Fluorochrome-Labeled Deoxyribonuclease: Specific Stain for Cell Nuclei

Abstract. *Nuclei of cells in tissue culture may be stained with deoxyribonuclease labeled with fluorescein isothiocyanate; this is a simple, rapid, and specific process which is useful in localizing DNA* in situ.

Specific enzyme-substrate reactions occur within cells, as shown by electron microscopy with ferritin-labeled chitinase-chitin systems (*1*) and in other systems in which fluorochrome-labeled enzymes are used (*2*). We now report another example, namely the specific staining of nuclei of cells in tissue culture with deoxyribonuclease labeled with fluorescein isothiocyanate.

Deoxyribonuclease-fluorescein isothiocyanate (*3*) is conjugated by the standard methods for fluorescent labeling of antibodies (*4*). Unconjugated free dye is removed by dialysis against phosphate buffer and passage through a fine-mesh Sephadex G-25 column (*5*).

The Feulgen reaction was modified from Gurr (*6*). The photographs showing fluorescence were taken through a Reichert Zetopan microscope, with ultraviolet illumination; a Yashica Lynx 5000, 35-mm camera mounted on a Unitron Model ACA photomicrography set was used. Photographs of preparations stained by the Feulgen method were taken through a Nikon Model M inverted microscope, with the Nikon 35-mm camera back.

The L cell, chick embryo cell, and the HeLa cell tissue-culture lines used in this study were grown on microscope slides in Leighton tubes (*7*). The staining procedure consists of washing the cells in Earle's basic salt solution (*8*) and overlaying them with deoxyribonuclease-fluorescein isothiocyanate. A cover glass placed over these preparations was sealed with paraffin, and the cells were viewed immediately with the fluorescent microscope.

In preparations both mounted and viewed in labeled deoxyribonuclease, the selective fluorescence in the nuclei of cells, as opposed to the absence of fluorescence in the cytoplasm (Fig. 1), indicates that the conjugate has a specific affinity for the cell nucleus.

That deoxyribonuclease is enzymatically active even after having been conjugated with fluorescein isothiocyanate is shown by the Feulgen reaction (Figs. 2 and 3), since the nuclear material (DNA) in the cells shown in Fig. 3 has been apparently digested by the tagged enzyme and the space normally occupied by the nucleus is now an unstained void which does not fluoresce. The conjugate of enzyme and dye is able to penetrate both the cytoplasmic and nuclear barriers to reach its substrate, DNA.

The fluorescent deoxyribonuclease staining method has the virtue of spec-

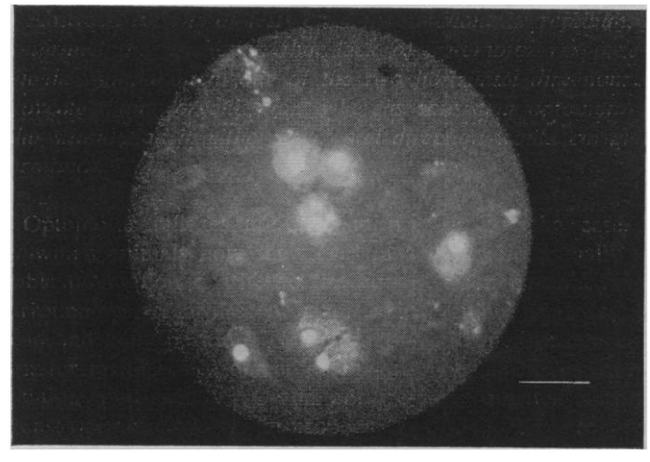

Fig. 1 (right). L cells mounted and stained in deoxyribonuclease-fluorescein isothiocyanate, viewed and photographed immediately (× 157). Marker represents 10 μ.

