Technical Papers

Meiosis in *Gymnosporangium* and the Cytological Effects of Certain Antibiotic Substances

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The cedar-hawthorne rust, *Gymnosporangium clavipes,* is a particularly suitable fungus for cytological study in that the teliospores when stored at a low temperature frequently remain viable for a long period of time, and the nuclei are relatively large, 8–10 μ in diameter. The experimental procedure was as follows: twigs of red cedar infected with the telial stage of *G. clavipes* were kept at a cool temperature in a dried condition and germinated over a period of 6 months. Each slide was prepared by placing a portion of a telial sorus in a drop of tap water on a slide, which is kept in a moist chamber. At appropriate intervals in basidial development, the gelatinous mass of germinating teliospores was smeared on the slide, and killed and fixed in a modified Carnoy's fluid containing chloroform, and then stained by the propionocarmine technique. The entire process of teliospore germination, basidial development, and basidospore formation takes place in 5–6 hr.

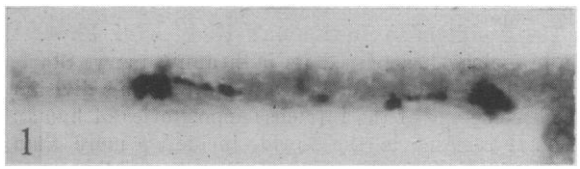

FIG. 1. Unusually elongated spindle of Anaphase I with lagging chromosomes, after treatment with 1000 ppm of streptomycin. × 1600.

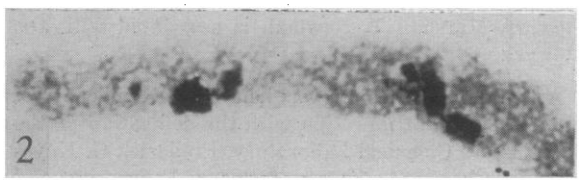

FIG. 2. Chromatic clumps representing unequal groups of chromosomes during the first meiotic division. Treated with 1000 ppm of streptomycin. × 1600.

Studies of normal cytology indicate that the diploid number of chromosomes as found in the unreduced nucleus in the basidium is 16 (8 bivalents), while the haploid number, found passing to each pole in the first and second divisions of meiosis, is 8. The chromosomes range in size from 1.0–4.7 μ at metaphase I of meiosis, and there is present a distinct nucleolus chromosome. Chromosome number determinations have also been made at metaphase I for *G. transformans, G. nidus-avis* and *G. juniperi-virginianae.* In all 3 of these species the haploid number of chromosomes is the same as in *G. clavipes,* namely 8. This appears to represent the first clear demonstration of chromosome numbers in the genus. The same number was found in *Cronartium* by Colley (*1*) and in *Coleosporium* by Olive (*2, 3*).

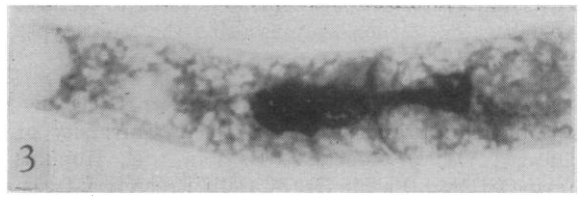

FIG. 3. Transverse septum in basidium cutting across a spindle, indicating a failure in synchronization between septum formation and nuclear division. Treated with 1000 ppm of streptomycin. × 1600.

The writers became interested in testing the effects of certain antibiotics on basidial development in *G. clavipes,* and the following 3 substances were used: penicillin G, streptomycin, and cycloheximide (Actidione), all kindly supplied by A. J. Whiffen of the Upjohn Co. In these experiments the telial sori were germinated in different concentrations of the antibiotic at various stages in basidial development and processed in the same manner as the control material previously described. The observable effects of these 3 antibiotics are often morphological as well as cytological. In certain cases fairly specific cytological effects can be produced. The following observations were made after periods of treatment varying from 1–5 hr.

The effective range of concentrations of penicillin G which showed consistent results was between 2000–5000 ppm. The most striking morphological effect of penicillin is to cause a forking of the basidium, or of the sterigma, depending on the time of application of the antibiotic. The nucleus has a tendency to remain in the teliospore or possibly to migrate back into it, again depending on the time of treatment. So far, in our work, the nuclei do not stain as well as in normal material and nuclear details are obscured.

Streptomycin has been the most rewarding and interesting of the 3 substances studied. The range of concentrations most effective was relatively high—between 1000–10,000 ppm. One of the interesting effects of streptomycin is to induce the basidia to form side arms superficially resembling clamp connections. Also there frequently occurs a back migration of one of the nuclei into the teliospore following the first meiotic division. Streptomycin also affects the synchronization between nuclear divisions and cross-wall formation in the basidium, which often results in the formation of a wall cutting across a nucleus or spindle (Fig. 3). This may be due to a retardation or cessation of the nuclear divisions induced by application of the antibiotic. This interference with nuclear division occa-

sionally results in the formation of several chromatic clumps, apparently representing unequal groups of chromosomes (Figs. 1 and 2). These groups may sometimes be found still connected to each other by chromatic strands.

