such a gradient by combining the BN-Chamber with a complex arrangement of mixing reservoirs, which also allowed the control of the composition of the solvent along the gradient.

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1 F. GEISS AND H. SCHLITT, *Chromatographia*, 1 (1968) 392.
2 A. NIEDERWIESER, *Chromatographia*, 2 (1969) 23.
3 A. NIEDERWIESER, *Chromatographia*, 2 (1969) 362.

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Thin-layer chromatography of cyclic adenosine 3′, 5′-monophosphate on tetraborate-impregnated silica gel layers

A number of procedures for the separation of cyclic adenosine 3′,5′-monophosphate (c-AMP) from other adenine derivatives on thin-layer chromatograms have been published[1–3]. These procedures generally employ cellulose thin layers and a multi-component solvent system, *e.g.* *n*-butanol–acetone–acetic acid–ammonium hydroxide–water.

The tendency of borate ions to form complexes with the 2′,3′-*cis*-diol grouping on simple sugars[4] was utilised to develop a simple and efficient procedure for the separation of c-AMP from other adenine derivatives.

Methods

Plates were prepared using a slurry made up of Silica Gel GF_{254} (Fluka) (30 g) and 5% (w/v) aqueous sodium tetraborate ($Na_2B_4O_7 \cdot 10\ H_2O$, 60 ml). Film thickness was 250 μ and the plates were heated at 110° for 30 min. Substrates (10 μl, 0.05% solution in 50% aqueous ethanol) were applied using a microsyringe. The developing solvent was 50% aqueous ethanol, and the development time was approximately 4 h for a 20 × 20 cm plate. After development the plates were dried at room temperature and viewed under UV light.

Results

The use of tetraborate-impregnated layers gave a very satisfactory separation of c-AMP from other adenine derivatives, as shown in Table I. c-AMP and theo-

TABLE I

TLC OF ADENINE DERIVATIVES ON TETRABORATE-IMPREGNATED LAYERS

Developing solvent: 50% aqueous ethanol.

Compound	R_F *value*
Adenosine	0.34
c-AMP	0.75
5′-AMP	0.06
3′-AMP	0.46
5′-ADP	<0.01
5′-ATP	<0.01
Theophylline	0.67

phylline can be further resolved by two-dimensional TLC, using water-saturated *n*-butanol as the developing solvent in the second direction. The R_F values of c-AMP and theophylline in this latter system are 0.05 and 0.44, respectively.

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1 H. BÄR AND O. HECHTER, *Anal. Biochem.*, 29 (1969) 476.
2 J. R. TURTLE AND D. M. KIPNIS, *Biochemistry*, 6 (1967) 3970.
3 H. SHIMIZU, J. W. DALY AND C. R. CREVELING, *J. Neurochem.*, 16 (1969) 1609.
4 J. D. SMITH, in E. CHARGAFF AND J. N. DAVIDSON (Editors), *The Nucleic Acids*, Vol. 1, Academic Press, New York, 1965, pp. 283–284.

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