in another extensive study (unpublished) we failed to find any significant changes in the peripheral LH concentration after semispaying of hamsters. Thus, our observations do not support the hypothesis that semispaying causes ovulatory compensation by a decrease in the negative feedback of oestrogen with a consequent rise in the secretion of LH.

[9] We thank Mr. D. J. Watson for his valuable assistance in this work.

Résumé. L'ovariectomie unilatérale pratiquée chez les hamsters au premier jour du cycle fut suivie d'un accroissement significatif de la sécrétion d'oestradiol mesurée dans le sang de la veine de l'ovaire restant, sans aucune variation du taux de progestérone pendant la durée du cycle.

H. S. Joshi and A. P. Labhsetwar[9]

The Worcester Foundation for Experimental Biology, Shrewsbury (Massachusetts 01545, USA), 24 August 1973.

Pigment Formation in a Colourless Strain of *Trichophyton mentagrophytes* after Phosfon D treatment

It is well established that several dermatophytic fungi form, on the underside of their thallus, coloured substances, occasionally diffusing into the culture medium[1–3]. The pigments vary from species to species and sometimes even among strains of the same species[4]. Several studies have shown that the pigmentation is due to a mixture of compounds, many of which are of quinone structure[5–8] and presumably similar to xanthomegnin, the only pigment in dermatophytes so far structurally defined[9]. Qualitative analysis of these pigments by chromatographic methods is a generally accepted criterion for the identification of a given dermatophyte[10–12]. The physiological significance of these coloured molecules remains to be established.

During investigations on the action of Phosfon D[13] on dermatophytes, we observed an inhibition of growth and the appearance of red hyphal strands on the underside of the thallus of a colourless strain of *Trichophyton mentagrophytes*. This phenomenon has been studied and the data obtained are reported in the present paper. The newly formed pigments have been extracted and analyzed by thin-layer chromatography and similarities with coloured substances of other dermatophytes have been established.

Material and methods. *Trychophyton mentagrophytes* (Rob.) Blanchard, strain No. 560.66 (Centraal Bureau voor

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[13] Phosfon D (Virginia-Carolina Chemical Corporation, Richmond, Virginia, USA) is a chemical commonly employed for retarding growth in higher plants. The compound has been shown to inhibit the gibberellic acid synthetic pathway both in higher plants and in gibberellic acid producing fungi (see West and Fall[14] for additional details).