Cycloheximide, like streptomycin, is an antibiotic produced by *Streptomyces griseus,* and has marked fungicidal properties. Whiffen (*4*) recently showed that the treatment of the sporophytes of *Allomyces arbuscula* with cycloheximide in very low concentrations resulted in the development of gametophytic outgrowths, thus indicating somatic reduction. However, this effect was observed only after 10–14 days, in contrast to the early observable effects upon the basidia of *Gymnosporangium.* According to Wilson (*5*), the substance has been shown to have interesting cytological effects in onion-root tips, in which it may induce the formation of "reductional groupings" of chromosomes during somatic mitosis. In our work with *Gymnosporangium,* the effective range of concentrations of cycloheximide has been found to be between 5–500 ppm. At the higher concentrations basidial development is almost completely inhibited and the nuclei become very large, with much extended chromosomes. Cycloheximide also causes a forking of basidia similar to that induced by penicillin, and in some instances there occurs a lagging of the chromosomes during meiosis. Lower concentrations cause a return of one of the nuclei to the teliospore, as was found in penicillin- and streptomycin-treated material. The most striking effect of cycloheximide is observed in the occurrence of many basidia with 2 nuclei closely associated, or still connected to each other by chromatic strands, in sharp contrast to the usual long distance between daughter nuclei following anaphase. This may be due to malfunction or disintegration of the spindle and might result occasionally in the reconstitution of a single diploid nucleus rather than two haploid ones.

References

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Effects of Hyaluronidase on Human Gingival Epithelium[1]

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As part of a study of the mechanism by which accumulations of gingival bacteria produce marginal inflammation, we were able to demonstrate the presence of a "spreading factor" in cell-free extracts of gingival debris. This fact, together with the known presence of the enzyme hyaluronidase in saliva (*1*) and the demonstration of high production of hyaluronidase by certain oral bacteria (*2*), made it seem important to examine the effect of this enzyme applied topically on human gingival tissues. The connective tissue reactions stimulated by hyaluronidase have been described previously (*3, 4*). However, these descriptions do not discuss the changes brought about in the epithelium or the manner by which the enzyme can reach the underlying connective tissue. Apart from general interest, this problem has special importance in gingivitis because it is not known whether the hyaluronidase reaches the connective tissue as the result of trauma, through a weak epithelial attachment, or by passage through the epithelium itself.

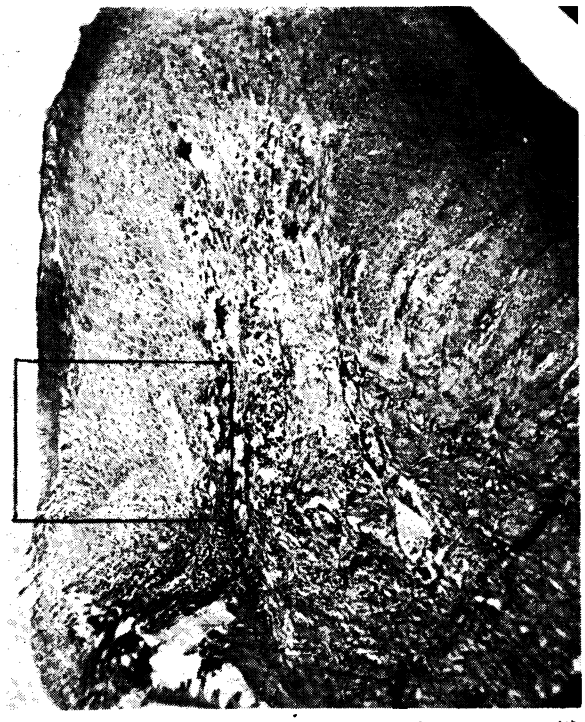

FIG. 1. Gingival tissue after 2 days of treatment with hyaluronidase. Paraffin embedding; section thickness 6 μ; stained with Masson's Trichrome stain. Area of dissolution of the pocket epithelium indicated. The connective tissue presents an irregular appearance.

Biopsies of human gingiva exposed to the action of hyaluronidase (Wydase) were chosen as a means of determining the tissue reactions. Both experimental and control tissues were taken from each of 36 individuals' normal and inflamed gingiva. The hyaluronidase solution was always used in a fresh state as 150 TR.U./cc in sterile triple-distilled water. It was introduced into the gingival crevice by a small syringe; extreme care was taken to avoid injury to the tissue. The delivery rate was about 12 TR.U./min. When inactivated hyaluronidase was used, the enzyme was either inactivated by heat or by addition of heparin to the solution. After the experimental period, the treated gingival tissues were carefully removed by biopsy. After fixation in Carnoy's fluid the tissues obtained were embedded in paraffin and sectioned. A

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