acetic acid–water (4:1:1) are also shown as we have found these to be valuable alternate solvent systems for proving the identity of substances and/or for performing two-dimensional TLC on polyamide for the purpose of resolving a mixture of metabolites as found in an extract of urine.

We have found the sequential use of two sprays to be a valuable adjunct in identifying the components of mixtures of unknown substances. Table II indicates the color development for each substance when studied sequentially.

Similarly, if large amounts of the substance are available they can be analyzed on pre-coated silica gel plates, (using the same solvent systems and sprays), as indicated in Table III. However, color development with the diazotized sprays will be different. With sulfanilic acid all of the substances in Table III will be orange in all solvent systems. With *p*-nitroaniline they will be red in the isopropanol–ammonia system and dark orange in the butanol–acetic acid system. In the isobutanol–cyclohexane–acetic acid system the metabases will be red and the others yellow with diazotized *p*-nitroaniline.

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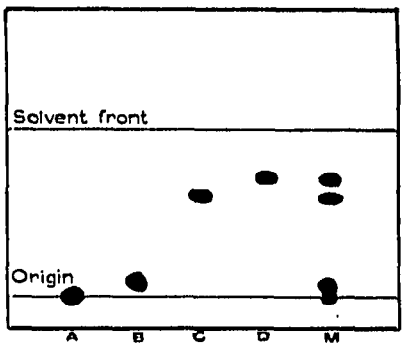
Chromatography of cyclic 3′5′-adenosine monophosphate on silica gel glass microfiber sheets

In the process of devising an assay for adenyl cyclase[1], it became apparent tha, a thin-layer chromatographic system for the rapid separation of the reaction product cyclic 3′5′-adenosine phosphate (cAMP) from its precursor adenosine triphosphate (ATP) and other adenine nucleotides would be highly desirable. A satisfactory system using silica gel glass plates has been described[2], but running time is rather long, and scraping and extraction of the gel is necessary for radioactive analysis. This report is concerned with the separation of cAMP from ATP, 5′-AMP and adenosine (Ado) on silica gel glass microfiber sheets. The procedure provides for rapid development (about 25 min) and ease of counting radioactive samples by scintillation spectroscopy.

Methods and materials

Glass microfiber sheets (20 × 20 cm Gelman ITLC type SG) were spotted with

5 μl of aqueous solutions (1 mg/ml pH 7.0) of the required compounds and air dried. The papers were developed in glass chromatographic tanks containing appropriate solvent mixtures. The compounds on developed chromatograms were visualized by means of a Spectroline Model SL-2537 short wave U.V. lamp. Chromatograms were traced, redrawn and photographed for presentation. In the studies using tritiated cAMP, the chromatograms were spotted and developed as usual and the papers were cut into 1 × 3 cm sections, starting at the origin and placed in scintillation vials containing 15 ml of scintillation fluid consisting of 0.4% 2,5-diphenyloxazole and 0.05% 1,4-bis-[2-(5-phenyloxazole)]-benzene in toluene. The samples were then counted in a Packard Tricarb Scintillation Spectrometer. All solvents were of the highest purity commercially available. ATP, cAMP, 5'-AMP and Ado were obtained from Nutritional Biochemicals Co., Cleveland, Ohio and [^{3}H]cAMP was obtained from Schwartz Bioresearch, Orangeburg, N.Y. The silica gel papers were obtained from the Gelman Instrument Co., Ann Arbor, Mich. and silica gel precoated glass plates from Brinkmann Instruments, Inc., Westbury, L.I., N.Y..



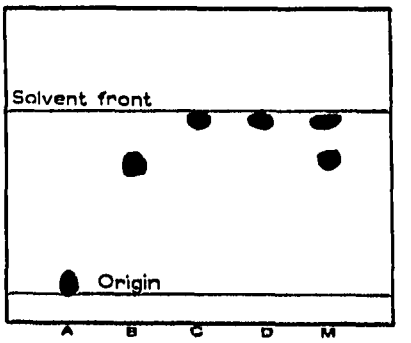
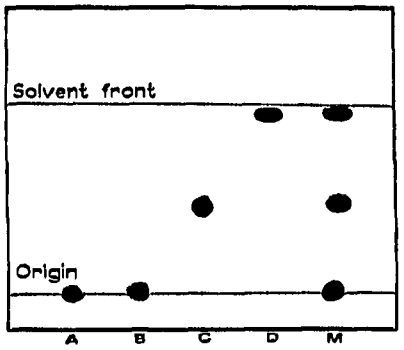


