finie s^{20}_0 est de 4,15 S. Ces deux techniques confirment la pureté de cette préalbumine. L'ensemble de ces résultats permet d'affirmer la réalité de la préalbumine.

Ses caractères de solubilité sont les suivants: elle est précipitée par l'acide trichloracétique à 5%, par l'éthanol à 30%, par l'ébullition. Son coefficient d'absorption à 278 mμ $E^{1\,cm}_{1\%} = 12{,}5$.

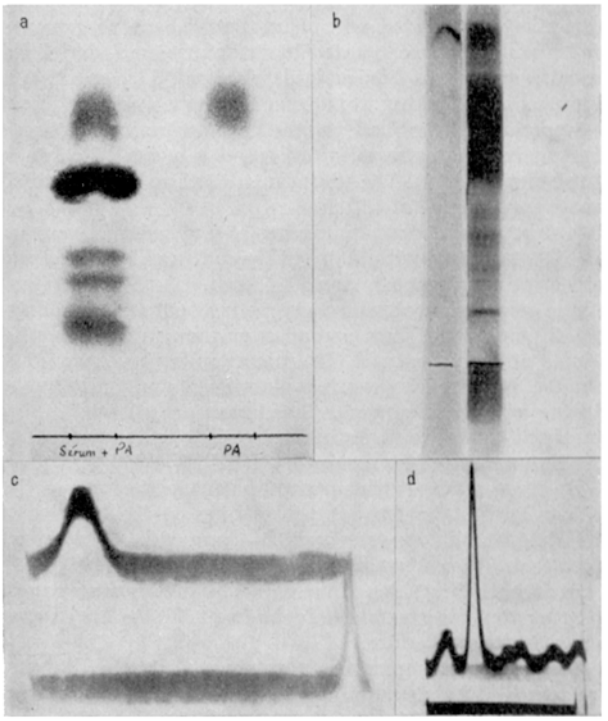

Fig. 1. Electrophorèses de la préalbumine. (a) Electrophorèse sur papier à pH 8,6: préalbumine pure (droite) – Sérum enrichi en préalbumine (gauche). (b) Electrophorèse sur gel d'amidon à pH 8,9: préalbumine pure (droite) – Sérum enrichi en préalbumine (gauche). (c) Electrophorèse libre de la préalbumine pure à pH 8,6. (d) Electrophorèse libre à pH 8,6 du sérum enrichi en préalbumine.

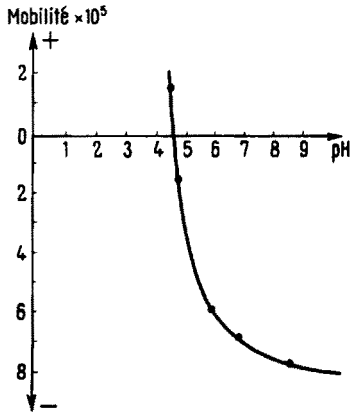

Fig. 2. Courbe de mobilité électrophorétique de la préalbumine en fonction du pH. Force ionique 0,1.

Ses principales propriétés chimique ont été déterminées: 14,2% d'azote par la microméthode de Kjeldahl, 96% de peptide par le biuret, 0,9% d'osamines après une hydrolyse de 4 h à 100°C par HCl 3*N*, 2,3% d'hexoses et 2,3% d'acide sialique; elle ne contient pas de phosphore.

SCHULTZE et al.[1] ont déterminé quelques caractéristiques physico-chimiques de la préalbumine qu'ils avaient étudiée en 1956. Ils donnent une mobilité électrophorétique de 50% supérieure à celle de l'albumine pour la préalbumine purifiée et ils admettent que la valeur de la mobilité augmente avec la purification, les préparations peu purifiées ayant une mobilité de 20% supérieure à celle de l'albumine.

