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Note

Separation of 8-azaadenine metabolites on columns of Sephadex G-10

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Recent investigations in several laboratories have demonstrated that columns of Sephadex G-10 can be successfully used for separating bases[1–5], nucleosides[1,2,4–9] and nucleotides[1,2,4,5,10]. These procedures can also be applied to de-salting of purine and pyrimidine samples. All these techniques are based on the "adsorption retardation" of the bases on dextran columns, ring nitrogens being of great importance[1]. Advantage has been taken of the fact that 8-azaadenine (the antimetabolite used) has an extra nitrogen atom in position 8 of the purine ring system to separate the compound and its metabolites from natural purine bases and from each other.

EXPERIMENTAL

The techniques of Sweetman and Nyhan[1] and Gorbach and Henke[2] were combined to analyze UV-absorbing compounds which accumulate[11] in the culture medium of *Neurospora crassa* during incubation of the fungus with the antimetabolite. All analytical separations were carried out on Sephadex G-10 (Pharmacia, Uppsala, Sweden) in 1.3 × 148 cm columns with a glass filter sealed to the bottom.

The Sephadex was suspended in distilled water and stirred overnight. Small particles were removed from the suspension by repeated decantations. Subsequent treatments were essentially the same as those described by Sweetman and Nyhan[1] (suspending, settling and decanting in 0.1 *M* sodium hydroxide, distilled water, 0.1 *M* hydrochloric acid, distilled water, 0.1 *M* sodium hydroxide and twice in 0.05 *M* sodium dihydrogen phosphate buffer solution adjusted to pH 7.05 with sodium hydroxide solution). The gel slurry in the buffer was then de-aerated with a water-pump and packed into the column. A filter paper disk was placed on top of the bed. After the gel had settled under buffer flow for 2–3 days, the column was used for the separation. Flow-rates were kept constant using an MA-62 micropump. Samples in phosphate buffer were applied on the drained top of the column. The maximum sample volume was 5 ml. Approximately one volume of the solvent was used to wash the sample. Chromatographic runs were performed at room temperature.

Fig. 1 shows a typical separation of the UV-absorbing compounds which accumulate in the culture medium during the growth of *Neurospora crassa* in the presence of 8-azaadenine (50 mg/l). Equally good results were obtained in a series of experiments with culture media resulting from incubations of different duration.

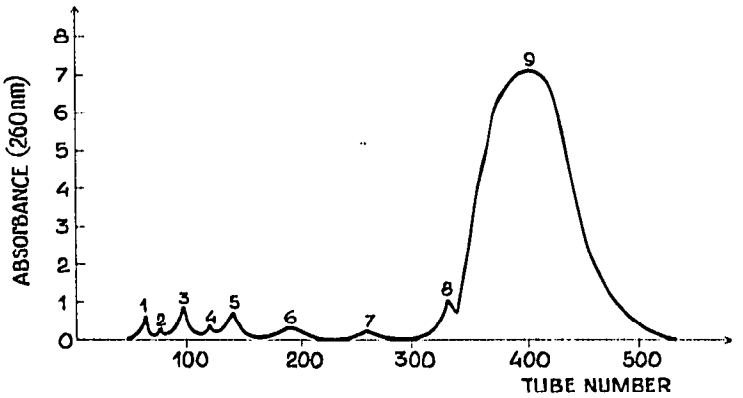

Fig. 1. Separation of the UV-absorbing compounds of the culture medium of *N. crassa* on a Sephadex G-10 column. Fractions of 2.2–2.5 ml were collected. Peaks 1–5 = unidentified compounds; 6 = 8-azahypoxanthine; 7 = 8-azaisoguanine; 8 = 8-azaxanthine; 9 = 8-azaadenine.

Separations were not affected by changes in the amounts of the UV-absorbing compounds loaded (200–800 absorbance units at 260 nm). The quality of separation also did not suffer when the concentration of 8-azaadenine present in the medium far exceeded that of the other compounds.

Elution positions of the compounds on Sephadex do not change significantly, regardless of whether or not the sample applied contains salts. Resolution of the peaks strongly depends on the flow-rate of the buffer. At low flow-rates the column was more efficient, the optimal being approximately 25–30 ml/h. With this flow-rate one could obtain better resolved and sharper peaks.

As peaks 6–9 in Fig. 1 represent single compounds, they were readily identified. Transparency of the buffer in UV light ensured adequate absorption spectra of the compounds in a tube with maximal absorbance. 8-Azahypoxanthine, 8-azaisoguanine, 8-azaxanthine and 8-azaadenine were all identified this way as single constituents of peaks 6–9. Other compounds in peaks 1–5 were not identified as they contained complex mixtures.

Finally, it should be noted that the method reported above has been extensively used in this laboratory (from 1969) for studying anabolic and catabolic transformations of 8-azaadenine in the metabolism of *N. crassa*. The results of this study will be published elsewhere.

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