those for the rats, because after sacrifice some of the caeca in both series of chicks were found plugged. The chick infections did not result in immunity.

At the present time it is not possible to say how liver acts in retarding the development of coccidian infections. The two possibilities that seem most plausible are that liver lacks the coccidium-growth-promoting substances and that liver contains a coccidicidal, or at least coccidistatic, material. Further investigation will be conducted along these suggested lines.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE USE OF HOLLOW GROUND SLIDES MADE WITH THE DENTAL ENGINE

THE method so aptly described by Dr. Marshall Hertig in a recent issue of SCIENCE[1] for making hollow ground slides with the dental engine has been in use in part by the present writer for several years in preparing slides for the mounting of diatoms, but the possibility of its more general application was not recognized. It is thought that the following supplementary suggestions and critical analysis from his own experience might add somewhat to the usefulness of the method.

Dr. Hertig mentions as part of the necessary equipment a flexible shaft or hand piece, normally part of the dental engine. This should be emphasized as essential. Slides first prepared by the writer were made with a drill press, using spherical carborundum points. Besides the greater difficulty of controlling the work, the constant angle maintained between the tool and the slide causes the carborundum point to wear unevenly, which produces an annular surface to the slide depression, visible and consequently detrimental under the lower powers of the microscope. Changing this angle while cutting the cavity produces a smooth curvature to the bottom of the depression, giving a clear and evenly lighted field, which can be done with a flexible shaft.

On first using such slides a question occurred to the writer, which may cause some misgivings in the minds of others also. Despite almost complete obliteration of the cavity when filled with balsam, due to similarity of refractive index of the balsam and the glass, there is usually enough difference in index to give some slight effect, and the question was to what extent the spurious deflection of light from a ground surface so close to the object might interfere with a critical image or produce artifacts under certain conditions.

To test possible undesirable effects various objects were examined under different powers and conditions, more significant being a critical examination of the classical test object, *Amphipleura pellucida,* under oil immersion with oblique illumination. It was thought that with a narrow beam of great obliquity, any spurious extraneous light from the ground surface of the cavity might diminish the resolution or definition of so critical an object. There proved to be no noticeable effect in any case, probably explainable by the overwhelming intensity of the controlled as compared with the diffused light, incident to any particular point on the object. It is recommended, however, that the object be fixed to the cover (as pertains in the work here described), and hence suspended over the cavity, which should be ground reasonably deep; for where the object and the bottom of the cavity fall simultaneously within the visible range of focus of the objective used the ground surface is detrimental. Also, no noticeable effects obtained in photographing objects mounted in this way, barring possible uneven lighting if the cavity was not sufficiently deep. Fortunately for this purpose the depth of focus in the higher power objectives is very shallow. With higher index media, of course, obvious and more unsatisfactory effects are produced. These tests should dispel the doubts of any who contemplate using the method according to the conditions here presented.

Some benefits may accrue from the ground surface in a possible slight whitening and brightening of the field, and in sharpness of image from diffuse rays incident to the object more close to the critical angle.

In the production of hollow ground slides with the carborundum point the writer finds very advantageous the use of a shield in the form of a block of hard wood or metal, slightly larger than a slide and about one quarter of an inch in thickness. This has on the under side a 3 by 1 inch depression into which a slide may fit. In the center of this depression there is a hole through the block one fourth to three eighths inch in diameter, countersunk from the top. This tool has the following advantages: (1) It makes it easier to hold the slide while drilling; (2) it facilitates centering the cavity on the slide; (3) it restrains the lubricant from running off the slide while drilling, and (4) it protects the slide from unsightly scratches across it if the point should slip, as well may happen, especially when first starting the cavity.

[1] Marshall Hertig, SCIENCE, 83: 2144, 110, January 31, 1936.

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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[1] Paper from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, No. 199. Published with the approval of the Director of the Station.

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