Fig. 1. Thin-layer chromatogram of various adenine nucleotides on silica gel glass plate. Solvent was acetone–*n*-butanol–isopropanol–0.03 *M* NH_4HCO_3 pH 8.6 (2:2:3:2). A = ATP; B = 5'-AMP; C = cAMP; D = Ado; M = mixture of A–D. Detection was U.V. light. Running time was 1 h 45 min.

Fig. 2. Thin-layer chromatogram of various adenine nucleotides on silica gel glass microfiber sheet. Solvent, detection and abbreviations are the same as in Fig. 1. Running time was 30 min.

Results

Fig. 1 shows the chromatographic separation of the compounds studied on a silica gel glass plate using a solvent system consisting of acetone–*n*-butanol–isopropanol–0.03 *M* NH_4HCO_3 pH 8.6 (2:2:3:2) as previously described[2]. Running time was approximately 1 h and 45 min. In Fig. 2, the same solvent system was used with the silica gel glass microfiber sheet. It can be seen in the latter example that both cAMP and Ado run with the front and are not separated. The results of developing the chromatograms in a new solvent system consisting of *n*-heptane–acetone–isopropanol–0.03 *M* NH_4HCO_3 pH 8.3 (4:2:8:0.5) are shown in Fig. 3. It can be seen that a very satisfactory separation of cAMP from ATP, 5'-AMP and Ado can be achieved in this system which has a running time of only 20–30 min. The R_F value of cAMP is apparently 0.5 while that of Ado is about 1 and the other nucleotides is 0. Fig. 4 shows a chromatogram of these compounds using the solvent system without 0.03 *M* ammonium bicarbonate, and in Fig. 5 the result of addition of one part ammonium



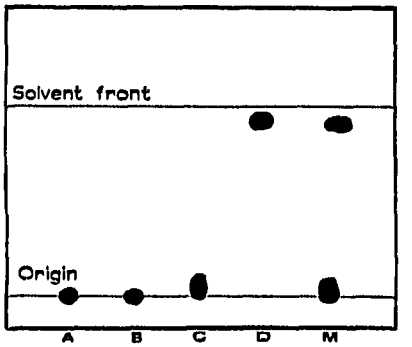


Fig. 3. Thin-layer chromatogram of various adenine nucleotides on silica gel glass microfiber sheet. Solvent was *n*-heptane–acetone–isopropanol–0.03 *M* NH_4HCO_3 pH 8.3 (4:2:8:0.5). Standards are represented by letters as in Fig. 1. Running time was 24 min.

Fig. 4. Thin-layer chromatogram of various adenine nucleotides on silica gel glass microfiber sheet. Solvent system was *n*-heptane–acetone–isopropanol (4:2:8). Detection and abbreviations as in Fig. 1. Running time was 23 min.

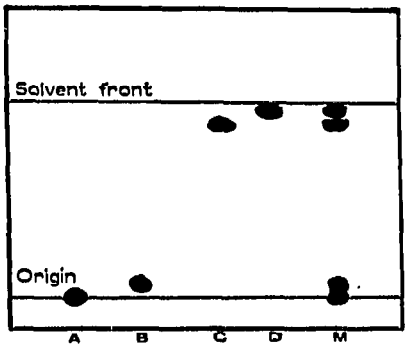
bicarbonate to the same solvent system is shown. In the former case, cAMP remains on the origin, and in the latter it moves to the solvent front. Table I illustrates the dependence of the R_F value of cAMP upon the amount of 0.03 *M* ammonium bicarbonate in the solvent system. It can be seen that increases in ammonium bicarbonate solution between 0 to 1 part result in proportional increases in the R_F value of cAMP. The separation of [^{3}H]cAMP from a mixture of ATP, 5'-AMP and Ado is compared with the chromatography of [^{3}H]cAMP alone in Fig. 6. In this chromatogram 5 μl containing 5 μg of [^{3}H]cAMP (4×10^4 d.p.m.) and 5 μl containing 5 μg of [^{3}H]cAMP (4×10^4 d.p.m.) and 5 μg each of unlabeled ATP, 5'-AMP and Ado were spotted, dried and developed in the solvent system containing 0.5 parts of 0.03 *M* NH_4HCO_3. It can be seen that the radioactive peak resulting from the mixture corresponds to the peak of cAMP alone. The R_F values of both spots in this area, visualized with U.V. light, was approximately 0.54 as expected for cAMP under these conditions. The data plotted were not corrected for counting efficiency or possible quenching.

