PRO EXPERIMENTIS

## Separation of the Constituent Nucleotides of Nucleic Acids on Ion-Exchange Thin-Layers[1]

We wish to report a new technique for complete separation of the constituent nucleoside monophosphates of RNA and DNA. The nucleotides are chromatographed on thin-layers of the anion-exchanger PEI-cellulose[2-5]. Figures 1 and 2 demonstrate typical examples. The separation is achieved according to the following procedure.

*Experimental.* A suspension of cellulose powder for TLC in a poly(ethyleneimine) hydrochloride solution is prepared as described[3,4]. The suspension is homogenized in an electric mixer for 15–30 sec and then coated on degreased glass plates in the usual way[3] (slit width of the commercial spreader 0.5 mm). In order to avoid edge effects, all plates are separated from each other immediately after coating. After drying overnight at room temperature dividing lines are scratched through the layer at a distance of 4–5 mm from the edges, and parallel lines are scratched into the bottom part of the layer, see Figure 1. Each plate is given a preliminary development with 10% NaCl solution (up to 5 cm from the bottom edge), then with $H_2O$ up to the top edge without intermediate drying. The plates are given a second development with $H_2O$ up to the top edge in order to remove impurities which would interfere with the subsequent chromatography. The plates are dried at room temperature and stored in the cold.

Ascending chromatography is carried out in closed rectangular jars filled with solvent to a height of 0.7–0.8 cm. The mixture of compounds is applied at S (3 cm from the left-hand and bottom edges), see Figure 1. Solvents used:

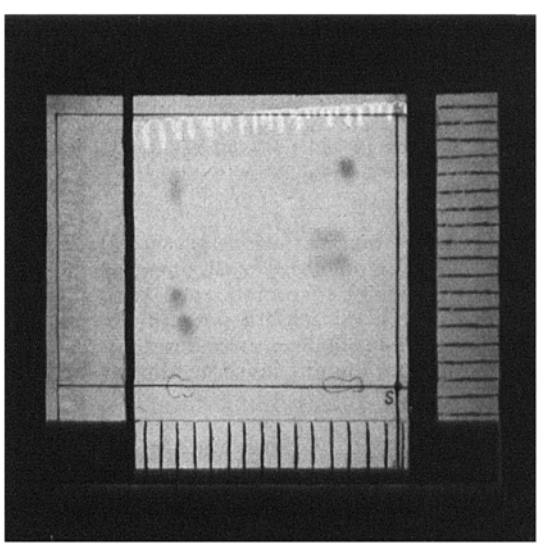


Fig. 1. Two-dimensional thin-layer chromatogram of an alkaline hydrolysate of yeast RNA. Yeast RNA (5 mg) was incubated with 1.0 ml 0.3*N* KOH for 20 h at 37°C, the solution brought to pH 5 with 30% (w/w) $HClO_4$, and insoluble $KClO_4$ removed by centrifugation. 5 µl of the clear supernatant solution was spotted at S. The chromatogram was developed as described in the text. First dimension from right to left, second dimension from bottom to top. Compounds (from top to bottom): left-hand group = 2′- + 3′-CMP, 2′-AMP, 3′-AMP; right-hand group = 2′- + 3′-UMP, 2′-GMP, 3′-GMP. Photography in short-wave (254 mµ) ultraviolet light.

First dimension: $H_2O$ (up to the start); 1.0*N* HCOOH (up to a line drawn 10.0 cm above the start). The plate is transferred from the tank containing $H_2O$ to a second tank containing 1.0*N* HCOOH without intermediate drying. The development takes about 60 min.

Second dimension: 60% saturated $(NH_4)_2SO_4$ solution (up to a line drawn 8.0 cm above the start of the second dimension). This development takes about 45 min. Before carrying out the second development all parts of the layer which are not needed for elution with $(NH_4)_2SO_4$ solution are removed from the plate (black areas of Figures 1 and 2). Subsequently the plate is laid for 15 min in a flat dish (25 × 25 cm) filled with 800–1000 ml methanol. After drying and scratching parallel lines into the bottom part (Figure 1) the compounds are eluted with the $(NH_4)_2SO_4$ solvent.

*Results and Discussion.* As can be seen from Figure 1, a complete separation of the major nucleotide constituents of RNA is obtained by the two-dimensional TLC procedure. In addition, the isomeric (2′- and 3′-) purine nucleotides are resolved, whereas the corresponding pyrimidine

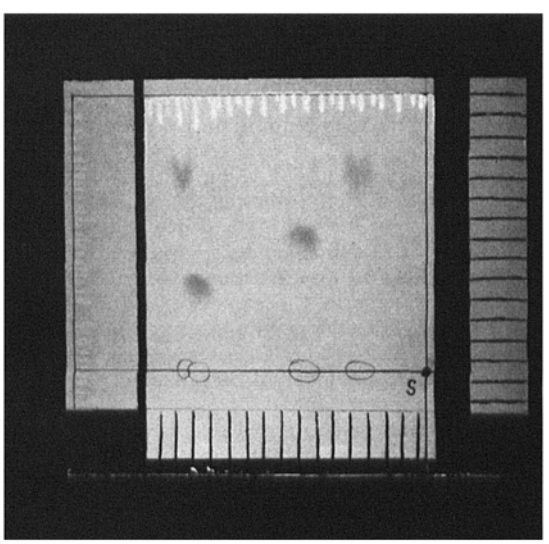


Fig. 2. Two-dimensional thin-layer chromatogram of deoxyribonucleoside-5′ monophosphates. 20 µl of a solution containing deoxy-AMP, deoxy-GMP, deoxy-CMP, and deoxy-TMP (15 mµmoles each) were applied at S. The chromatogram was developed as described in the text. First dimension from right to left, second dimension from bottom to top. Compounds (from top to bottom): left-hand group = deoxy-CMP, deoxy-AMP; right-hand group = deoxy-TMP, deoxy-GMP. Photography in short-wave ultraviolet light.