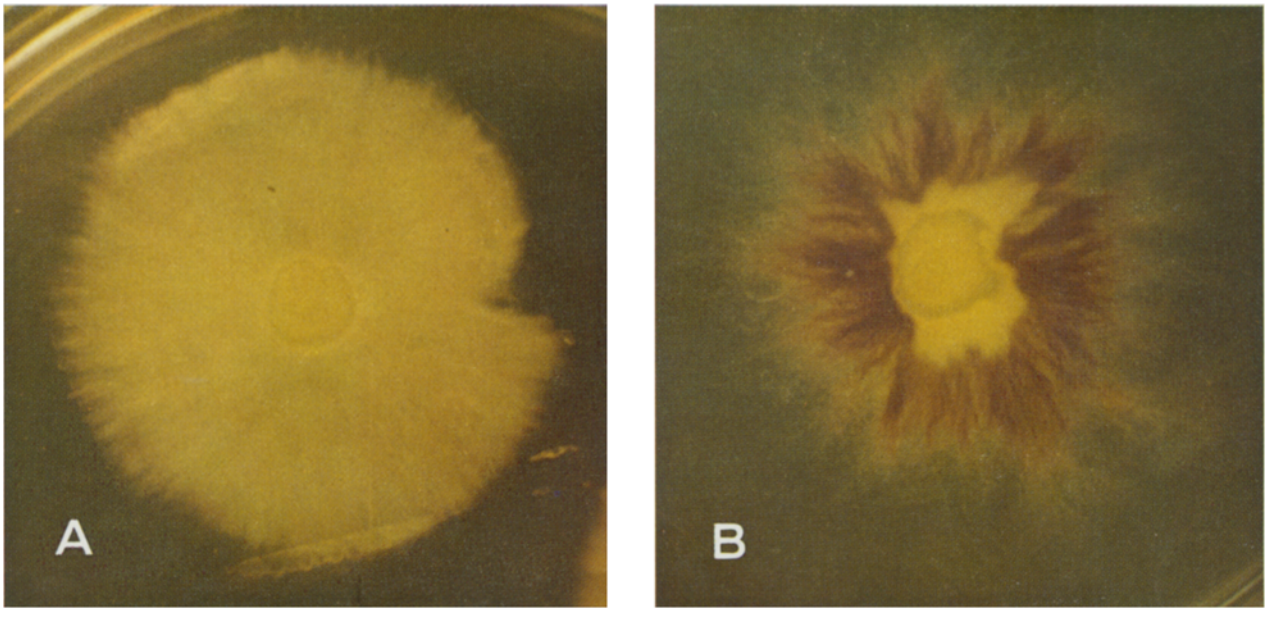

Fig. 1. Inferior surface of cultures of *Trichophyton mentagrophytes* CBS 560.66, photographed through the growing medium: A) Control culture, ×1; B) Phosfon D treated culture, ×1.8.

Schimmelcultures, Baarn, The Netherlands), was cultivated at 28 °C on Sabouraud's maltose agar, used as such or containing 200 µg/ml of Phosfon D (2,4-dichlorobenzyl-tributyl-phosfonium chloride). In order to facilitate the harvesting of the culture and to avoid washing procedures, mycelia were grown on a thin sheet of cellophane, laid over the growing medium[15]. With this technique, which does not modify the macro and microscopic aspects of the mycelium, it is possible to avoid almost completely the diffusion of the pigment from the thallus into the medium.

After 2 weeks of culture, mycelia mats were removed from the cellophane sheet, freeze-dried, and subsequently repeatedly extracted for 10–12 h with petroleum ether to remove lipids[12]. After centrifugation the solvent was discarted by suction, and the residual pellet was extracted with acid acetone (0.1% HCl)[11]. The extracts were concentrated to dryness under vacuum, carefully washed with petroleum ether to remove residual lipid traces, dissolved in small aliquots of chloroform and finally submitted to thin-layer chromatography[12].

Results and discussion. The macroscopic aspects of *Trichophyton mentagrophytes*, cultured in the absence and presence of Phosfon D, is shown in Figure 1. It is evident that, while the control culture shows a colourless lower surface, the Phosfon D treated fungi show under the thallus (the diameter of which is reduced by the treatment and is 50–60% smaller than the normal) red hyphal strands, which, because of the presence of the membrane of cellophane over the medium, cannot penetrate into it. The same finding was consistently observed in all cultures treated. It has to be noted that in both cases the culture medium did not show pigments of any kind in detectable amounts.

The chromatographic pattern of the chloroform extracts from control and Phosfon D treated fungi is shown in Figure 2. 3 distinct yellow spots are clearly visible in the treated samples. After spraying with 2*N* NaOH, the spots turned to red-violet colour and back again to yellow after H_2S vaporisation. This oxido-reducing process can be repeated several times in the same preparation. Such behaviour is typical of quinone pigments[2,11,16–18].

In control samples, even when 10 times the normal amount was chromatographed, the 3 spots were undectable. The Rf values of the 3 newly synthesized spots are 0.54, 0.44 and 0.32, respectively. The same results have been obtained in all determinations. Similar Rf values have been demonstrated by ITO et al.[12] for the pigments aurosporin, xanthomegnin and citrosporin obtained from 8 different dermatophytes, including a coloured strain of *Trichophyton mentagrophytes*, and in similar experimental conditions. We have been unable to obtain from other laboratories purified xantomegnin to use as internal standard in pigment chromatography; nevertheless, on the basis of the identity of the Rf values, we hypothesize that the coloured molecules synthesized in our experimental conditions correspond to the 3 pigments identified by ITO et al.[12].

