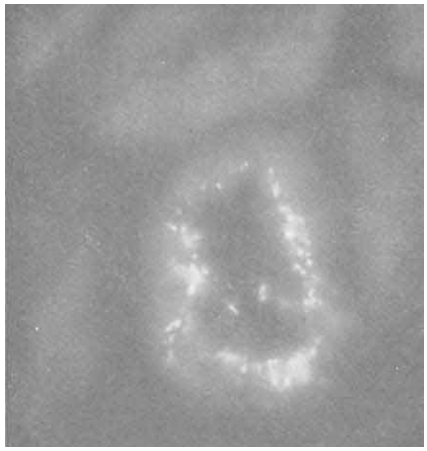

Fig. 2. 8-$\mu$ cold microtome section of mouse kidney, 18 hr. after intraperitoneal injection of ovalbumin labelled with a fluorescent dichloro-triazinyl dye. (× 1,100)

The labelling of proteins with reactive dyes is simple and offers considerable advantages over current methods of conjugating proteins through azo[5] and ureido[6] linkages. In a series of preliminary tests with fluorescent or coloured dyes containing other reactive groups (sulphon-β-chloroethylamide, sulphon fluoride, thiocyano, chloroacetylamino, γ-chloro- β-hydroxypropyl) the superiority of the dichloro-triazinyl group appears to be established. These studies are being continued and extended.

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## Reversal of Antibiotic Action of Cycloheximide (Actidione) by Bivalent Metal Ions

Cycloheximide, an antibiotic with selective inhibitory effect on many fungi[1,2] but inactive against bacteria, has received increased attention during recent years, but few reports are concerned with its mode of action. Cycloheximide inhibits aerobic utilization of many intermediates of carbohydrate metabolism by *Tetrahymena pyriformis*[3], and it is active against higher plants[4]. Kerridge[5] has observed that fungistatic doses of cycloheximide (0·5–1·0 μgm./ml.) have no marked effect on fermentation and respiration of *Saccharomyces mandshuricus*, but synthesis of protein and deoxyribonucleic acid is completely blocked. On the other hand, Greig *et al.*[6] have recently announced that in the case of *Saccharomyces cerevisiae* the same antibiotic shows an inhibitory effect on fermentation.

This communication presents the results of a study of the inhibitory action of cycloheximide on growth and respiration of a sensitive strain of *Candida krusei* and a resistant strain of *Candida albicans*. Previous authors employed agar plate techniques for measurement of growth; in the present work growth was estimated turbidimetrically. Cultures were incubated for 22 hr. with forced aeration at 28° C. in a liquid medium containing glucose and mineral salts.

The results of experiments on growth show that 0·5 μgm./ml. of cycloheximide has a marked antibiotic effect while 2 μgm./ml. inhibit completely the multiplication of *C. krusei*. The growth of the resistant *C. albicans* was not altered by concentrations of antibiotic up to 100 μgm./ml. The activity of antibiotic remained unchanged when ammonium sulphate in the medium was replaced by other nitrogen sources, such as amino-acids, peptides and protein hydrolysates. Curiously enough, the only exception was peptone which, in a concentration of 1 per cent, almost completely suppressed the inhibitory action of cycloheximide. The degree of inhibition depended on the origin of the peptone employed and was due not to the organic components but to iron. When the heavy metals in peptone were precipitated by hydrogen sulphide the reversal of inhibition did not occur; when ferrous iron alone was added to the mineral medium with inorganic source of nitrogen, the inhibitory effect of cycloheximide was suppressed.

The reversal of inhibitory activity was also demonstrated in manometric experiments with washed suspensions of *C. krusei*. Cycloheximide at a concentration of 0·5 μgm./ml. caused 40 per cent inhibition of the rate of oxidation of glucose by intact cells. In the presence of $2 \times 10^{-4}$ *M* $FeSO_4.7H_2O$ and 0·5 μgm./ml. of cycloheximide the respiration was even faster than in the control without antibiotic. Manganese or zinc, like iron, antagonized the inhibitory effect of antibiotic; calcium, magnesium or cadmium were less active and copper had no detectable effect. Respiratory activity of washed cells of *C. albicans* was not influenced by cycloheximide.

These observations suggest that cycloheximide forms chelates with bivalent metals and that these complexes are antibiotically inactive. This phenomenon of chelate formation is not unique among antibiotics, and the subject has been well summarized in a review by Weinberg[7]. He, as well as Whiffen[2], did not report any influence of metals in the case of cycloheximide; but Greig *et al.*[6] observed potentiation of the inhibitory effect of this antibiotic on fermentation by *Saccharomyces cerevisiae*. This apparent discrepancy concerning the mode of action of metals on antibiotic potency of cycloheximide seems to be due not only to the use of different organisms but also to different criteria and methods employed.

Details of these experiments will be published elsewhere.

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