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 WELCH[1]. 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 system[2–4]. 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

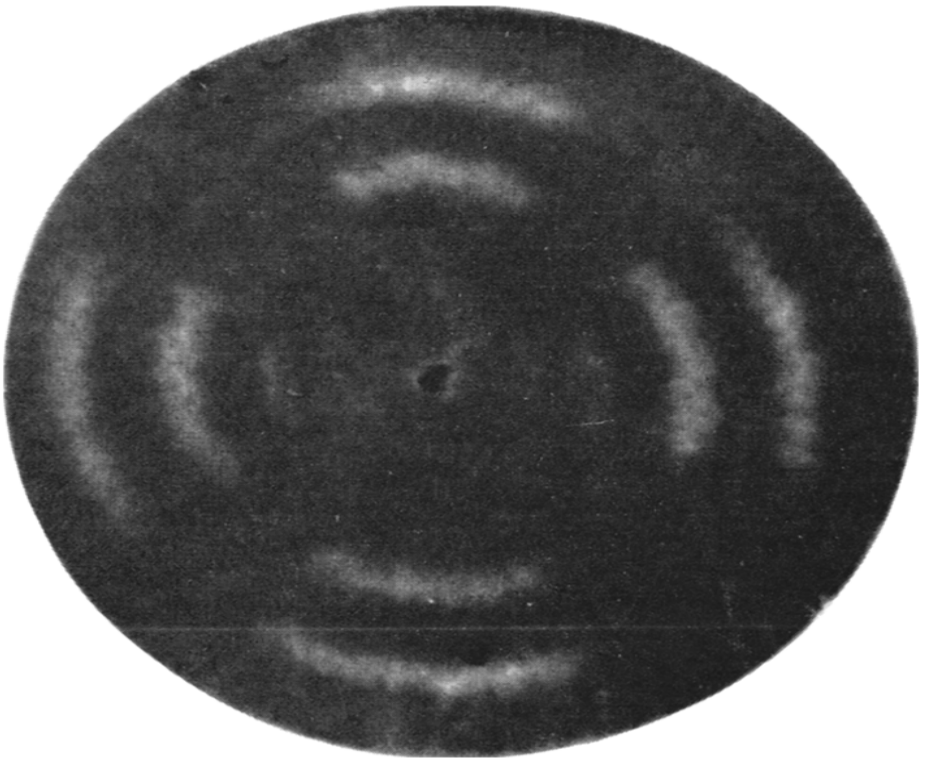

Fig. 1. Contact photocopy of a circular chromatogram. Top and bottom: a mixed standard; left and right: a sample of the fermentation medium. 5th hour of fermentation, AU is still untransformed.

