one of them showed identical Rf values in 7 solvents when co-chromatographed with myricetin 3-glucoside isolated from tea (Table I) and, therefore, was tentatively identified as myricetin 3-glucoside. The other unidentified glucoside has Rf values higher than monoglucosides and its position in the chromatograms suggests a diglucoside, probably 3-gentiobioside or 3-sophoroside. The 2 other glycosides were examined by standard procedures and identified as myricetin 3-arabinoside, only reported once before[2] in plants, and the more common myricetin 3-rhamnoside (Table I). The occurrence of myricetin 3-arabinoside in *L. punctata* (Primulaceae) has chemotaxonomic interest since it was previously reported[2] in *Vaccinium macrocarpon* (Ericaceae). Both families belong to orders with such fairly close affinities that many authors placed them in the series pentacyclic Gamopetalae[3].

Resumen. Miricetina-3-ramnósido y miricetina-3- arabinósido se identificaron en *L. punctata* (Primulacea). Otros dos glicósidos parecen ser miricetina-3-glucósido y miricetina-3-soforósido o 3-gentiobiósido.

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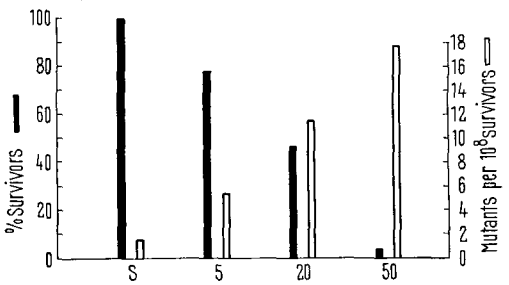
Department of Plant Biochemistry, C.S.I.C., Santiago de Compostela (Spain), 11 August 1969.

[2] O. Puski and F. J. Francis, J. Food Sci. *32*, 527 (1967).
[3] This work was carried out at the Botany Department of the University of Liverpool while the author was a recipient of a Fundación J. March fellowship. Thanks are due to Prof. B. Casaseca, Botany Department, University of Salamanca, Spain, for valuable information, to Dr. J. B. Harborne for the facilities given and to the Fundación J. March for financial support.

Griseofulvin Resistance in Dermatophytes

By introducing the antifungal antibiotic griseofulvin (GF), a decisive change in the therapy of dermatomycoses was brought about. However, from the history of chemotherapy, it is known that the efficacy of every antibiotic is inhibited by the occurrence of resistant cultures[1]. For this reason, the question of the further perspective of applying GF has been systematically studied from this aspect[2,3]. In this brief communication the knowledge concerning the frequency and properties of GF-resistant mutants is summed up.

Two compatible monsporic strains 155 and Z of the dermatophyte *Microsporum gypseum*[4] were used in these experiments. Z is the wild type with typical cinnamon-brown colony, 155 is a spontaneous mutant with cream colony (*cre*). GF-solution in dimethylformamide was added to Sabouraud dextrose agar. The resulting concentration in orientation experiments amounted to 10–30 µg GF/ml, in quantitative experiments to 50 µg GF/ml. On this selective medium, a spore suspension of a standard concentration was pipetted and after cultivation for 7 days all colonies grown were isolated. After 4 transfers on medium without GF, the sensitivity of the colonies to GF was evaluated by the mycelial growth test[5]. Besides these spontaneous mutants, others were prepared by means of UV-radiation. The procedure with UV was described in a previous communication[6].



Frequency of griseofulvin resistant mutants. S, spontaneous mutants; 5, 20, 50, UV-induced mutants (time of irradiation in sec).

From the macroconidial strain Z, no mutants could be obtained; from the spores of the microconidial strain 155, 13 spontaneous and 134 UV-induced mutants were isolated. Loci for resistance to GF were designated *grf*. The frequency of the mutants is shown in the Figure. Each value represents the mean obtained in at least 3 experiments. The frequency of spontaneous mutants varied at about 1.5×10^{-8}. By using UV the frequency of the

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[6] K. Lenhart, Z. allg. Mikrobiol. *5*, 222 (1965).

Table 1. Results of crossing between several GF-resistant mutants (*cre grf*) and sensitive wild strain Z (*cre*$^+$ *grf*$^+$)

Mutants in crossing	Locus for resistance	N_i	*cre*$^+$ *grf*	*cre*$^+$ *grf*$^+$	*cre* *grf*	*cre* *grf*$^+$	χ^2 for 1:1:1:1	P
VIII/1	*grf*-1	156	41	33	37	45	2.05	0.50–0.60
X/2	*grf*-1	146	31	41	35	39	1.7	0.60–0.70
X/3	*grf*-1	173	48	39	44	42	1.0	0.80
X/5	*grf*-1	194	45	52	40	57	3.5	0.30–0.40
X/8	*grf*-1	240	62	54	69	55	2.4	0.40–0.50
IX/1	*grf*-1	176	43	38	49	46	1.5	0.60–0.70
XI/2	*grf*-1	128	34	28	35	31	0.9	0.80–0.90
XI/3	*grf*-2	154	0	73	81	0	(1:1) (0.42)	0.50–0.60

N_i, total number of colonies isolated and tested.