[1] Abbreviations used: AMP, GMP, CMP, UMP, TMP = monophosphoric acid esters of adenosine, guanosine, cytidine, uridine, and thymidine. The prefixes 2′-, 3′-, and 5′- indicate the position of the phosphate residue. RNA = ribonucleic acid. DNA = deoxyribonucleic acid. PEI-cellulose = a cellulose anion-exchange material obtained by impregnating unmodified or modified celluloses with poly(ethyleneimine) (molecular weight 30,000–40,000)[2]. TLC = thin-layer chromatography.

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compounds travel together. The 5'-ribo- and deoxyribonucleoside monophosphates obtained by enzymatic hydrolysis of RNA and DNA behave similarly, see Figure 2. A mixture of nucleoside monophosphates can also be separated one-dimensionally on PEI-cellulose[6] and DEAE-cellulose layers[7,8]. The two-dimensional procedure results, however, in a much more distinct resolution.

The $(NH_4)_2SO_4$ solvent was chosen for the second dimension because of excellent spacing between purine and pyrimidine nucleoside monophosphates. In Figure 3 are plotted the Rf values of 5'-AMP and 5'-UMP against the $(NH_4)_2SO_4$ concentration of the solvent. It is to be seen that adsorption on the ion-exchanger of both nucleotides is increased at high $(NH_4)_2SO_4$ concentrations. This anomalous behavior is probably due to a salting-out effect[9].

The separation of nucleoside monophosphates by paper chromatography is very difficult[10,11]. No solvent system for one-dimensional paper chromatography is known which permits the separation and subsequent spectrophotometric estimation of all four major mononucleotides of RNA. They may, however, be resolved by rather laborious two-dimensional paper chromatographic methods or by paper electrophoresis[12–14]. The latter technique, followed by elution and quantitative estimation, has been a standard procedure in nucleic acid analysis for many years. We feel that the method described in this paper has distinct advantages over two-dimensional paper chromatography and paper electrophoresis. The quantitative estimation of the nucleic acid components after elution from the layer will be described in the near future[15].

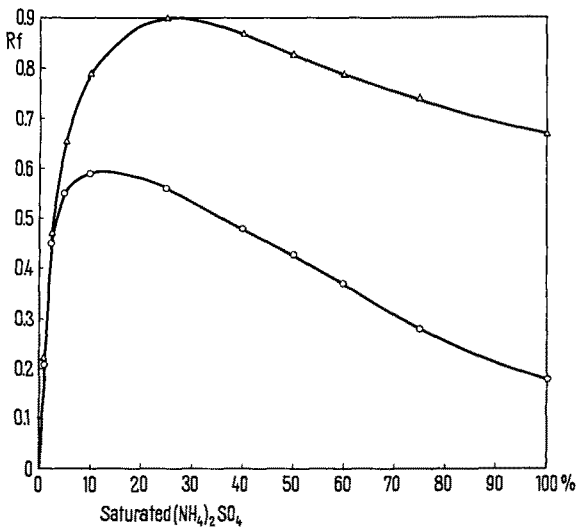


Fig. 3. Relationship between Rf values of ribonucleoside-5' monophosphates and $(NH_4)_2SO_4$ concentration of the solvent. Δ–Δ = 5'-UMP. o—o = 5'-AMP.

*Zusammenfassung.* In der vorliegenden Untersuchung wird gezeigt, dass die monomeren Bausteine von hochmolekularer Ribo- und Desoxyribonucleinsäure an Anionenaustausch-Dünnschichtplatten (PEI-Cellulose) getrennt werden können. Die Vorteile des neuen Trennverfahrens gegenüber bisher üblichen Methoden werden charakterisiert.

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[6] Unpublished experiments.
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[15] *Acknowledgments.* I wish to thank Professor P. C. ZAMECNIK and Professor H. M. KALCKAR for their generous support during the course of this work. This investigation was supported by grants-in-aid from the U.S. Atomic Energy Commission, the U.S. Public Health Service, the National Science Foundation, and the Burroughs Wellcome Fund. This is publication No. 1168 of the Cancer Commission of Harvard University.

## Aluminium-Paper Covers for Tubes or Flasks in Microbiology

The classic cotton stoppers used to close tubes and flasks in bacteriological laboratories now tend to be replaced by various substitutes, each offering its own special advantages. The threaded plastic cap is easy to handle and affords an excellent seal, but is expensive. There is the tubular stainless cover, but it fits tubes of one dimension only. And there is the sheet of paraffin paper, which effects a hermetic seal but once removed cannot be sealed again.

The caps used in our laboratory are made of aluminium foil (0.035 mm) cemented to paper, cut into discs 8 cm in diameter (AP discs)[1]. This is the type of ready-made material commonly used for Christmas wrapping and decorations.

As shown in the Figure (A, B, C) the metallic side is applied to the mouth of the tube and the disc is pressed down with the palm of the hand, while with the help of the fingers it is moulded about the mouth of the tube.

In experiments with this type of closure for tubes of triptose broth and thioglycolate culture medium, the following was noted:

Twenty tubes were kept on the laboratory table for two weeks and incubated for another week at 37°C. All media remained sterile.

[1] Patent pending.