From the data reported in this paper it is evident that Phosfon D induces the appearance of 3 pigments in cultures of *Trichophyton mentagrophytes* (Rob.) Blanchard CBS 560.66, while these pigments are absent, or present in undetectable amounts, in untreated cultures. The pigment induction by Phosfon D seems to be specific, since the use of several other chemicals known to inhibit the growth of dermatophytes, including nifuratel, miconazole and coumarin, did not result in pigment synthesis.

The formation of the 3 pigments clearly requires the presence of enzymes catalyzing their synthesis. Consequently Phosfon D either activates these enzymes, inactive in normal conditions or, more probably, the chemical de-represses the normally repressed genes for these 'colour' enzymes. It has been suggested that several

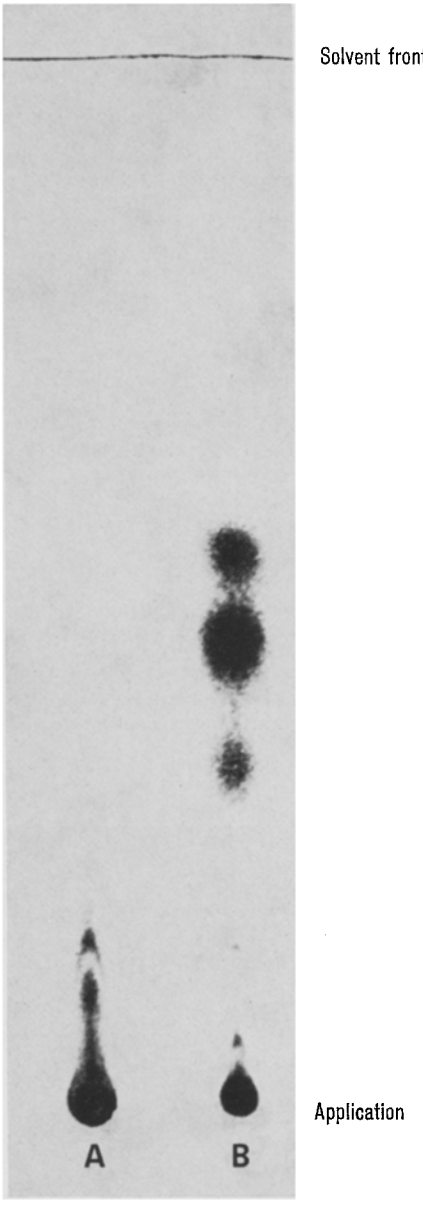

Fig. 2. Thin-layer chromatography pattern of the chloroform extracts from control (A) and Phosfon D treated (B) *Trichophyton mentagrophytes* cultures. In the control sample an aliquot of extract ten times higher than in the treated sample was applied. Adsorbing system: Silica Gel G (E. Merck, Darmstadt, Germany). Developing system: benzene-acetone 3:1 (v/v). The chromatogram was photographed after spraying with 2 *N* NaOH to reveal quinone pigments[12].

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Trichophyton mentagrophytes strains, normally coloured, become colourless on repeated subculturing on Sabouraud's medium[7]. As shown by the present results, this could occur also in our strain since we have shown a phenomenon indicating the reversible repression of the genes for the 'colour' enzymes.

Our observations are of interest in that they suggest the existence in fungi of genes that can be alternatively repressed and de-repressed, depending on the environmental conditions. Our system therefore represents a model for studying the still unknown controls of the genes expression in fungi.

Riassunto. Il Phosfon D, composto ritardante la crescita nelle piante superiori, induce la comparsa di pigmenti chinonici in un ceppo incolore del fungo dermatofita *Trichophyton mentagrophytes*. Viene ipotizzato che il Phosfon D dereprima i geni per gli enzimi catalizzanti la sintesi dei pigmenti neoformati.