mutants was increased about 10 times. All mutants were characterized by the same resistance to GF: their values ED 50 varied about 102 µg GF/ml (ED 50 of wild strain 155 = 1.4 µg GF/ml). In all mutants also the character of the colony, its micromorphology, spore production, growth rates on Sabouraud dextrose agar and on minimal medium were evaluated. In most resistant mutants no changes were found. 35 resistant mutants were crossed[4] with the sensitive strain with opposite mating type. In all crossings the ratio 1:1 for resistant and sensitive random isolates was obtained. In each mutant the resistance to GF was apparently controlled by a single gene. In our group of mutants, at least 2 different loci for resistance could be found (Table). Locus *grf*-1 segregated independently with a gene *cre*, locus *grf*-2 (mutant XI/3 in the Table) was markedly linked with this gene. By further crossings it was proved that the recombinants *cre*$^+$ *grf*-2 occurred with a frequency of about 0.8%. However, by both the loci the same increase of resistance was caused.

Thus the genetic control of resistance to antifungal drug has been proved in dermatophytes for the first time.

Zusammenfassung. Häufigkeit und Eigenschaften der griseofulvinresistenten Mutanten von *Microsporum gypseum* wurden geprüft. 2 Loci, durch welche die Resistenz gesteuert wird, wurden in verschiedenen Kopplungsgruppen identifiziert und im Vergleich mit dem Ausgangsstamm die Resistenz bei allen Mutanten hundertfach vergrössert.

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PRO EXPERIMENTIS

Innovations in Processing and Exposure Control in Radioautography

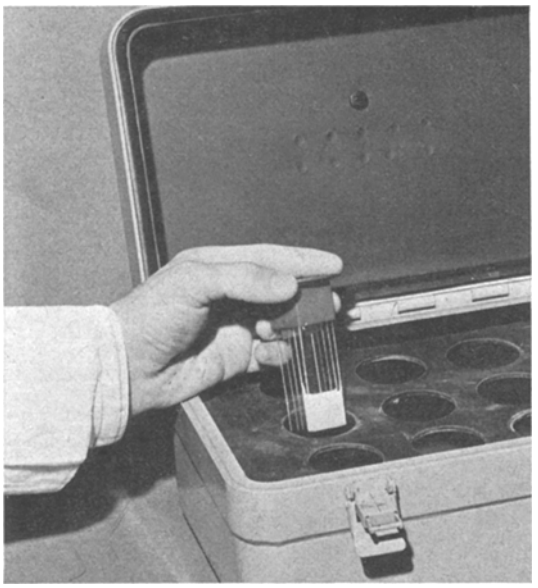
Most radioautographic procedures call for transferring slides, after dipping, to a suitable drying location and then into small slide boxes for exposure[1,2]. When a large number of slides are to be processed, these darkroom manœuvers become burdensome.

Water molecules in the emulsion absorb radioactive particles and increase the exposure time. Dry-rite or another suitable drying agent is usually placed in the slide boxes to lower the humidity. Unless the boxes are air-tight, large variations in the humidity occur. Many techniques also call for predrying at about 80% humidity before the slides are stored in the slide boxes. KOPRIWA and LEBLOND[2] suggest that for long exposure times one should check or change the drying agent about once a month. These changes in humidity that occur between different exposure boxes lead to variations in the degree of exposure of the emulsions.

The innovations described here eliminate the need for individual manipulation of each slide and provide for controlled moisture content of the emulsion during exposure.

Methods. Parraffin is removed from tissue sections with xylene. Sections are hydrated through decreasing percentages of alcohol and water, washed in deionized water and dried. The slides are placed in Peel-A-Way slide holders[3] and coated with photographic emulsion. This is done by dipping the slides into a Coplin jar (with a screw-on lid) containing liquid Kodak type NTB-3 photographic emulsion at 40 °C. The emulsion is stored in the Coplin jar and heated by placing it in the darkroom sink filled with water at 40 °C. The slides, still in the Peel-A-Way slide holders, are placed in a light-tight, air-tight box[5] (Figure). The box is fitted with a shelf containing holes into which the slide-holders are placed. This box is then refrigerated (about 5 °C) until the emulsion is suitably exposed.

Initially a container of Dry-Rite was placed within the exposure box to remove the excess moisture. With a large box and a large amount of drying agent, the slides do not need to be predried. Extremely dry air produced with Dry-Rite caused the emulsions to crack or wrinkle when the exposure time was long (several months). This results in slight movements of the emulsion and the exposed silver grains may be dislocated from the exact site of radioactive isotope location. These problems were



A Peel-A-Way slide holder containing 5 slides is being placed into a light-tight, air-tight box used for exposing radioautographs.

1 N. L. JERRY, J. biol. photogr. Ass. *35*, 73 (1967).
2 B. M. KOPRIWA and C. P. LEBLOND, J. Histochem. Cytochem. *10*, 269 (1961).
3 The Peel-A-Way slide holders were purchased from: W. Glenn Wunderly Co., 1800 Floradale Ave., South El Monte, California 91733, USA.
4 The exposure box is made by TA Mfg. Corp., Instrument Case Div., Los Angeles, California, part number DR05-05-04M1, serial number 5895.
5 R. G. HALL JR., Stain Technology, in press.