which extends throughout the entire anterior portion of the head and seems to terminate in the posterior third of the sperm head. (Fig. 1 a.) The reasons for the difference in density within the sperm head may be twofold. The head may be flattened anteriorly thus offering less resistance to the electron beam which must penetrate it. Thus the anterior portion of the head would appear less dense than the region caudal to it which may be thought to be spherical in shape. Another explanation may be that the ovoid body occupying the anterior part of the head may consist of material less dense than the caudal region of the head. Completely surrounding this structure (nucleus?) is a narrow zone of even lighter density which stands out very clearly in stained preparations under the electron microscope but which is difficult to reproduce photographically. (Fig. 1 c.) Outside these regions of different densities the internal structure of the head appears to be homogeneous and no stains used up to now have shown any structures or regions of density which might suggest accumulation of chromosomes in either diffuse or condensed state.

In no case, even when the sperm was stained with silver to demonstrate lipoidal materials, was a structure observed that could be recognized as the acrosome. Correlating with optical microscope studies, the dense band seen in the lower half of the sperm head (Fig. 1) may be interpreted to delimit the acrosome, however, a more convincing electron microscope picture is needed.

The protoplasmic cap seems to envelop the whole head and in the lower half of the head it clings to the head outline rather closely.

The tail is connected with the head by means of 2 or more threadlike structures which become apparent when the tail breaks away from the head. (Fig. 3.) This condition frequently appears after centrifuging and staining.

The middle piece is uniformly dense and no mitochondria were demonstrable. The constriction between the middle piece and the end piece is easily seen and is essentially as observed under the light microscope.

The end piece (or main piece) in the unstained specimen appears hollow (Fig. 4) which may be due to the fact that the electron beam penetrates the central structure more easily than the thicker peripheral layer of cytoplasm surrounding it. In the stained tail in which the density of the central fibers has been increased over that of the enveloping cytoplasm, a very clear-cut axial filament is observed. This is very apparent in silver preparation. (Fig. 5.) In regions in which the cytoplasmic sheet has been broken one can see the naked filament exposed. (Fig. 5.)

The extremity of the tail (naked filament) in many preparations of fresh sperm appears brush-like in nature (Fig. 4) which suggests that the axial filament may be composed of a trunk of very thin but long fibers lying side by side and becoming exposed where the cytoplasm terminates. This is further substantiated by photographs of regions in which the end piece has broken, allowing the axial filament to flare out in a brush. (Fig. 6.) It is possible that the brushes observed at the

[3] McKenzie, F. F., Miller, J. C., and Bauguess, L. C., *Mo. Agr. Exp. Sta. Res. Bul.* 279, 1938.

extremity of the tail are due to such flaring out of the filament exposed by the breaking of the end piece, although in some cases such a break must have occurred very near the tip of the tail.

The fact that the filament and the extreme tip of the tail are composed of individual fibrils has been observed as early as 1886 and pictured by Ballowitz.[4] Since then this information has been disregarded by workers in the field of sperm morphology. Ballowitz calls these fibers the "Elementar-fibrillen" and ascribes to their presence the ability of sperm to move. He states, however, that in both birds and mammals the number of fibrils varies between 2 and 4. The electron microscope reveals that there are actually many more.

In stained preparations this brush phenomenon is never observed, possibly because the stain encases the fibers, cementing them into a rigid cylinder, thus not permitting them to flare out. In such preparations the naked terminal piece is clearly seen to be continuous with the axial filament of the end piece. (Fig. 7.) Usually following staining the very tip end of the tail appears clearly tapered although not brushed.

In the chicken sperm the head appears very dense, an acrosome is seen and the tail ends in a mass of long delicate fibers of sub-light-microscope dimensions. In chicken sperm also the middle piece breaks easily and releases a mass of fibers. This suggests that in the cock as well as in the bull the axial filament is made up of a bundle of many fibers.

We could not confirm the observation of Seymour and Benmosche[5] that the sperm head (human) has a suction disc nor could we find any "joints" in its middle pieces of any of the sperm types studied.

Summary. Fresh, unstained and unfixed samples of sperm from many fertile bulls studied under the electron microscope have shown that the anterior portion of the sperm head is always enveloped by a protoplasmic cap which appears damaged or disappears altogether if sperm are stained or fixed. This suggests that, contrary to results obtained with the optical microscope, the protoplasmic cap is not a sign of immature or abnormal sperm but is typical of normal sperm when these are examined without being exposed to solvents usually present in stains.

The tails end in a brush consisting of many free and very long filaments. Breaks in the main or end pieces of the tail have also shown flared brushes which make it seem likely that the axial filament consists of a bundle of fine fibers rather than a single relatively thick thread.

[4] Ballowitz, E., *Arch. f. Mikr. Anat.*, 1888, **32**, 401.

[5] Seymour, F. I., and Benmosche, M., *J. A. M. A.*, 1941, **116**, 2489.

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Utilization of Asparagus Juice in Microbiological Culture Media.

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This laboratory has a series of investigations in progress which relate to the possible usefulness of waste vegetable juices as microbiological culture media. The observation that asparagus-butt juice undergoes extraordinarily rapid microbiological spoilage, together with the interest now being shown in the production of antibacterial agents for use in the treatment of certain disease and wound infections, has led us to investigate the possibility of utilizing this juice as a medium for culturing some of the organisms that produce