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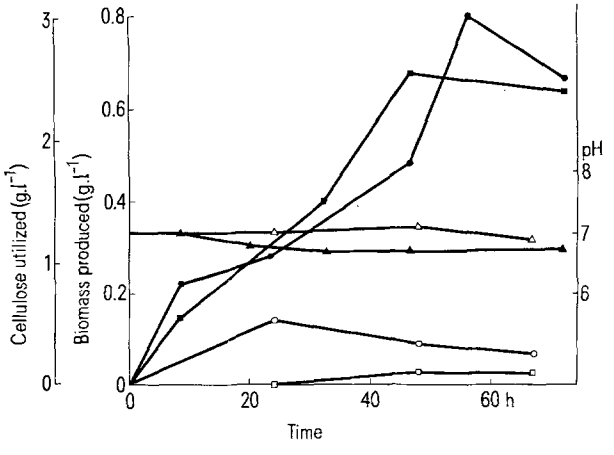
[21] The authors are indebted to Prof. F. CONCONI for helpful discussion.

Production of Single-Cell Protein from Waste Paper by a Mixed Culture

The industrial development of Latin America has revealed new problems, such as the exodus of the rural masses toward urban centers in search of employment[1]. This has contributed considerably to a spectacular increase in the municipal solid wastes, a fact especially observed in Mexican cities where in the last 10 years the output has more than doubled. About 40% of that refuse is represented by paper and cardboard[2,3]. In high developed countries like the United Kingdom, the latter figure is close to 50%[4].

The necessity of maintaining or improving the environment, as well as the protein shortage, make imperative the research of processes for conversion of cellulose wastes into microbial foods. In the present paper, the results obtained with the direct transformation of newspaper to biomass will be given.

Materials and methods. Isolation of Y-11 cultured used in these experiments, consisting of 2 gram-positive and 1 gram-negative rods, has been previously described[3].



Biomass production, cellulose utilization and variation of pH using the mixed culture Y-11. The non-degraded newspaper from a first experiment was washed and dried at 55 °C and reused in a new fermentation run.

	Experiment 1	Experiment 2
Biomass produced (g/l^{-1})	●—●	○—○
Cellulose utilized (g/l^{-1})	■—■	□—□
pH	▲—▲	△—△

The fermentation medium contained (in g/l of tap water): finely ground Excelsior newspaper, 20; $(NH_4)_2SO_4$, 4.0; KH_2PO_4, 1.0; KCl, 0.5; $MgSO_4 \times 7H_2O$, 0.5; and corn steep liquor, 1.0. The fermentor used throughout this study was a 14-liter total capacity (Fermentation Design, Inc.) with a working volume of 9 l. The fermentor was autoclaved at 15 psig for 30 min and operated aseptically. Temperature was controlled at 37 °C, initial pH was 7.0 and agitation was at a speed of 400 rpm. Aeration was not at a constant rate, but was controlled by a dissolved oxygen probe[5]. On-off control was used and arranged so that the dissolved oxygen partial pressure did not fall below 0.10 atm. Inoculum was grown in 500-ml Erlenmeyer flasks containing 50 ml of medium and incubation was carried out for 1 day. In all cases 5% (v/v) inoculum was utilized. Each analyzed sample from the fermentor was usually composed of 3 subsamples taken one after each other with an interval of approximately 4 min between subsamples. Cell concentration was determined on a protein basis by a modified LOWRY method using the Folin-Ciocalteu reagent[6,7]. Cellulose was measured by a semimicro method[8].

Results and discussion. In experiment 1 (Figure) the highest cell mass production was attained at the 56th h of cultivation, declining after that time, probably due to lysis of the mixed culture. The utilization of the newspaper also tended to decline following the time period when 2.55 g/l^{-1} of cellulose had been consumed. The pH changed from 7.0 to 6.7. During the fermentation the approximate population ratio was constant. The bacterial cell count consisted of 99% of the 2 gram-positive rods and 1% of the gram-negative rod. Some of the characteristics of the Y-11 culture have already been published[3],

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