SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE USE OF MICRO-MANIPULATORS

SOME interesting variations in the use of the micro-manipulator have induced me to call attention to a point in its historical development. I developed a micro-manipulator in 1905[1] at the University of Pennsylvania for the purpose of dissecting the egg cases of certain animals and orienting the eggs in such a way as to be able to work out the cell lineage; and used the same apparatus for removing chromosomes from eggs. Since these eggs are heavy and would fall to the air-water surface of a hanging drop and be burst by surface tension effects, it was necessary to work from above even though the air-water surface might produce optical distortions while making the observations. A slightly modified micro-manipulator was used in cutting and stimulating protozoa.[2] This work was done entirely independently of the Barber pipette holder which was developed in Kansas simultaneously. I did not hear of the Barber pipette holder until many years later when it was developed by Chambers and others, who found it preferable to work in hanging drops. More recently Richards[3] has developed an instrument for working above the slide and McNeil and Gullberg[4] have described a similar instrument. As to the virtues of these different types, the rack and pinion used by myself gives very rapid manipulation at low powers of the binocular microscope. I used also a micrometer screw, working against a spring. This type gives a slower motion but if it guides a square tube sliding upon a square rod, there is liable to be some lost motion at right angles to the action of the spring unless further springs are used to tighten the bearings. This micrometer arrangement, as well as rack and pinion for rapid motion, is used by Richards. McNeil and Gullberg used the hinged joint as developed by Chambers. It seems possible that the hinged joint acting against a spring may be preferable for movements of short distances. More extensive movements would be complicated by the fact that the movement is in the arc of a circle and not in a straight line.

In my micro-manipulator, movement in the three dimensions was obtained by actuating three knurled heads side by side in the same plane and it was intended to develop reflexes by which movement in any direction could be performed by the fingers of one hand. A long period of education is necessary and it seems probable that a micro-manipulator made on the principle of the pantograph would be preferable in that time required to develop these reflexes would be saved.

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[1] *Biological Bulletin*, 12: 141, 1907.
[2] *Journal of Experimental Zoology*, 6: 87, 1909.
[3] *Journal of Biological Chemistry*, 87: 463, 1930
[4] SCIENCE, 74: 460, 1931.

RAPIDLY RIPENED HAEMATOXYLIN AND ITS USES

KOHL and James[1] have recently presented a rapid method for the ripening of haematoxylin in solutions by exposure to a powerful quartz mercury vapor light. The writer for several years has been using, with considerable success, another method suggested by Miss Ethel Thomas, of the Thomas Biological Supply Company, Charleston, Ill. The method applies principally to the ripening of the haematoxylin for Haidenhain's iron-alum stain, but may be adapted to other haematoxylin stains. It consists of merely adding the haematoxylin crystals (sufficient to make a one half per cent solution) to boiling distilled water. In making the solution the writer has found it best to bring the water to a vigorous boil. The fire is turned off and the crystals added before bubbling completely ceases. A sufficiently large container should be used so that the solution does not overflow during the effervescence accompanying mixing. The solution may be used as soon as cool, but is a little better if permitted to stand a few hours. It changes but little after several weeks in a flask with only a loose cotton plug.

In use, material to be stained is treated according to the usual Haidenhain method, with the exception that the writer uses intensifiers which have proved to be advantageous, although they may be omitted, the results being comparable to the usual ones with this method.

In intensifying the stain the material is removed from the haematoxylin, rinsed in distilled water and destained in 2 per cent, iron-alum until all undesirable precipitates are removed and the intensity of the stain is below that desired in the finished preparation. The material is then rinsed for one to three hours or even longer in two to six changes of distilled water. After the material is thoroughly rinsed to remove all traces of iron-alum, the stain is then intensified by immersion in a weak solution of sodium acid carbonate, magnesium carbonate, or lithium carbonate, until it assumes the desired intensity. In this way a greater degree of differentiation is attained than by the ordinary method. The writer has found lithium carbonate more satisfactory than sodium acid carbonate. Magnesium carbonate has not been used on a sufficient variety of material for its value to be estimated, al-

[1] E. J. Kohl, C. M. James, "A Method for Ripening Haemotoxylin Solutions Rapidly," SCIENCE, 74: 247, 1931.

though where it has been used it has given satisfactory results.

