They suggested that substances in the medium which are toxic to damaged cells could be removed by adsorption on metal precipitates or by co-precipitation with them, and that cations which are not precipitated could reduce the permeability of the damaged cell membrane and so reduce the inward diffusion of such toxic substances and the leakage from the cell of vital metabolites[6,7]. The protective influence of metallic cations, especially those of high valency, against the action of phenol has been observed in ref. 7.

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[1] Harris, N. D., and Richards, J. P., *J. App. Bact.*, **21**, 86 (1958).
[2] Curran, H. R., and Evans, F. R., *J. Bact.*, **34**, 179 (1937). Nelson, F. E., *ibid.*, **45**, 395 (1943). Flett, L. H., *et al.*, *ibid.*, **50**, 591 (1945). Jacobs, S. E., and Harris, N. D., *Congr. Intern. Microbiol.*, Rome, **1**, 603 (1953).
[3] Jacobs, S. E., and Harris, N. D., *J. Pharm. Pharmacol.*, **6**, 877 (1954).
[4] Jacobs, S. E., and Harris, N. D., *J. App. Bact.*, **23**, 294 (1960).
[5] Harris, N. D., and Jacobs, S. E., *J. App. Bact.*, **23**, (3), viii (1960). Richards, J. P., thesis (London, 1959).
[6] Gale, E. F., and Taylor, E. S., *J. Gen. Microbiol.*, **1**, 77 (1947). Bean, H. S., and Walters, V., *J. Pharm. Pharmacol.*, **7**, 661 (1955). Beckett, A. H., Patki, S. J., and Robinson, A. E., *Nature*, **181**, 712 (1958).
[7] Haydon, D. A., *Proc. Roy. Soc.*, B, **145**, 383 (1956).

Enzymic Inactivation of Trichothecin and Crotocin

Work on the inhibitory action of trichothecin[1] and crotocin (substance *T*, ref. 2) on the inductive amylase synthesis of *Penicillium chrysogenum*[3] suggested that these antibiotics may undergo enzymic inactivation in certain circumstances. The possibility of such an enzymic inactivation was first recognized by Darpoux and Faivre-Amiot[4].

Penicillium chrysogenum was grown on a synthetic medium as described previously[5]. Cultures for the experiments were prepared by inoculating 200 ml. synthetic medium with 10 ml. of a 72-hr.-old vegetative mycelium suspension. The fate of crotocin added was examined by two different methods. First, the antibiotic was added in a 1 mgm./ml. single dose after 48 hr. incubation. The level of the antibiotic in such a culture remained unchanged for a minimum of 24 hr. as revealed by the Bradler[6] agar-diffusion method. In the second series of experiments 10 μgm./ml. crotocin was added after 40 hr. and this was followed by the addition of 1 mgm./ml. of the same antibiotic after 48 hr. incubation. Under these experimental conditions a 70 per cent decrease in the antibiotic activity of crotocin was observed in 5 hr.

This phenomenon suggested the possible presence of a crotocin-inactivating enzyme in cultures pretreated with a low dose of the antibiotic. The enzyme appeared to be bound to the mycelia as the supernatant was inactive, while 1 gm. of washed mycelium (calculated on dry material) inactivated an average of 40 mgm. crotocin per hr. The preliminary examinations carried out up to now indicated that the enzyme was probably bound to the cell-wall of the mycelium.

It is known that trichothecin is an ester of trichothecolon and isocrotonic acid[7]. The alcoholic component of crotocin is very similar, though not identical, to that of trichothecolon. It seemed plausible that the decrease of the biological activity was a result of enzymic cleavage of the ester linkage. The validity of this assumption was later confirmed by isolating and chromatographing the split products.

After inactivation of crotocin by washed mycelia the mixture was filtered and the fluid phase was saturated with sodium chloride and extracted with chloroform at *p*H 10 (ref. 8). The extract was evaporated and the oily residue crystallized from benzene. The isolated substance had a melting point of 155·5° C., identical with the melting point of the alcoholic component obtained by alkaline hydrolysis of crotocin. Mixed melting points of the two materials obtained by the two different methods did not exhibit any depression. The acid component was identified by paper chromatography.

According to the above observations crotocin if applied in small quantities can act as an inductor. The enzyme thus induced is able to hydrolyse the ester linkage in the antibiotic which destroys its biological activity. On the addition of a single large dose of crotocin no inactivation of the antibiotic activity could be observed since this quantity inhibited also the synthesis of the inductive enzyme. Crotocin might be replaced by trichothecin, both as an inductor and a substrate.

Other fungi such as *Penicillium urticae*, *Aspergillus parasiticus* and *Aspergillus oryzae* failed to exhibit similar action. *Aspergillus niger*, however, when tested under the same experimental conditions, had an effect similar to that observed for *Penicillium chrysogenum*.

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[1] Freedman, G. G., and Morrison, R. I., *Nature*, **162**, 30 (1948).
[2] Gláz, E. T., Scheiber, E., Gyimesi, J., Horváth, I, Stecek, K., Szentirmai, A., and Bohus, G., *Nature*, **184**, 908 (1959).
[3] Horváth, I., and Szentirmai, A., *Nature*, **184**, 57 (1959).
[4] Darpoux, H., and Faivre-Amiot, A., *Atti del VI Congresso Internationale di Microbiologia, Roma*, **1**, 499 (1953).
[5] Horváth, I., Szentirmai, A., and Bajusz, S., *Nature*, **183**, 477 (1959). Horváth, I., Szentirmai, A., Bajusz, S., and Parragh, E., *Acta Microbiol. Hung.*, **7**, 19 (1960).
[6] Bradler, G., *Monatsber. Deutsch. Akad. Wissenschaften, Berlin*, **1**, 609 (1959).
[7] Freedman, G. G., and Gill, J. E., *Nature*, **166**, 698 (1950).
[8] Gyimesi, J., (unpublished results).

Detection of Gibberellic Acid in *Azotobacter* Cultures

It is known that *Azotobacter* cultures can affect the germination of seeds and growth of plants. It is assumed that this influence is due to the presence of physiologically active substances and vitamins in the cultures. Actually, indole-3-acetic acid has been detected in *Azotobacter* cultures[1,2]. In a previous paper[2] the detection of another growth-active compound, in addition to indole-3-acetic acid, was described. More details about this compound, which appeared to be gibberellic acid, are presented here.

Azotobacter chroococcum was grown in Burk's solution containing traces of molybdenum and boron, the same procedure as that used for growth of cultures when isolating indole derivatives[2]. The medium did not contain calcium carbonate, however. After ten days of incubation the culture was centrifuged, the supernatant evaporated *in vacuo* to a tenth of its original volume, made acid with 1 *N* hydrochloric acid (to *p*H 2·8–3·0) and extracted with *n*-butanol[3] or