Pour toutes nos préparations, nous avons trouvé sensiblement la même valeur de mobilité électrophorétique: 20% de plus que l'albumine. L'électrophorèse sur papier (Figure 1a) ou en veine liquide (Figure 1d) d'un sérum enrichit en préalbumine (20 mg/ml) confirme cette donnée: la frontière préalbumine, très apparente, a la même mobilité que celle obtenue lors de l'électrophorèse de la protéine pure. Notons que la position de la bande préalbumine dans l'électrophorèse en gel d'amidon est en accord avec cette valeur de mobilité légèrement plus élevée que celle de l'albumine, les deux protéines ayant des poids moléculaires voisins.

Le pHi, qui n'avait pas encore été déterminé, est proche de celui de l'albumine. La constante de sédimentation est en accord avec celle de SCHULTZE et al.[1]. Cependant, la courbe de variation de la vitesse de sédimentation en fonction de la concentration est une droite de pente négative ($s = 4{,}15\,S - 0{,}08c$) alors que SCHULTZE et al.[1] obtenaient une droite de pente nulle.

Quant aux propriétés chimiques, SCHULTZE et al.[1] trouvaient 1,1% d'hexoses, 0,15% d'hexosamines et absence d'acide sialique. Nous trouvons des pourcentages nettement plus élevés pour ces différentes substances glucidiques; ces valeurs sont confirmées par le taux d'azote relativement faible, le pourcentage de peptides, et par le fait que la préalbumine est légèrement colorée par le réactif de Schiff après oxydation périodique en électrophorèse sur papier.

Ainsi, la préalbumine obtenue par cette nouvelle méthode de préparation se présente comme une entité protéique; il s'agit, vraisemblablement de la protéine mise en évidence par SCHULTZE et al.[1], bien que certaines des caractéristiques physico-chimiques de ces deux protéines diffèrent d'une façon significative.

Summary. Prealbumin is prepared by a new method. Its homogeneity is demonstrated and its electrophoretic behaviour is discussed. Some new physico-chemical characteristics are determined.

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Thin-Layer Chromatography of Deoxyribo-Oligonucleotides

Chromatography has been used extensively as a tool for characterization of nucleotides. Despite much effort and many previous improvements in this field[1,2], the criteria of chromatography (efficient and rapid separations, high sensitivity, mildness and reproducibility) have not been completely met by the techniques so far developed.

[1] E. A. PETERSON and H. A. SOBER, J. Amer. chem. Soc. *78*, 751 (1956).

[2] R. V. TOMLINSON and G. M. TENER, J. Amer. chem. Soc. *84*, 2644 (1962).

Thin-layer chromatography (TLC) based on ion-exchange (DEAE- and ECTEOLA-cellulose) has been shown recently[3] to give good and rapid resolutions of ribonucleoside mono-, di-, and triphosphates. In the present communication we wish to describe a method for the separation of deoxyribo-oligonucleotides which appears to meet the above criteria optimally.

Although polyethylenimine (PEI) is known to be fixed substantively on cellulose fibers[4], PEI-cellulose has not been used until recently[5] in anion-exchange chromatography. Cellulose impregnated with PEI offers some distinct advantages as compared with the DEAE-cellulose hitherto used.

Experimental. Preparation of the deoxyribo-oligonucleotides. Thymidine-oligonucleotides were prepared and characterized according to the procedure of KHORANA and VIZSOLYI[6]. Removal of terminal phosphomonoester groups was carried out with alkaline bacterial phosphomonoesterase (Worthington): 0.2 μmoles of phosphomonoester groups, contained in a final volume of 0.04 ml water, 0.01 ml of 0.05 *M* trihydroxymethyl aminomethane buffer (pH 8.0), 4 μg of the phosphatase in 0.01 ml buffer. The dephosphorylation was complete after $3^1/_2$ h at 37°C. *d*-Adenylyl-(3′→5′)-*d*-adenylyl-(3′→5′)-*d*-adenosine-5′ phosphate was prepared according to RALPH and KHORANA[7].

