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].

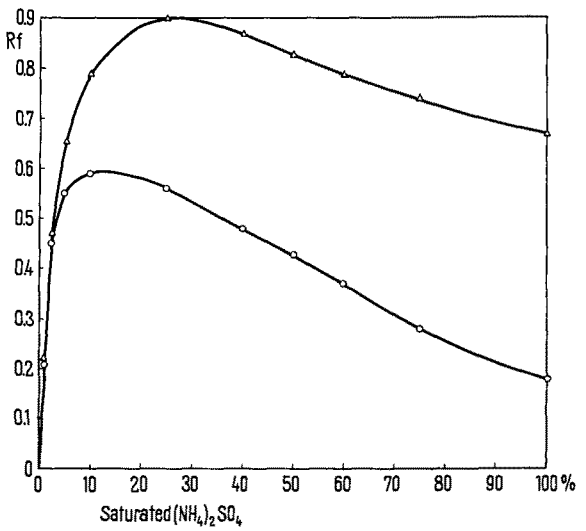

Fig. 3. Relationship between Rf values of ribonucleoside-5' monophosphates and $(NH_4)_2SO_4$ concentration of the solvent. Δ–Δ = 5'-UMP. o—o = 5'-AMP.

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.

K. RANDERATH

John Collins Warren Laboratories of the Huntington Memorial Hospital of Harvard University at the Massachusetts General Hospital, Boston (Mass. USA), April 6, 1964.

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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.

Twenty tubes were incubated for two weeks at 37°C, 10 closed with bacteriological cotton and 10 with our AP discs. Evaporation was 35% less for tubes sealed with AP discs as compared with cotton plug. When tubes sealed with AP discs were sterilized for 2 h at 200°C, the paper surface of the discs turned only slightly yellow. The cotton plugs turned dark brown and distillation substances from the cotton adhered to the inside of the tubes.

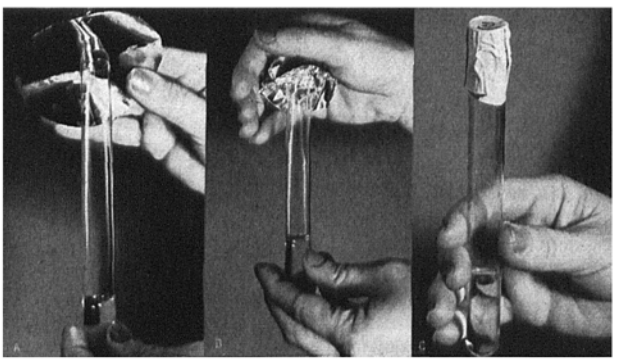
When tubes of liquid medium, closed with the AP discs, were sterilized at 30 pounds pressure for 1 h, and even though the pressure was released suddenly, all the caps remained in place, while more than half of the cotton plugs in the control tubes came out. The metallic cover permits vapours to leave the tube and equalize the pressure in tube and autoclave. (When the pressure drops in the autoclave it drops to the same degree in the tube, and the cover stays in place.)

Opening and closing of the tube on the occasion of seeding or withdrawing colonies is also easier than with a cotton plug. Since the metal cap does not adhere perfectly to the walls of the tube, it does not give rise, when removed, to that phenomenon of an aspiration which may bring about air infection of the medium.

The external surface, the paper side, of the AP disc is white, and serves for any desired annotations with ink or otherwise. Such notes are much more reliable than those made on the tube, which may be effaced by repeated handling or by sterilization in the autoclave. Besides, the clean tube represents an economy of washing time.

A single size of AP disc will fit a wide range of tube and flask sizes; they are much cheaper than cotton plugs, and require only $^1/_{100}$ the space for storage.

Discs of this material, aside from serving as caps, are very useful for holding material to be weighed on pharmaceutical or analytical scales, replacing the usual watch crystal in many cases.



A B C

A, The disc (metallic side) is applied to the mouth of the tube. B, it is pressed down with the palm of the hand. C, Ready.

Résumé. Un nouveau type de couvercle pour tube de microbiologie ou biochimie a été décrit. Il s'agit d'un disque d'aluminium doublé de papier. Il ferme plus étanchément que les bouchons, résiste à 200°C, conserve la stérilité, on peut écrire dessus et il est bon marché.

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STUDIORUM PROGRESSUS

The Mechanism of Action of Glyceraldehyde-3-Phosphate Dehydrogenase

In our recent work on the hydrolytic reaction catalysed by GAPD[1], it was assumed that owing to its nucleophilic character a particular thiol group has an essential role[2]. It was established that the formation of an intermediate acetyl-enzyme during hydrolysis of an acyl-compound depends both on the electrophilic carbonyl carbon atom of the acyl-moiety of the substrate and on the structure of the leaving group originally bound to the acyl carbonyl carbon atom. The present paper is an attempt to study the nature of the influence which the structure of substrate and coenzyme has on the reaction of GAPD, and to analyse in detail the catalytic process.

In the reaction with aldehydes, such as GAP, GA, or acetaldehyde, the formation of the intermediate acyl-enzyme is accompanied by dehydrogenation and is followed by phosphorolysis[3–7] as shown below.

$$E\text{–}S^{\ominus} + NAD^{\oplus} + \underset{\underset{H}{|}}{\overset{\overset{O}{\|}}{C}}\text{–}R \rightleftharpoons E\text{–}S\text{–}\overset{\overset{O}{\|}}{C}\text{–}R + NADH \quad (1)$$

$$E\text{–}S\text{–}\overset{\overset{O}{\|}}{C}\text{–}R + HOPO_3^{2\ominus} \rightleftharpoons E\text{–}S^{\ominus} + \overset{\overset{O}{\|}}{R\text{–}C}\text{–}OPO_3^{2\ominus} + H^{\oplus} \quad (2)$$

[1] Abbreviations: GAPD = glyceraldehyde-3-phosphate dehydrogenase; GAP = glyceraldehyde-3-phosphate; GA = glyceraldehyde; *p*-NPA = *p*-nitrophenyl acetate; IAA = iodoacetic acid; NAD = nicotine amide-adenine dinucleotide; NADH = reduced nicotine amide-adenine dinucleotide; AMP = adenosine monophosphate; IMP = inosine monophosphate.

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