Research Note

PREPARATION OF CYTOLOGICAL SPECIMENS USING GASTRIC JUICE OF AFRICAN GIANT SNAIL[1]

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Gastric juice extracted from African giant snail, *Acatina fulica* Bowd, was employed to remove the ascus walls of *Ascobolus immersus* for the chromosomal studies in our laboratory (Li *et. al.* 1967). The treatment greatly enhanced the staining of nuclear materials by acetocarmine. We have since used the same technique to prepare the explants of *Haplopappus gracilis* for karyological studies, The techique is described in detail here for those laboratories where commercial cellulolytic enzyme preparations are difficult, or too costly, to obtain.

The snails are cellected early in the morning when they are fully fed with vegetation. Yield of gastric juice is higher when the animals are fully fed. The gastric systems are collected and homogenized with a motar which is chilled with ice cubes. The homogenate is then centrifuged at 14,650 g for 20 minutes under refregeration. The supernatant, which contains various cellulolytic nzymes, is stored in a deep freezer untill used.

Haplopappus explants are prefixed with 1:3 acetic acid-alcohol for 12 hours. Excessive fixative is immediately rinsed off with distilled water.

A small piece of the fixed tissue, about $2mm^3$, is then placed on a slide and covered with a drop of the snail gastric extract (diluted two fold with distilled water) for approximately 30 minutes under room temperature. At the end of digestion, the gastric juice is removed with small pieces of filter paper followed immediately by smearing and staining.

Specimens treated in this manner are sufficiently macerated so that smear consisted of mostly single-cell-layer can be easily prepared by applying slight pressure on the cover glass.

Preliminary experiment has to be performed with each batch of gastric juice extract to determine the adequate dilution and length of treatment, since the enzyme concentration varies with each batch of aminals. Excessive ex-

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posure to the gastric extract, either by prolonging the treatment or by insufficient dilution, would result in the complets degradation of cell wall.

As shown in Fig. 1, spindle fibers are stained much more readily in the specimens treated with the gastric juice than in those prepared by conventional weak HCl hydrolysis (Jonsen 1962).

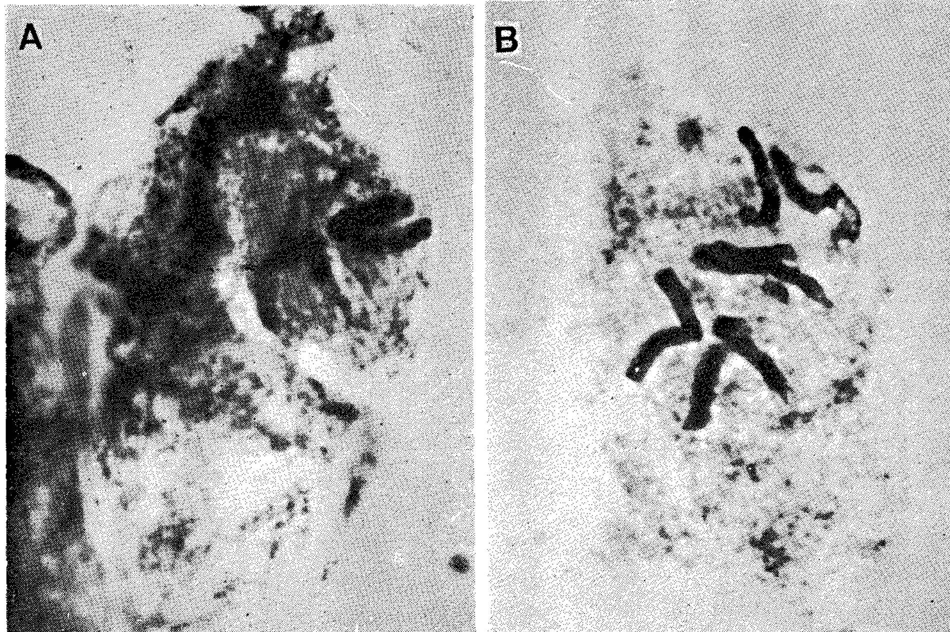

Fig. 1. Smear preparations from *Haplopappus gracilis* explants. A, treated with the gastric extract of African giant snail for 30 min. prior to acetocarmine staining, showing a diploid mitotic apparatus with prominent spindles. B, hydrolyyed with 0.1N HCl for 10 min at 60°C prior to similar acetocarmine staining, showing a polyploid metaphase plate with obscure spindles. Note that the gastric extract treated preparation gave much better contrast for the mitotic spindle.

Literature Cited

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