Preparation of PEI- and DEAE-cellulose layers. 3 g of a 50% (w/v) solution of PEI in water, which is commercially available[8] are diluted with 6 ml distilled water, neutralized by adding concentrated hydrochloric acid, and filled up with water to a final volume of 15 ml. This solution is dialyzed in a Visking 36/100 feet dialysis tubing against 3 1-portions of distilled water. The magnetically stirred dialysis bath is changed after 4 and 8 h, and the run is finished after 20 h. The solution is filled up with distilled water to a volume of 150 ml.

A slurry is prepared by mixing 15 g of cellulose powder MN 300 for TLC[9] with 90 ml of the dialyzed PEI-solution and shaking vigorously for 30–45 sec. 10–15 ml-portions of the suspension obtained are poured onto glass plates, 10×20 cm in size, and spread over them by means of a simple new applicator[10]. The plates must be thoroughly cleaned by brushing with a detergent and subsequent rinsing with distilled water. The layers are allowed to dry for about 15 h at room temperature. The drying procedure can be accelerated by means of a hair-dryer (50°C).

To avoid cracks, it has proved useful in the case of the PEI-cellulose layers to scratch in lines at the bottom of the plate, about 2 cm long and 0.5–1 mm wide, 5 mm apart from each other. Moreover, the layer is scraped off about 1 cm on both long sides of the plates by means of a spatula in order to get a straight solvent front. These are crucial points of the procedure.

Last traces of impurities, not removed by the dialysis, are removed by developing the plates in a jar filled up with distilled water to a height of 0.5–0.8 cm. Before carrying out the chromatography, the layers are dried with a hair-dryer (5 min, 60°C).

It is also feasible to modify the procedure so that the cellulose powder is impregnated with non-dialyzed polyethylenimine, washed with water, and dried. Such a product will be marketed in the near future[10]. DEAE-cellulose layers were prepared as recently described[3]. DEAE-paper was the commercially available Whatman product (DE 20-paper).

Chromatography. (1) PEI-cellulose layers. 2 μl of the oligonucleotide samples (concentration: about 6 O.D. units per ml) are applied from a pointed micropipet at a line 3.0 cm from the lower end of the plate. Ascending chromatography is carried out with sodium chloride solutions. The jars are filled with the solvents to a height of about 0.5 cm. The plates are immediately dried after chromatography in a stream of hot air. The spots are located by means of an ultraviolet lamp. (2) DEAE-cellulose layers. The chromatography is carried out as recently described[3]. (3) DEAE-paper. Ascending chro-

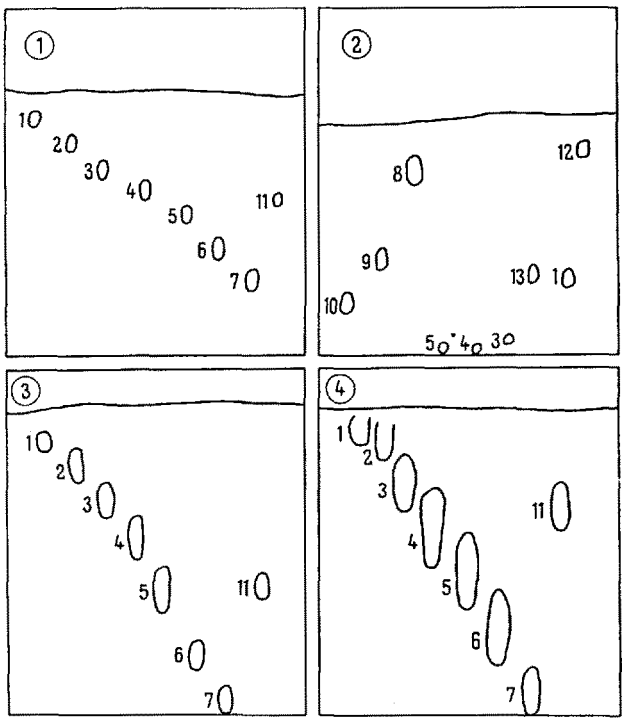

Fig. 1. PEI-cellulose thin-layer chromatogram. Thickness of the layer 0.5 mm. Applied quantities: 2 μl of the stock solutions. The plate was developed with 0.7 *M* NaCl for 5 min, then in the wet state immediately transferred to 1.0 *M* NaCl, after 15 min transferred from there to 1.2 *M* NaCl and left there for 30 min. Distance covered: 7.5 cm.

