LETTERS TO THE EDITORS

CHROMATOGRAPHY OF PURINES AND PYRIMIDINES ON STARCH COLUMNS

Sirs:

We have found that it is possible to obtain satisfactory resolution of the six bases, thymine, uracil, cytosine, adenine, guanine, and hypoxanthine, by chromatography on a single column of the type developed by Stein and Moore[1] for amino acids. On known mixtures recoveries have averaged 100 ± 4 per cent. The bases are easily identified by their absorption spectra which are characteristic, and not appreciably changed by passage through the column. The quantitative estimation of purines and pyrimidines by paper chromatography has been described by Vischer and Chargaff[2] and by Hotchkiss;[3] and Edman *et al.*[4] have reported the separation of adenine and guanine on a starch column.

The starch columns employed in our work are 30 cm. in height and 0.9 cm. in diameter, and are prepared according to the procedure recommended by Stein and Moore. The solvent is composed of *n*-propanol and 0.5 N HCl in the proportions of 2:1. A solution containing from 0.20 to 0.35 mg. of each base in 0.5 to 1.0 ml. of solvent is added to the top of the column. The effluent is collected in a regular series of 0.5 ml. fractions.[5]

The fractions are evaporated to dryness in groups of 50 to 80 in a vacuum desiccator warmed to about 40° by means of an infra-red lamp. Each residue is dissolved in 5 ml. of 0.1 N HCl. The extinction coefficients of the solutions are measured in the Beckman spectrophotometer at wave-lengths corresponding to the absorption maximum for each

[1] Stein, W. H., and Moore, S., *J. Biol. Chem.*, **176**, 337 (1948). Moore, S., and Stein, W. H., *J. Biol. Chem.*, **178**, 53 (1949).

[2] Vischer, E., and Chargaff, E., *J. Biol. Chem.*, **168**, 781 (1947).

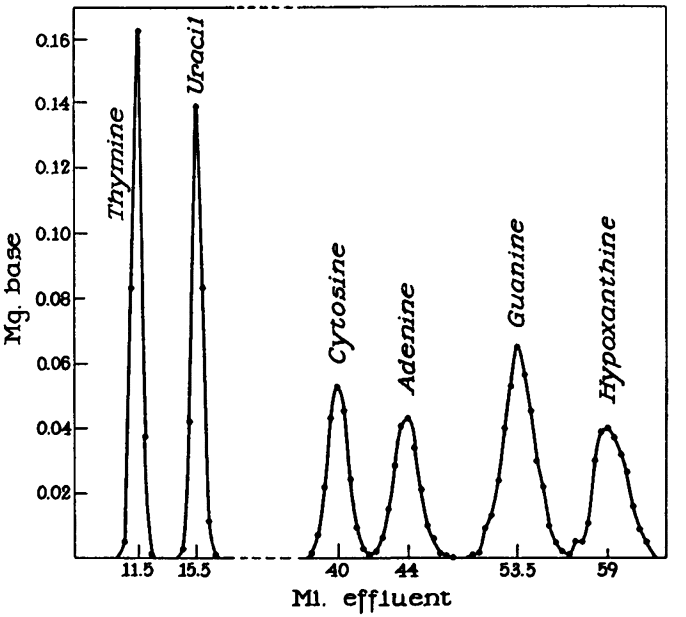
[3] Hotchkiss, R. D., *J. Biol. Chem.*, **175**, 315 (1948).

[4] Edman, P., Hammarsten, E., Löw, B., and Reichard, P., *J. Biol. Chem.*, **178**, 395 (1949).

[5] The procedure for the addition of the sample to the column and the collection of the effluent is the same as that described by Stein and Moore. The authors wish to acknowledge many helpful suggestions from Dr. Moore and Dr. Stein which have aided in carrying out this work.

base. The weight of purine or pyrimidine in each fraction is calculated from the absorption of known solutions of the compounds.

If the extinction coefficient or the weight of base in each fraction is plotted against effluent volume, a series of sharp, well separated peaks



is obtained (see the figure). The curves are integrated by the addition of the analytical values for the points on a given peak. For accurate plotting and integration, an average fraction ahead of or behind the peaks should be used as the blank for measurement of the extinction coefficients. The positions of the peaks are reproducible to ±10 per cent.

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