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

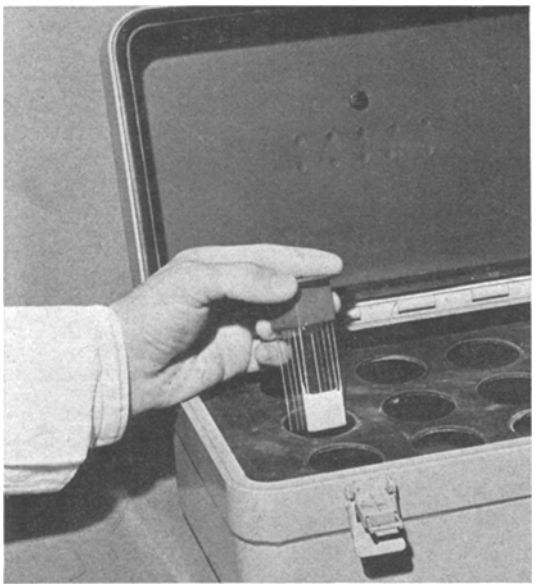
Most radioautographic procedures call for transferring slides, after dipping, to a suitable drying location and then into small slide boxes for exposure[1,2]. When a large number of slides are to be processed, these darkroom manœuvers become burdensome.

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.

solved by using a 95% glycerol solution to provide and regulate a low humidity within the exposure box. About 200 ml of the solution is placed in a container filled with cotton. The container is then placed in the exposure box. Under these conditions the humidity drops from about 80% to 30% in a day and is held constant at 30%. Predrying and excessive handling is eliminated.

Following exposure the radioautographs were developed by the method of KOPRIWA and LEBLOND[2]. The slides were usually stained with hematoxylin[2] or a saturated solution of indio-carmine. When mitotic cells were to be observed, the radioautographic slides were stained with a basic fuchsin and picro-indigo carmine staining procedure[5].

These radioautographic modifications using Peel-A-Way slide holders, large exposure box and the glycerol-water solution provide a more convenient and reproduciable technique than those described previously.

*Zusammenfassung.* Es wird eine einfache Methode für die Vorbereitung und Belichtung von Objektträgern zur Autoradiographie beschrieben. Eine individuelle Behandlung der Objektträger sowie deren Vertrocknung vor der Belichtung wird vermieden und die Feuchtigkeitskontrolle der Emulsion ist gesichert.

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## A Method for the Determination of Thrombocyte Aggregation in Circulating Rat Blood

Various methods have been developed to study the thrombocyte aggregation both in vivo and in vitro. However, so far, it has not been possible to measure the degree of aggregation in circulating blood. Using a microfilter, introduced by SWANK et al.[1] for in vitro experiments, we developed a method to measure the thrombocyte aggregation and des-aggregation in circulating blood continuously and during a prolonged period, without any loss of blood. The experimental set-up is shown schematically in Figure 1.

Via a polythene aorta-prosthesis, inserted between the spermatic- and iliolumbar arteries, the filter (F) (nickel, pore size 20 μm, ex Veeco, Eerbeek, The Netherlands) is connected to the blood circulation of rats. Before and behind this filter the blood pressure is measured. Solutions can be infused before the filter. Filter and connecting tubes are siliconized and kept at 37.5 °C. The blood stream can be led via a by-pass or through the filter by means of 3 clamps. Aggregation which occurs in the blood stream may obturate the filter, as a result of which the pressure behind the filter (the experimental pressure, EP) drops and the pressure before the filter (the reference pressure, RP) increases (see Figure 2). The degree of aggregation, designated by the aggregation-index A, is obtained by expressing the minimal ratio of the experimental and reference pressure after ADP administration ($EP_2 : RP_2$) as a percentage of the ratio between experimental and reference pressure at the beginning of the

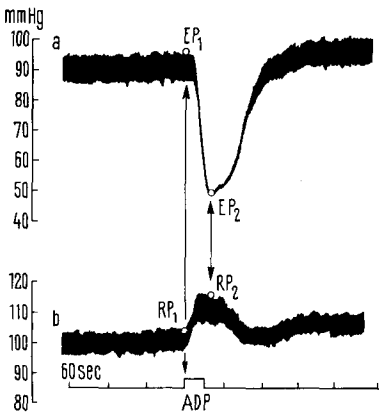


Fig. 2. Recording of an aggregation-measurement. (a) Experimental pressure; (b) reference pressure.

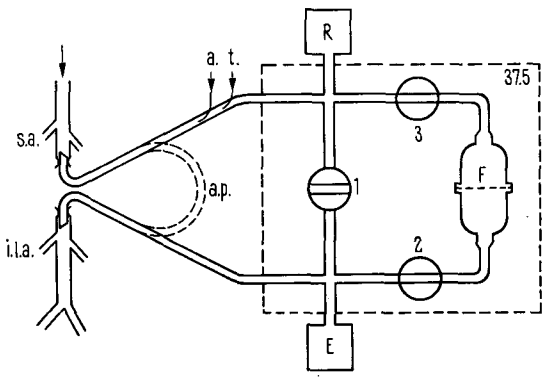


Fig. 1. Scheme of set-up for measuring thrombocyte aggregation. a.p., Aorta-prosthesis (cut through); s.a., spermatic artery; i.l.a., iliolumbar artery; R, recording of reference pressure; E, recording of experimental pressure; F, filter; a.t., administration of test solutions; 1, 2 and 3, clamps.

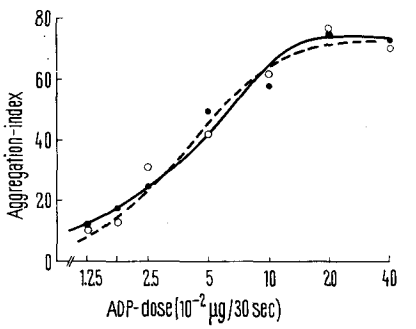


Fig. 3. Relation between ADP-dose and aggregation index at the first (full line) and second (dashed line) measurement (mean of 10 experiments per dose).

[1] R. L. SWANK, J. G. ROTH and J. JANSEN, J. appl. Physiol. *19*, 340 (1964).