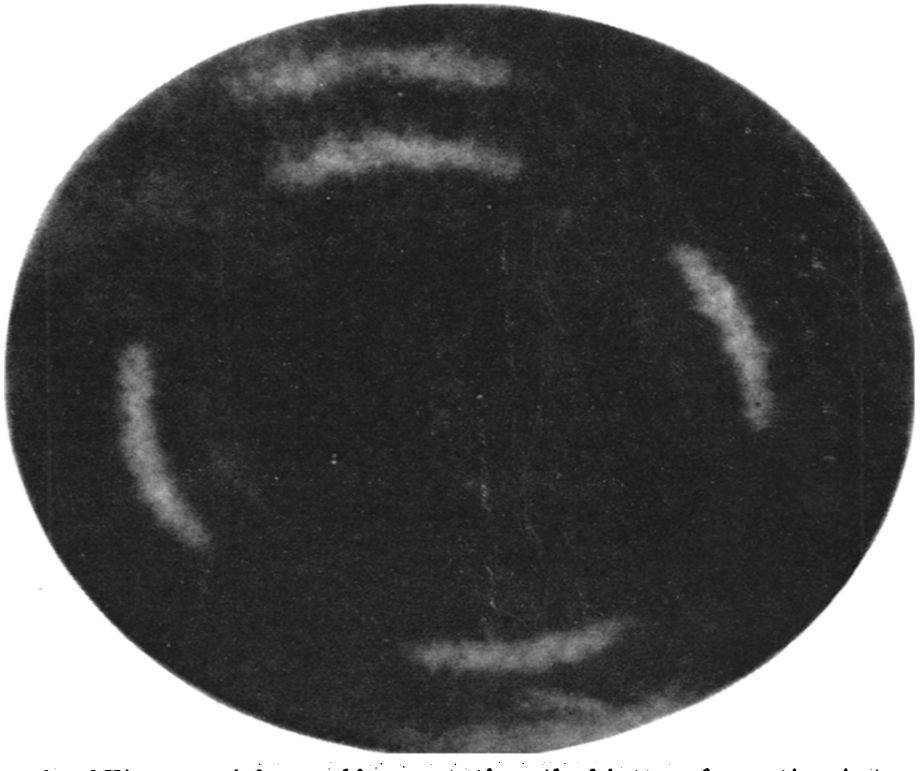

Fig. 2. Same sample of Fig. 1, 10th hour of fermentation, the biotransformation is terminated. Close to the starting point traces of the side-product (orotic acid) are visible.

from the centre alternatively with 10 μl aliquots of aqueous "mixed standard solution" (0.01 *M* AU and 0.01 *M* AUR) and with sample solutions. Fermentation fluid samples were warmed for 5 min in a test tube on a boiling water bath, cooled, centrifuged and the clear supernatant was directly spotted. The spots were dried in a current of hot air and the paper was positioned horizontally between two lids of Petri dishes (15 cm diameter). The developing solution was introduced to the centre of the paper by means of a paper wick, dipping in a small vessel containing the mixture. The inside of the Petri dishes was saturated with the developing mixture at least 4 h before starting the development. When the front of the solution was near to the circumference of the dish, the paper was taken out, dried and the spots were either directly observed in U.V. light (Hanau Mineralite), or the chromatograms were superimposed on photographic paper and prints were made by exposing them to a U.V. light source. This latter variant is described in detail below. In either case, the results are known within 1 h from starting the development of the chromatogram, which allows the biologist to follow the gradual conversion of AU to AUR in the course of the microbiological transformation (Figs. 1 and 2).

