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A Method of Demonstrating Corneal Mitosis (After the Method of Gay and Kaufmann)

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procedure to increase sensitivity. The optimal pH is that of normal fresh skim milk.

The addition of hydrogen peroxide to a level of 0.3 per cent appeared to stimulate activity, and no activation was realized by the addition of reducing agents. This anomalous behavior remains to be elucidated.

The addition of formaldehyde to the reaction mixture was inhibitory, but infected material exposed to formalin vapors sufficient to inhibit spore germination still retained enough enzymatic action to yield a positive field test based on the peptonizing activity. The curdling activity responded in a similar fashion to the effects of formaldehyde and peroxide.

The practical application of these results to the diagnosis of American foulbrood disease of bees was discussed.

INTRODUCTION TO RESEARCH PROBLEMS FOR UNDERGRADUATES

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A METHOD OF DEMONSTRATING CORNEAL MITOSIS (AFTER THE METHOD OF GAY AND KAUFMANN)

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For this demonstration the mouse eye was used because of the thin character of the cornea.

Procedure: The mouse is killed by dislocating one of its cervical vertebrae, crushing its spinal cord.

The entire eye is then ennucleated and placed in a solution which simultaneously fixes and stains it. This solution is prepared in the following manner:

> Three parts of a saturated solution of orcein in acetic acid (2 gr. orcein in 100 ml. glacial acetic acid; reflux for 6 hrs., add water during refluxing if necessary; filter with a Buchner funnel). Eleven parts saturated solution of orcein in 95 per cent ethyl alcohol (2 gr. orcein in 100 ml. alcohol; let stand for a day; filter with a Buchner funnel).

This solution can either be kept mixed or with the two solutions in separate containers for an indefinite period of time.

The entire eye is kept in the complete solution for 45 minutes or slightly longer.

The eyeball is then placed in a freshly prepared solution of two parts acetic acid and eleven parts alcohol. In this solution the cornea is removed, the iris removed from the cornea, and four equidistant cuts from the circumference toward the center of the cornea are made to flatten it out.

This operation should not take longer than three minutes. If more time is desired the work should be done in a 95 per cent alcohol solution because the tissue readily destains.

The cornea is rinsed in 95 per cent alcohol and placed face up on a slide and mounted in euperal and covered with a no. 1 or 00 cover glass.

This eliminates the use of a microtome. Therefore, all stages of mitosis can be seen from all angles. Mitotic figures and the cell walls can also be seen very well.

The mitotic figures are very distinct. Because cytoplasm also stains relatively deep, this procedure appears unsatisfactory for counting chromosomes.

Therefore, this method of demonstrating mitosis is satisfactory for an animal with a corneal thickness of one cell, but fails to differentiate the chromosomes sufficiently for a count.