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HEAVY GLASSWARE IN THE LABORATORY

THIS note was suggested by that of Dr. J. Howard Brown who recently described in these columns the use of milk bottles with a crown seal for bacteriological laboratory purposes. In the laboratory with which the writers are connected the replacement of the fragile and expensive laboratory glassware by heavy or commercial products has been constantly increasing. The type commonly known as a prescription bottle, in the 6 oz. and 12 oz. sizes, has been found particularly desirable. The modern heavy glass bottle is so well annealed that it will stand repeated heatings in the autoclave with no clouding of the glass and with a negligible amount of breakage.

Instead of the crown seal, we have used the screw cap seal with excellent results. The screw caps can be obtained with various types of liners, of which the cardboard-waxed paper combination has proved the best. Such caps can be placed on loosely in situations where ventilation is desired. For many purposes an unlined cap is best. It permits of practically no evaporation on long storage and the closure is sufficient to prevent all contamination. The easy removal of the screw cap and its re-use an indefinite number of times is an advantage over the crown seal.

A prescription bottle, which has one side nearly flat, is used in this laboratory for a variety of purposes. It serves admirably for the storage of bacteriological media, for the water used as dilution blanks in quantitative bacteriological work, and in many instances in place of the familiar petri dish. About 15–20 cc of agar is sterilized in the bottle, which is placed with the flat side down for the agar to harden, when the bottle is used in place of a petri dish. When used in the student laboratories in place of the petri dish for isolation purposes or for quantitative work, there is less difficulty from contamination and much less desiccation of the agar than with the petri dish.

The screw cap when placed loosely on the bottle permits of sufficient diffusion of oxygen to support the growth of the obligate aerobic organisms and serves to keep out contamination as well if not better than a cotton plug. In moist situations there is no danger of molds growing through the seal as often occurs with cotton plugs.

These bottles are very satisfactory for the growth of mass cultures of bacteria for the preparation of vaccines and antigens. During the past three years many thousand cultures of the legume-nodule bacteria have been grown and distributed to the farmers of the state in such bottles. Before the adoption of the screw cap, bottles with cork finish were used. The cork-finish bottles were plugged with cotton during the incubation period and this cotton plug was replaced with a paraffined cork for shipment. With the screw-cap bottle, the cap is placed on loosely during the incubation period and then tightened for shipment. The use of the screw cap has materially lessened the labor of preparation and has actually resulted in a lower percentage of contamination. The inoculation of the agar for these cultures is done by a spray process similar to that employed in applying lacquer or paint. An ordinary atomizer with supply pipe dipping into a suspension of the inoculant is supplied with compressed air which passes through a sterile three-foot length of 2 inch iron pipe which is packed with cotton. Two men can easily inoculate 800 bottles an hour with this apparatus.

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SPECIAL ARTICLES

A PRELIMINARY NOTE ON THE SIGNIFICANCE OF THE PHOSPHORUS INTAKE IN THE DIET AND BLOOD PHOSPHORUS CONCENTRATION, IN THE EXPERIMENTAL PRODUCTION OF CARIES-IMMUNITY AND CARIES-SUSCEPTIBILITY IN THE RAT[1]

INTRODUCTION

RECENTLY Hoppert, Webber and Canniff[2] have reported that rats fed stock diets develop caries[3] of the molars at three months of age. The composition of the stock diet as published consists of whole ground corn 60 parts, whole milk powder 30 parts, linseed oil 6 parts, alfalfa 3 parts and sodium chloride 1 part.

When the 60 parts of corn were omitted from the diet and replaced by either 60 parts of oatmeal or 60

[1] The Biochemical Laboratory, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland.

[2] C. A. Hoppert, P. A. Webber and T. L. Canniff, SCIENCE, 74: 77, July 17, 1931.

[3] Dental caries in the rat means a breaking down of tooth structure in the molars of rats resulting in a cavity formation such as has been described in a previous communication (H. Klein, *J. Dental Research*, 11: 151, February, 1931). The use of the term "dental caries in the rat" means exactly what the term implies and does not carry with it any implication or suggestion that such caries in the rat is the same as that known as human dental caries.