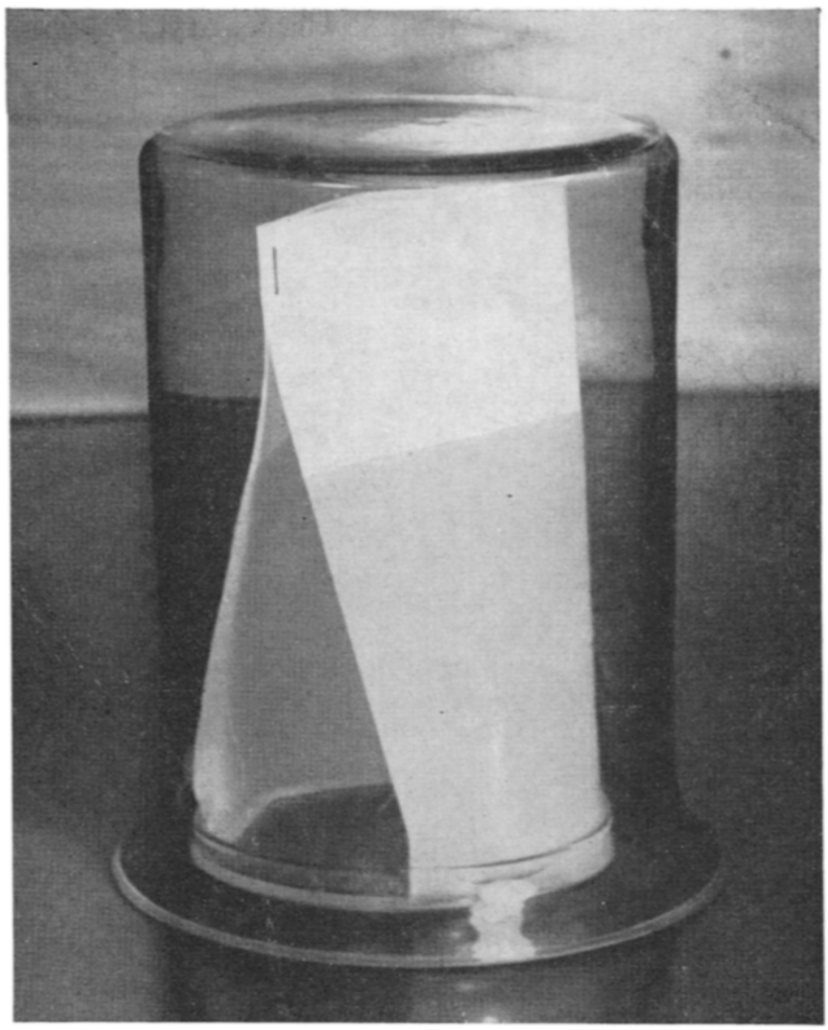

Fig. 3. General arrangement for semiquantitative chromatography.

(2) *Semiquantitative separation of AU and AUR by ascending paper chromatography*. For semiquantitative determination of AU and AUR in mixtures the ascending technique was used on 21 × 19 cm Whatman No. 1 paper sheets, folded in the shape of a cylinder. These sheets were placed in small Petri dishes, containing the developing mixtures (same as above), the whole assembly was put in an all-glass chamber (Fig. 3). Sample and "mixed standard solution" were applied in the same concentration as for the circular technique; the spottings were made in volumes of 20 μl 3 cm from the lower margin of the paper; the development took 3–4 h. After drying, photographic prints of each sheet were made and the prints were compared with a standard print, made from a chromatogram, to which a series of mixed standards was applied in molar percentual ratios AU to AUR of 100:0,90:10 ...10: 90, 0:100. In this way the ratio of AU to AUR in an unknown mixture can be determined with 5 % accuracy.

