Though the use of such unpolished hollow ground slides may not conform entirely to the principles of theoretical microscopy, nevertheless they prove highly practicable for the most critical work.

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PREPARATION OF AVIAN SPERM SMEARS FOR MICROSCOPY[1]

EXCELLENT smear preparations of mammalian sperm (bovine, equine and human) may be made according to the method of Savage, Williams and Fowler.[2] The approximately globular heads of sperm of these species can be fixed by heat with little difficulty. The elongated heads of avian sperm, however, present technical difficulties which make dry smear preparations extremely difficult. As the smear dries the elongated heads become contorted and twisted and often assume a spiral or corkscrew shape. In other cases rupture of the surrounding membrane and complete disintegration of the head occurs. In addition to these difficulties of fixation, differential staining by means of aniline dyes is extremely difficult.

The following procedures are recommended and will be found to produce excellent results, particularly after the operator has had some experience. The first method is the more reliable and may be used for pigeon, dove or fowl sperm. The second or dry smear method can be relied on only in the case of fowl sperm.

MOUNTED PREPARATIONS

The pure semen is diluted with two to four times its volume of Ringer's solution and a drop placed on a slide that has previously been smeared with a very thin film of albumen fixative. Allow the drop to spread over an area which may be conveniently covered with a cover-slip. Drain off any excess with blotting paper. The smear is then fixed by suspending the slide in osmic acid vapor (osmium tetroxide) for thirty minutes to one hour, although longer exposures to the vapor are not injurious. A small airtight jar or chamber such as a stender dish or coplin jar may be used for a fixing chamber. A two per cent. solution of osmic acid is placed in the bottom of the chamber. Some arrangement, such as a bit of broken glass tubing in the bottom, must be made to keep the slide out of the solution. After fixation the slide is washed in several changes of water and placed in ferric alum mordant for one half hour and thence to Heidenhain's hematoxylin for one hour or more. Extract the stain from the acrosome, middle piece and tail by immersing for a few seconds in ferric alum. Considerable experience is necessary in this destaining process, since it is rather easy to under- or overdo it. The slide is now run up through the alcohols to 95 per cent. and counterstained in a one per cent. solution of light green. Light green is a very deep and rapid cytoplasmic stain and should not be confused with methyl green, which is a nuclear strain. Rinse off in 95 per cent. alcohol and run rapidly through absolute alcohol to a mixture of xylol and absolute alcohol and thence to xylol. The preparation is now mounted in balsam under a cover-slip.

It is rather difficult to secure preparations with well-stained tails. The tail absorbs the stain very poorly and loses it rapidly when rinsed and in absolute alcohol. Hence the necessity of passing quickly through these. Much of the success of the preparation depends on the completeness with which the hematoxylin has been extracted from the tails, providing, of course, that it was not also extracted from the nucleus. Because of the fact that the counterstain is readily lost, it may be found preferable to pass the slide directly into carbol-xylol from 95 per cent. alcohol and mounted directly.

Formalin vapor may be used as the fixative in place of osmic acid vapor, in which case a 15 to 20 per cent. solution of formalin (6 to 8 per cent. formaldehyde), which is somewhat stronger than that used for tissue fixation, should be employed. If osmic acid is procurable, however, it is to be preferred.

DRY SMEARS

In cases where available equipment is limited, fairly good dry-smear preparations of fowl sperm may be made by the following method.

After diluting the semen with normal saline, place one drop near one end of the slide and quickly draw out to a very thin film with a slip of paper. The film must be thin enough to dry instantaneously on the warm slide; otherwise the heads will become contorted. Fix overnight in an oven at 100° C. Stain for one-half to one hour in a freshly made preparation of carbol-fuchsin (Ziehl-Neelson's). Wash and counterstain for a few seconds to one minute (depending on microscopic control) with an aqueous solution of methylene blue, previously warmed to 40° C. If it is desired to preserve these dry smears indefinitely they may be mounted in balsam under a cover slip when dry.

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[2] A. Savage, W. W. Williams and N. M. Fowler, *Trans. Roy. Soc. Can.*, 21: 425, 1927.