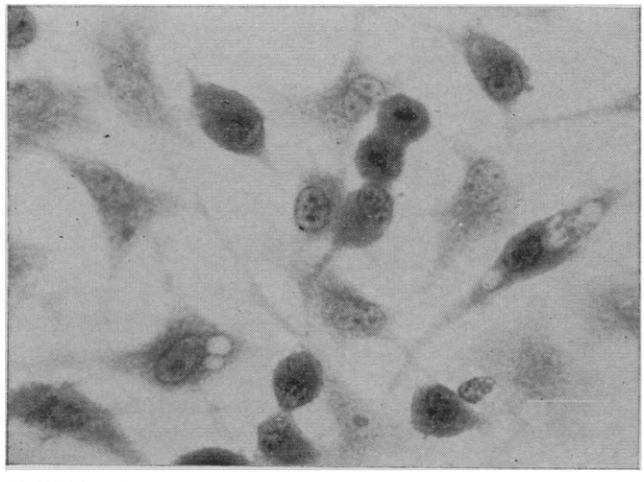

Fig. 2 (bottom left). L cells stained by the Feulgen reaction (× 196). Marker represents 10 μ.

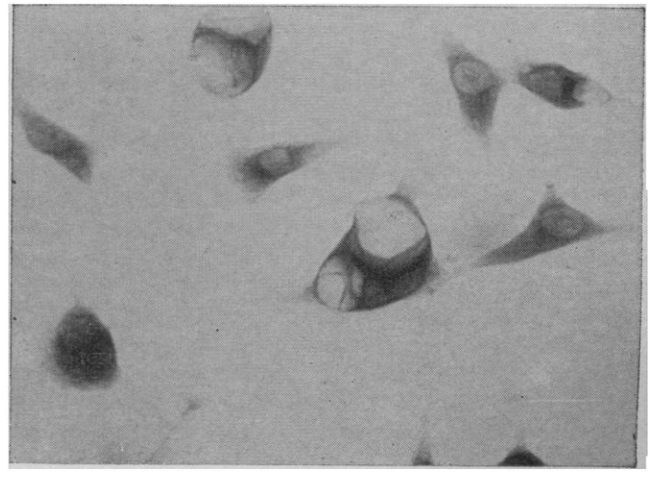

Fig. 3 (bottom right). L cells stained by the Feulgen reaction after 15 minutes in deoxyribonuclease-fluorescein isothiocyanate (× 196). Marker represents 10 μ.

ificity inherent in enzyme-substrate reactions and should therefore be useful as a cytochemical technique.

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References and Notes

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3. Pancreatic deoxyribonuclease, electrophoretically purified and free of ribonuclease was obtained from Worthington Biochemical Corp., Freehold, N.J. Fluorescein isothiocyanate was obtained from Nutritional Biochemicals, Cleveland, Ohio.
4. R. C. Nairn, *Fluorescent Protein Tracing* (Livingston, London, ed. 2, 1964), pp. 14–24.
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8. Grand Island Biological Co., Grand Island, N.Y.

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Propanil Hydrolysis: Inhibition in Rice Plants by Insecticides

Abstract. *Hydrolysis of the herbicide propanil (3',4'-dichloropropionanilide) by rice plants is inhibited by insecticides. The inhibitory activity of an organophosphate such as paraoxon in vivo and in vitro is significantly stronger than that of an organothiophosphate such as parathion. The injury to rice plants by insecticides sprayed on them with propanil seems to be caused by the inhibition of the propanil detoxifying enzyme.*

Propanil (3',4'-dichloropropionanilide) is a highly selective herbicide which discriminates between rice and certain weed plants, especially barnyard grass (*Echinochloa crusgalli*). There is reason to believe that the tolerance of rice plants to propanil, and hence its selectivity, rests on the ability of the rice plant to metabolize or detoxify the chemical. McRae *et al.* (*1*), Adachi *et al.* (*2*), and Ishizuka *et al.* (*3*) reported the existence of an enzyme capable of hydrolyzing the anilide into 3,4-dichloroaniline and propionic acid in rice plant homogenates. Still and Kuzirian (*4*) have described the metabolism in more detail. The purification and properties of a rice aryl acylamidase (aryl-acylamine amidohydrolase, E.C. 3.5.1.13) which could hydrolyze the anilide have been reported recently (*5*).