The method can be also applied for the separation of AUR derivatives. We show

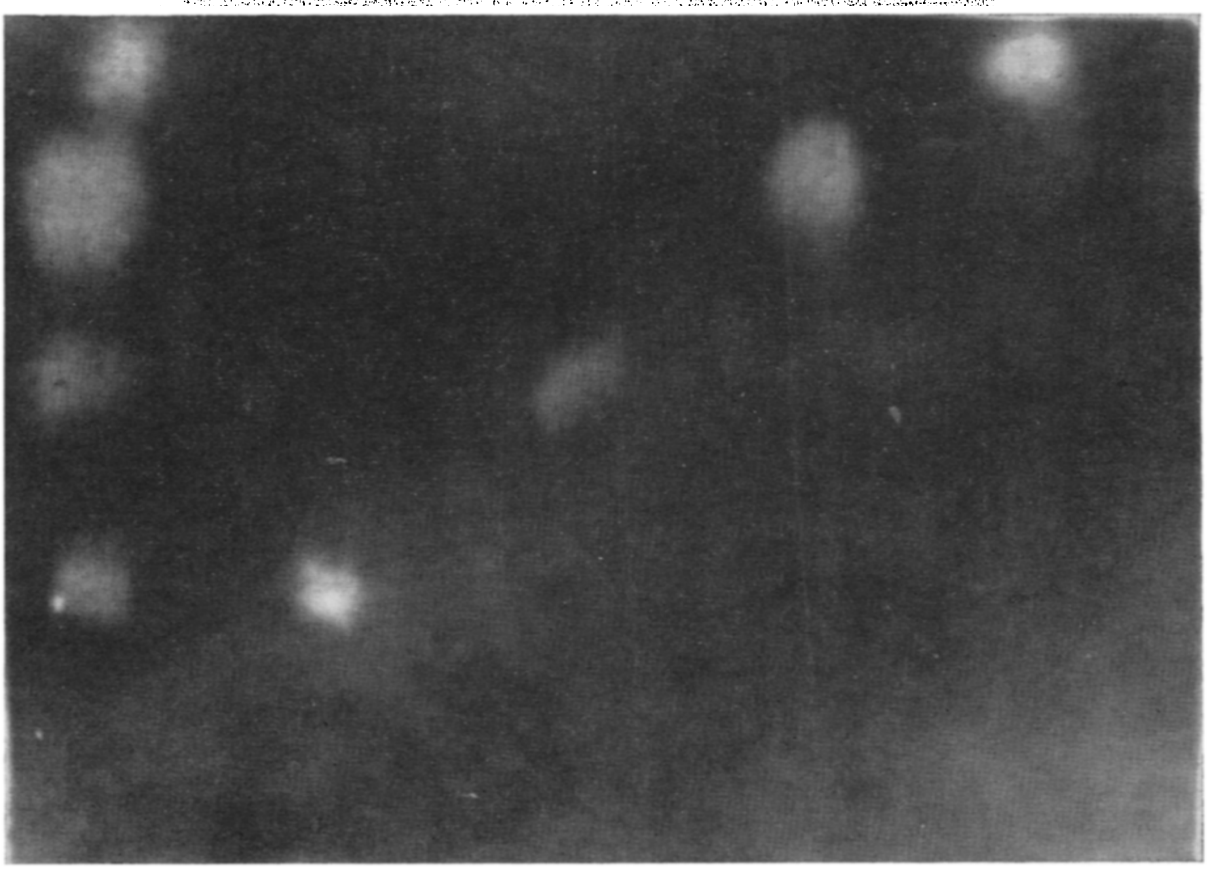

Fig. 4. Chromatogram of azauracil derivatives. 6-Azauridine, R_F 0.32; 6-azauracil, R_F 0.71; bromazauracil, R_F 0.88; 6-azauridine triacetate, R_F 0.96.

for example a photocopy of a mixture of 6-azauridine triacetate, bromazauracil, 6-azauracil, and 6-azauridine (Fig. 4).

Detection technique

Chemical reactions suitable for detection of purine and pyrimidine bases, nucleotides and nucleosides have been described by several authors[5–8]. Some reactions for the

