LETTERS TO THE EDITORS

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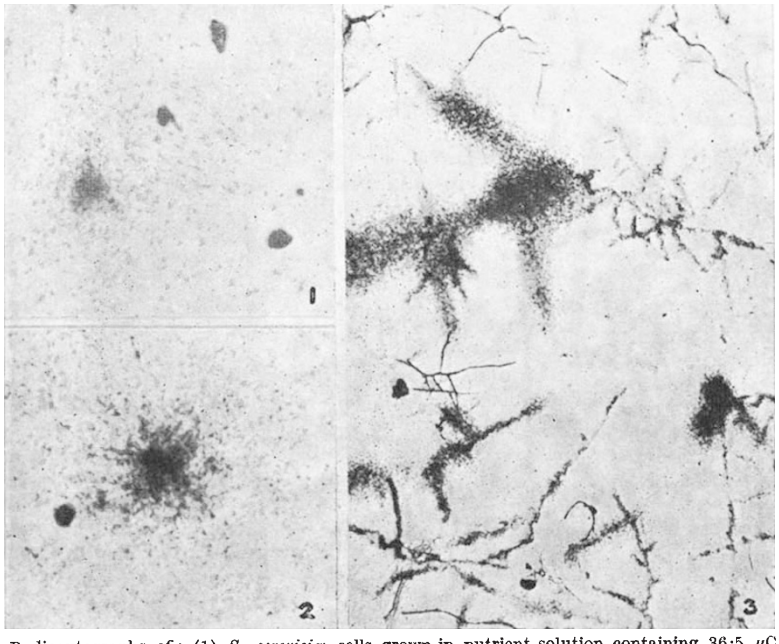
Detection of Radiophosphorus in Cells and Spores of Fungi by Radioautography

THE employment of radioautographic techniques has been of much value in botanical studies in demonstrating the presence and localization of tracer elements within plant organs and tissue. In this respect the work of Arnon *et al.*[1], Colwell[2], and Harrison *et al.*[3], may be cited as typical. Pearson *et al.*[4] prepared radioautographs of fungus cultures, but stated that because of the limitations of the materials and methods employed it was not possible to obtain radioautographs of single cells or hyphæ. Boyd *et al.*[5], however, were able to obtain single cell autographs of leucocytes and erythrocytes. More recently, Fitzgerald *et al.*[6], using tritium, obtained radioautographs of single yeast cells and *Paramecia*.

In the method employed here, phosphorus-32 was added to the nutrient medium in the form potassium dihydrogen phosphate. *Aspergillus* sp., *Hormodendrum* sp. and *Penicillium* sp. were grown on nutrient agar ('Difco') containing 5 per cent glucose. *Saccharomyces cerevisiæ* was grown in nutrient broth ('Difco') containing 5 per cent glucose. Cells harvested from these media were washed and placed directly upon the surface of a photographic emulsion. Best results were obtained with a special emulsion (*NTB* plates) developed by the Eastman Kodak Research Laboratories, Rochester, N.Y. After exposure, the plates were developed in a 'fine grain' developer.

The accompanying photographs are from representative radioautographs obtained in this work. It seems of interest to note that localization of activity occurred not only in mycelium but also among conidia from the same test-tube culture, some producing radioautographs and others not. Perhaps conidia of different ages vary in their phosphorus content. A like condition was noted among cells of *S. cerevisiæ* from a single broth culture. This is in agreement with the results of Boyd and Levi[7], who reported finding evidence of non-uniform uptake of phosphorus-32 by yeast cells.



Radioautographs of: (1) *S. cerevisiæ* cells grown in nutrient solution containing 36·5 μC. phosphorus-32. (× 540.) (2) *Aspergillus* sp. conidia from nutrient agar containing 13·8 μC. phosphorus-32. Exposure period, 14 days on Kodak *NTB* emulsion. (× 540.) (3) *Penicillium* sp. mycelium from a nutrient solution containing 250·0 μC. phosphorus-32. Exposure period, 30 days on Kodak *NTB* emulsion. (× 120)

This work was conducted in the Department of Botany, McMaster University, Hamilton, Ontario.

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[2] Colwell, R. N., *Amer. J. Bot.*, **29**, 798 (1942).
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[5] Boyd, G. A., Casarett, G. W., Altman, K. I., Noonan, T. R., and Salomon, K., *Science*, **108**, 529 (1948).
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Effect of Œstradiol Benzoate and Orchidectomy on the Toxicity of Trypan Blue

PRISELKOV[1] found that the toxic effect of trypan blue was manifested in guinea pigs by loss of weight and hair, apathetic appearance, fall in hæmoglobin content, accumulation in the blood of large numbers of polychromatophiles and normoblasts and the appearance of erythrocyte decomposition products in many organs. Nicol[2] pointed out that some animals appeared to be more susceptible to the toxicity of the dye during œstrus. The toxic effect of injections of trypan blue on the tissues has been studied histologically by Downey[3], who observed that the cells of connective tissue first showed diffuse staining of the cytoplasm and the nucleus which he considered to be the appearance of dying cells. Many cells died, but some were able to eliminate the dye and recover, except the eosinophiles. The reticulo-endothelial cells, which specifically store the vital dye, are affected in the same manner, and it is probable that the resulting inhibition of the functional capacity of the reticulo-endothelial system is partly responsible for the toxic manifestations of trypan blue.

In view of the stimulating effect of œstrogens on the reticulo-endothelial system[2,4], it was considered desirable to ascertain their influence on the toxicity of trypan blue.

In the present experiments, the toxicity of trypan blue, judged