Fig. 2. PEI-cellulose thin-layer chromatogram. Thickness of the layer 0.5 mm. Applied quantities: 2 μl of the stock solutions. Solvent: 0.25 *M* NaCl. Distance covered: 6.5 cm in 30 min.

Fig. 3. DEAE-cellulose thin-layer chromatogram. Thickness of the layer 0.5 mm. Applied quantities: 2 μl of the stock solutions. Solvent: 0.2 *M* NaCl. Distance covered: 8.7 cm in 20 min.

Fig. 4. DEAE-paper chromatogram. Applied quantities: 10 μl of the stock solutions. Solvent: 0.2 *M* NaCl. Distance covered: 8.7 cm in 35 min.

List of Compounds: (Abbreviations as currently adopted by the J. biol. Chem.) 1 = $(pT)_1$, 2 = $(pT)_2$, 3 = $(pT)_3$, 4 = $(pT)_4$, 5 = $(pT)_5$, 6 = $(pT)_7$, 7 = $(pT)_9$, 8 = TpTpT, 9 = TpTpTpT, 10 = TpTpTpTpT, 11 = $(pdA)_3$, 12 = Thymidine-3′,5′-cyclic phosphate, 13 = Cyclo-pTpTpT

[3] K. RANDERATH, Angew. Chem. *74*, 484 (1962), internat. ed. *1*, 435 (1962).
[4] H. WILFINGER, Das Papier *2*, 265 (1948).
[5] K. RANDERATH, Angew. Chem. *74*, 780 (1962), internat. ed. *1*, 553 (1962); Biochim. Biophys. Acta *61*, 852 (1962).
[6] H. G. KHORANA and J. P. VIZSOLYI, J. Amer. chem. Soc. *83*, 675 (1961).
[7] R. K. RALPH and H. G. KHORANA, J. Amer. chem. Soc. *83*, 2926 (1961).
[8] Badische Anilin- und Sodafabrik, Ludwigshafen/Rhein (Germany).
[9] Macherey und Nagel, Düren (Germany).
[10] Obtainable from Serva-Entwicklungslabor, Heidelberg (Germany). Of course, the applicator described by STAHL[11] can also be used.
[11] E. STAHL, Chemiker-Z. *82*, 323 (1958).

matography is carried out with sodium chloride solutions. Details see legend to Figure 4.

Results. As can be seen from Figures 1, 3, and 4, PEI-cellulose TLC offers considerable advantages as compared with DEAE-cellulose TLC and with DEAE-paper chromatography. The PEI-cellulose layer gave sharply delineated spots, whereas the spots on the DEAE-cellulose layers are more diffuse. The least satisfactory results are obtained with DEAE-paper. The sensitivity decreases in the order: PEI-cellulose layer, DEAE-cellulose layer, DEAE-paper, being for the latter at least ten times smaller than for PEI-cellulose. Radioactive labeling of the compounds will of course further increase the sensitivity of the PEI-cellulose technique. The anion-exchange procedures have, as compared with partition chromatography of oligonucleotides[12], the additional advantage of requiring a minimum of time. As it seems to us, an important feature of the method is the remarkable mechanical stability of the PEI-cellulose layer which permits writing on it like on a sheet of paper. Moreover, the nitrogen content of the impregnated cellulose can be varied over a relatively wide range so that the chromatographic conditions can be adapted to the analytic requirements.

In the case of the impregnated PEI-cellulose there is apparently a principal difference as compared to the substituted DEAE-cellulose. Compounds with free phosphomonoester groups are on PEI-cellulose, in contrast to DEAE-cellulose, more retarded than can be predicted from the net charge of the molecule (Figure 2). For example, compare TpTpTpTpT (No. 10) with $(pT)_3$ (No. 3), both having a net charge of four, or $(pT)_1$ (No. 1) with TpTpT (No. 8), both having a net charge of two. Base differentiation between adenine and thymine derivatives of the same type is also observed on PEI-cellulose (see Figure 1: $(pT)_3$ and $(pdA)_3$).