TABLE I

THE EFFECT OF SEVERAL PROPORTIONS OF 0.03 *M* NH_4HCO_3 ON THE R_F VALUE OF cAMP ON SILICA GEL THIN-LAYER GLASS MICROFIBER SHEETS

Procedure is described in *Methods*. Solvent was composed of *n*-heptane–acetone–isopropanol (4:2:8) and the stated proportion of 0.03 *M* NH_4HCO_3 pH 8.3.

Parts of 0.03 M NH_4HCO_3	*R_F value*			
	ATP	*5'-AMP*	*cAMP*	*Ado*
0	0	0	0	0.92
0.2	0	0	0.26	0.95
0.4	0	0	0.47	0.93
0.6	0	0	0.60	0.97
0.8	0	0.06	0.79	0.97
1.0	0	0.88	0.88	0.95



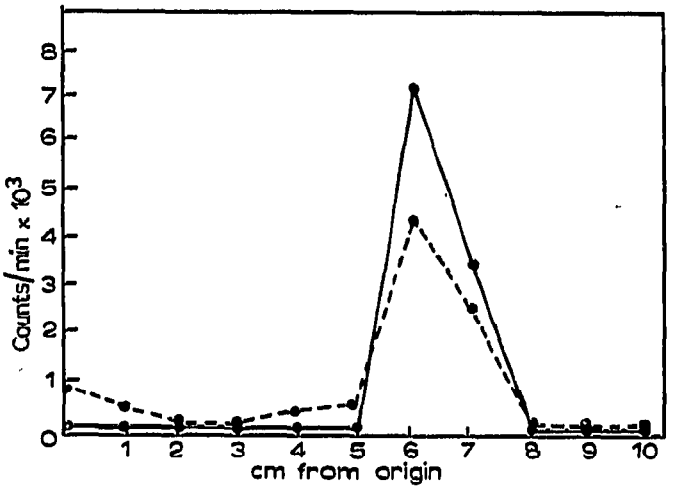


Fig. 5. Thin-layer chromatogram of various adenine nucleotides on silica gel glass microfiber sheet. Solvent system was *n*-heptane–acetone–isopropanol–0.03 *M* NH_4HCO_3 pH 8.3 (4:2:8:1). Detection and abbreviations as in Fig. 1. Running time was 25 min.

Fig. 6. Migration of [^{3}H]cAMP on a silica gel glass microfiber sheet. Procedure is described in *Methods*. Solid line: migration of [^{3}H]cAMP alone. Broken line: migration of [^{3}H]cAMP spotted in a mixture of ATP, 5′-AMP and Ado. Solvent was *n*-heptane–acetone–isopropanol–0.03 *M* NH_4HCO_3 pH 8.3 (4:2:8:0.5).

Discussion

The chromatographic system described provides for rapid separation of cAMP from other adenine nucleotides and ease of counting radioactive samples directly from developed chromatograms. The recent interest in the enzyme adenyl cyclase as direct participant in mediating certain hormonal actions[1] has led to variations in assay methods for this enzyme[2,3]. It is felt that the system for separation of cAMP described here will simplify and speed up determinations of adenyl cyclase and other assays involving cAMP. Although 5 μg samples of the components were used in this study, quantities as low as 0.5 to 1.0 μg can easily be detected using U.V. light. The radioactive studies indicate that about 35% of spotted radioactivity can be accounted for without appropriate corrections for efficiency and quenching. For optimum separations, spotted samples should be free of excess salts and other tissue constituents.

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