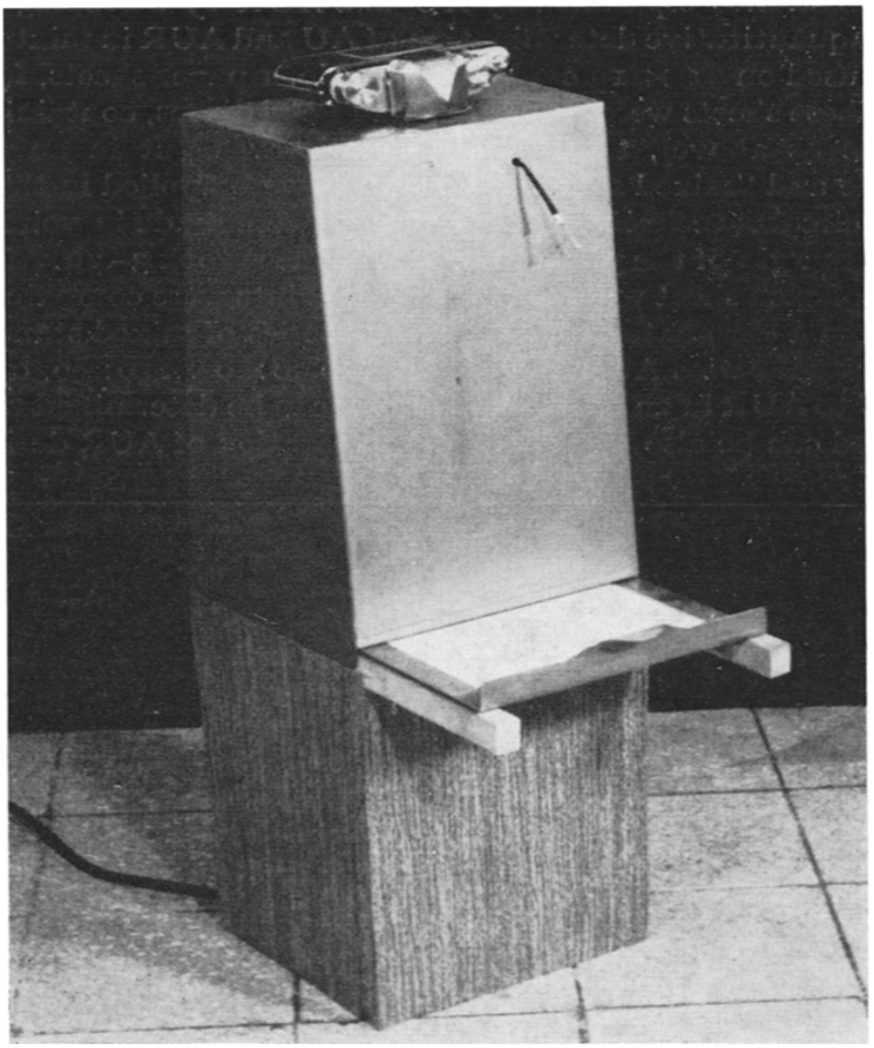

Fig. 5. Apparatus for detection by photography of the chromatograms in U.V. light. Distances: camera–chromatogram, 45 cm; chromatogram–U.V. lamp, 35 cm.

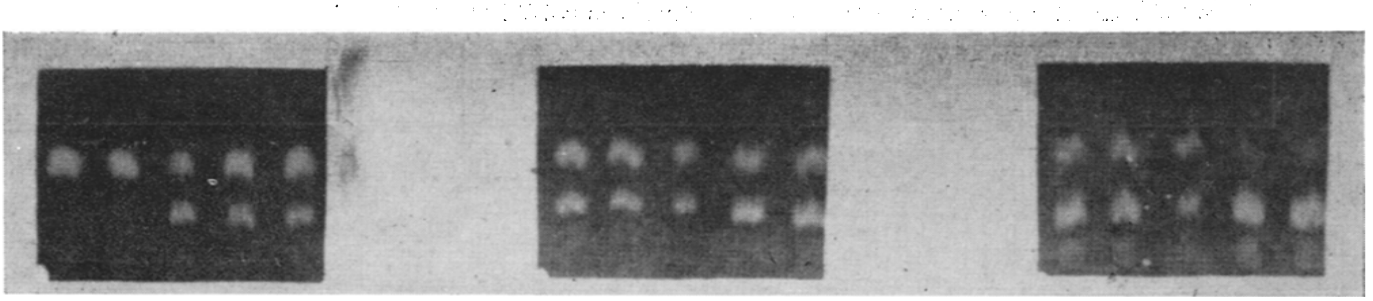

Fig. 6. Photograph showing a cine film strip. Semiquantitative estimation of the AU biotransformation process (0,3,5,7,9 and 10 h, "mixed standard solution" in the middle of each sheet).

phosphorus or sugar compounds may also be used[9], but chemical detection has several drawbacks. It is more laborious, less sensitive and it alters the chemical character of the detected substances which then cannot be used for other tests. The detection with U.V. light is therefore preferable. Low pressure mercury arc-lamps (*e.g.* Hanau Mineralite) are suitable for this purpose as long as they emit sufficient light in the wavelength range near 260 mμ.

During our work we tested several light sources and found that almost all low-pressure mercury arc-lamps yield adequate U.V. light quantities in the region of 260 mμ within a few seconds following ignition. This short time lapse is not long enough for visual perception and marking of the spots but it proves adequate for the exposure of the photographic paper. This revelation was utilized for the development of the contact photocopy detection method.

Under nonactinic light the dried chromatograms were superimposed on a sensitive photographic paper, weighted with a metal frame and exposed to the light of a mercury arc-lamp (Fig. 5). The exposure time and focal distance of the lamp must be checked and determined according to the sensitivity of the paper and the intensity of the lamp. If normal photographic papers for enlargement and a 250 W lamp are used, the focal distance is 2 m and the exposure time 4 sec. The exposed papers were developed by means of a hard contrast developer.

More convenient still, and less time consuming is the detection by photographing on a cine film (Fig. 6). The prerequisite necessary for the development of this method was again a suitable light source. Several available U.V. lamps were tested using the spectrophotometer Unicam SP 700 and the one-beam technique. The lamp under test served as light source and the intensities of the lines were measured using several split-widths for comparison. The germicidal lamp TUW 30 was found to be best for our work. The photographic apparatus used was Exacta Varex, cine film 17/10 din, exposure time 2 sec. The required quartz lens-set was made in the Research Institute for Monocrystals, Turnov.

The filing of chromatographic documents thus obtained is very convenient. The size and intensity of the zones can best be read by means of a slide-projector on a screen.

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