The results make it clear that PEI-cellulose TLC may be a valuable analytical tool in nucleic acid chemistry. The possibility of applying this method in column chromatography is under current investigation[13].

Zusammenfassung. In der vorliegenden Untersuchung wird gezeigt, dass Dünnschichtchromatographie von Desoxyribo-oligonucleotiden an Cellulose, welche zuvor mit Polyäthylenimin imprägniert worden war, hinsichtlich Empfindlichkeit und Schnelligkeit der Trennung den bisher bekannten Methoden überlegen ist.

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Institut für organische Chemie, Technische Hochschule, Darmstadt (Germany), October 11, 1962.

[12] G. WEIMANN and H. G. KHORANA, J. Amer. chem. Soc. *84*, 419 (1962).

[13] *Acknowledgment.* We wish to thank Professor F. CRAMER for his interest in this work.

Strip-Transfer Method Applicable in Two-Dimensional Paper Chromatography

Normally the two separations in two-dimensional paper chromatography are carried out on one sheet of filter paper. Only a rather narrow strip, however, is necessary in the first development, whereas the rest of the paper can be omitted. It should be possible, therefore, to start with one-dimensional chromatography on a strip of filter paper. Then the developed chromatogram can be extended in two opposite directions with rectangular sheets of filter paper of appropriate size. One sheet serves to absorb the solvent in the second separation, which separation is carried out perpendicular to the one-dimensional direction in the second sheet.

Advantages of this method are: (1) It allows the use of smaller chromatography boxes than those usually applied in two-dimensional chromatography. (2) A considerable decrease of the amount of solvent necessary in the first separation. (3) The possibility of carrying out both separations in the same fibre direction of the paper. (4) A one-dimensional separation by paper electrophoresis can be combined with a second separation by means of paper chromatography in a direction perpendicular to the first one. (5) The possible influence of the solvent from the first run on the second separation will be decreased, since only a small part of the two-dimensional chromatogram has been in contact with the first solvent. (6) Two different types of filter paper can be used for the two subsequent separations.

Methods for attaching segments of a chromatogram to another sheet of filter paper have been described mainly in one-dimensional chromatography, e.g. by sewing[1,2], or by pressing between a folded sheet[3], or by inserting into a rectangular hole[4]. These methods, however, are rather laborious. SCHLÖGL and SIEGEL[5] have pressed segments of one-dimensional chromatograms to sheets of filter paper by means of two glass plates covering the segments totally. This method can also be applied in a subsequent two-dimensional separation of a one-dimensional chromatogram. The procedure, however, did not appear to be generally applicable, as will be shown below.

We have developed the following method: A segment of a one-dimensional paper chromatogram or an electropherogram of 26 × 2.5 cm containing the larger part of the compounds separated is attached to two sheets of filter paper by means of two pairs of glass bars as shown in Figure 1. We used a wooden planchet provided with a groove having the same form as the glass bar, see Figure 2. After first putting one bar in this groove one of the sheets is put upon the bar with its appropriate edge, covering about 4 mm of this bar. Subsequently the other sheet is placed in the opposite direction covering the first sheet over a length of about 3 mm. Finally the two papers are pressed together by a second bar and clamped by two clamps, see Figure 3. One can make use of weights to prevent the sheets moving from their positions during handling. The method has been tested both with Whatman No. 1 and with Whatman No. 541 filter paper. The resulting chromatograms were comparable with normal two-dimensional chromatograms. The compounds however, are not removed quantitatively from the strip, if the edges of the sheets are not completely covered by the bars.

[1] L. A. BOGGS, Anal. Chem. *24*, 1073 (1952).
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[5] K. SCHLÖGL and A. SIEGEL, Z. physiol. Chem. *292*, 263 (1953).