In paddy fields the combination of certain insecticides with propanil causes injury to rice plants (*6*). Both organophosphorus insecticides and carbamate insecticides extremely enhance the herbicidal activity of propanil against rice. Since propanil is used as a selective herbicide on rice plants, the enhancement of toxicity by insecticides used to protect the rice is very important in crop management. However, the synergistic action of the insecticides with propanil is being utilized in a combination of propanil and carbaryl for the control of crabgrass (*Digitaria adscendens*) in citrus orchards in Japan. This combination takes advantage of the fact that the insecticide inhibits the high enzymatic activity of the crabgrass, thus permitting action of the propanil.

Two different types of experiments were performed to investigate the mechanism of the interaction of the organophosphorus and the carbamate insecticides in the phytotoxicity of propanil.

A homogenate was prepared from rice plants at the five-leaf stage (Variety: Nôrin 29) by grinding 5 g of fresh tissue with a small amount of 0.05*M* phosphate buffer (*p*H 7.0) with 10 g of quartz sand for 15 minutes. The resultant paste was diluted with additional buffer to 50 ml, filtered through two layers of cheesecloth, and centrifuged at 1000*g* for 10 minutes. The supernatant was then used as the enzyme solution. The reaction was carried out with 5 ml of enzyme solution, 5 ml of 0.1*M* phosphate buffer (*p*H 8.3), and 5 ml of $1.5 \times 10^{-4}M$ propanil and inhibitors. Initially both propanil and insecticides were dissolved in ethanol. The final concentration of ethanol in the reaction mixture was lower than 0.33 percent. At the end of 2 hours at 30°C, the reaction was stopped by the addition of 1.5 ml of cold 50 percent trichloroacetic acid, the mixture was centrifuged, and the supernatant was used for determination of 3,4-dichloroaniline by the method of Goto and Sato (*7*). The inhibitors used in this study included parathion, paraoxon, sumithion (fenitrothion), and sumioxon (Fig. 1). The concentrations required to produce 50 percent inhibition were calculated (Table 1).

The inhibitory patterns for both the propanil-hydrolyzing enzyme in rice plants and the acetylcholinesterase in insects are very similar. It is speculated, therefore, that the propanil detoxifying enzyme and acetylcholinesterase may resemble each other. Carbaryl (1-naphthyl-*N*-methylcarbamate), an insecticide which inhibits acetylcholinesterase, is also a strong inhibitor of the propanil detoxifying enzyme (*5*). On the other hand, BHC (benzene hexachloride), an insecticide having no effect on acetylcholinesterase also had no effect on the rice enzyme. In the

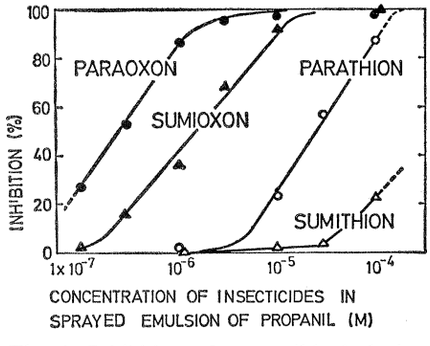

Fig. 1. Inhibition of propanil-hydrolyzing enzyme in rice plants by insecticides.

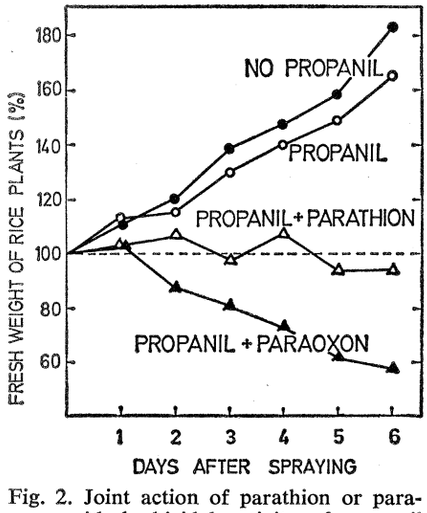

Fig. 2. Joint action of parathion or paraoxon with herbicidal activity of propanil in rice plants.