

calculated as indicated by WEISS *et al.*². The spots produced by this technique are sharper than those obtained with ordinary one- or two-dimensional chromatography.

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¹ B. WEISS AND G. V. ROSSI, *Nature*, 195 (1962) 178.

² B. WEISS, G. V. ROSSI AND L. A. REBER, *Nature*, 197 (1963) 280.

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Temperature control in paper chromatography

In order to overcome the considerable variations in R_F values of paper chromatograms and the even more marked effects on reverse phase systems that occur with temperature variation, it is usual where this technique is employed extensively to resort to temperature controlled rooms. However, where it is used less frequently such measures may be inconvenient.

To achieve similar control, we have found that a suitably sized box, in our own experience accommodating three tanks of usual size, may be maintained at an even temperature over long periods, by simply using a hair drier (fitted with the usual heating coil) in series with a mercury contact thermometer. The general arrangement is illustrated in Fig. 1.

The provision of a collapsible side finishing flush with the bottom of the box, allows for ease of removal of a tank and for the side to be used as a small bench if required.

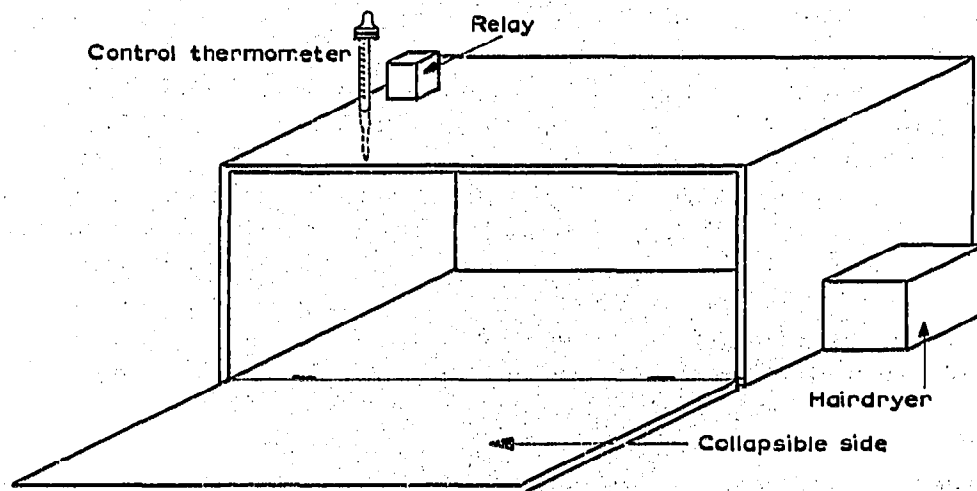


Fig. 1. Temperature control box.

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This system has the advantage that as it is required heat is dissipated through the container by the blower. It is clear that the work required of the drier can be reduced by insulation and as a further contribution in this direction we have standardised on two temperatures, 20° and 25°, according to ambient temperature, with preference for the lower setting where possible.

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Chromatography and photographic detection in ultraviolet light of 6-azauracil and its derivatives

For the purpose of analytical control of the biochemical transformation of 6-azauracil (3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazine) to its riboside by *E. coli*, a rapid method was needed which could indicate the progress of the biotransformation. In order to be able to control the process (possible duration of a typical run is 8-10 h) the analysis should not take more than 1 h. A rapid qualitative chromatographic method on circular paper was found to meet this requirement, using either visual or photographic detection in U.V. light. The latter might be also used for a more elaborate semiquantitative method by the ascending technique.

Chromatographic technique

Paper chromatographic separation of purine and pyrimidine bases, nucleosides and nucleotides, was studied by HANDSCHUMACHER AND WELCH¹. Generally, these compounds are first adsorbed on active charcoal, eluted by ammonia in methanol and after concentration developed by ascending paper chromatography in a butanol-water system²⁻⁴. We have found in model experiments that the rate of adsorption on charcoal is different for 6-azauracil (AU) and for 6-azauridine (AUR). In samples with low AUR content this fact can lead to considerable errors (up to 20%). Polarographic studies in the course of the biotransformation process show that AUR in the fermentation fluid is strongly bound to high-molecular compounds, but that such complexes can be broken up by deproteinisation agents or simply by boiling. In samples treated in this way the charcoal step is no longer necessary and the samples can be directly spotted and developed with reproducible R_F values.

After extensive experimentation with several developing systems we have chosen the mixture *n*-butanol-acetic acid-water (12:1.5:5) which gives good resolution of the reaction components in a short time (R_F AU = 0.66, R_F AUR = 0.26).

Experimental

(1) *Rapid qualitative chromatographic separation of AU and AUR on circular paper.* Circular chromatographic paper Whatman No. 1 (diameter 15 cm) was spotted 1.5 cm