Apyrases of Moulds

THE mycelial mats of *Aspergillus niger* and *Penicillium chrysogenum* possess apyrase activity. The organisms were grown as surface cultures on synthetic media, and at regular intervals the mat was analysed for apyrase activity and for '7-minutes phosphate' in the acid-soluble fraction. During the first phase of metabolism, when sugar was being rapidly consumed, the enzymic activity and the content of labile phosphate were high. When autolysis set in there was a marked drop and sometimes even a complete disappearance of both apyrase activity and labile phosphate. Apyrase probably plays an important part in the metabolism of the mould.

Estimation of enzymic activity in culture solutions was rendered difficult because of the presence of orthophosphate; but at no time was the enzyme concentration high.

Considerable variation existed in the apyrase activity of different strains. The *p*H – activity relationship was different for *Aspergillus* and *Penicillium*.

Apyrase activity of the mycelium could be extracted by grinding with water in the Waring blendor. The apyrase of *Aspergillus niger* has been concentrated about 450-fold by fractionation with ammonium sulphate. A colourless solution was obtained which was free from glucose oxidase. The enzyme preparations were inhibited by both calcium and magnesium ions at a final concentration of $M/100$. This is in contrast to the marked stimulation of potato apyrase by added calcium ions[1]. Tests with inorganic pyrophosphate showed that the purified enzyme possessed powerful pyrophosphatase activity also. The relative speed of dephosphorylation of inorganic pyrophosphate and adenosinetriphosphate remained fairly constant without any sharp break during the various stages of purification of the enzyme.

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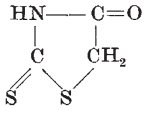
[1] Krishnan, P. S., *Arch. Biochem.*, **20**, 272 (1949).

Mildew-preventing Activity of Rhodanine Derivatives

ALTHOUGH rhodanine (I) has some fungicidal activity[1], it was found to be ineffective as a mildew- or rot-preventing agent on cotton cloth. Through condensation of rhodanine with aldehydes and



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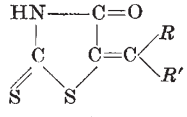

II

ketones, derivatives (II) having marked mildew-proofing activity may be obtained. More than a hundred such derivatives have been prepared and tested in our laboratory. It is interesting to note that the new mildew-proofing agents contain the structure —N—C(=S)— present in many plant fungicides (tetramethylthiuram disulphide and the salts of dithiocarbamic acid), as well as a carbonyl group conjugated with an ethylenic linkage, found in another class of fungicides[2].

Uniform strips of dyed herring-bone cotton twill were impregnated with 2 per cent of the fungicide under examination and incubated for two weeks on mineral salts agar with pure cultures of *Aspergillus niger* and *Chœtomium globosum*. Other such strips were buried for two weeks or four weeks in soil. In each case the percentage loss in tensile strength was determined.

The majority of the compounds of type II tested were effective in preventing deterioration of the cloth in the pure culture tests (less than 5 per cent loss in tensile strength). Eighteen of the compounds afforded similar protection in the two weeks soil burial test. Under the same conditions cloth strips impregnated with chloranil lost 80 per cent or more of their tensile strength.

In four weeks soil burial tests, the most active five compounds were the 2-thenylidene-, *p*-methylcyclohexylidene-, α-*n*-amylhexylidine-, *o*-methylcyclohexylidene- and *p*-chlorobenzylidene-rhodanines. All these prevented tensile strength losses greater than 25 per cent.

Details of this work, which was supported by a contract with the Medical Division, Chemical Corps, U.S. Army, will be reported elsewhere.

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[1] U.S. Patent 1,962,109.
[2] Geiger and Conn, *J. Amer. Chem. Soc.*, **67**, 112 (1945).

Improved Sampling Method for Demonstration of Local Antibodies in the Vagina

IN infections with pathogens of low virulence strictly localized to a few or single organs, it is often difficult, if not impossible, to obtain reliable diagnostic findings by means of tests for antibodies circulating in the blood stream. This holds true in particular if only some parts of a mucous membrane are involved. In such cases, however, it may prove practicable to utilize the local antibody production for diagnostic purposes.

Serological examination of vaginal mucus has been employed by Pierce[1] for the diagnosis of bovine trichomoniasis, and by Stegenga and Terpstra[2] for the diagnosis of vibriosis (due to *Vibrio fœtus*). The local production of antibody has also been used to detect bulls with brucellosis localized to the genitals[3].

The procedure hitherto employed for collection of vaginal mucus by suction with a glass tube is cumbersome as well as time-consuming; besides, it is applicable only to cows with sufficient secretion in the vagina. On the other hand, on account of its great dilution, the usually abundant œstral discharge is less suitable for the demonstration of antibodies. Therefore, this sampling technique is not very serviceable for routine examination.

A gauze tampon (about 15 cm. × 19 cm., weighing 1·1 gm.) placed in the fundus of the vagina will absorb on an average about 3 gm. of secretion, and even when the vagina is 'dry' the tampon will take