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# RNA FRACTIONATION ON MODIFIED CELLULOSES

## I. ECTEOLA-, ECTHAM-, AMINO-ETHYL-, NUCLEIC ACID-, AND NITRO-CELLULOSE

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### 1. INTRODUCTION

The interest in nucleic acids has resulted in an intensive search for alternate methods for their fractionation. These methods include the use of calcium phosphate and hydroxyapatite gels; starch, agar and polyacrylamide gels; Sephadex gel and its modified forms; different reversed-phase columns; Kieselguhr columns with their several modifications; modified celluloses; etc.

In particular, the modification of pure and fine particles of natural cellulose by substituting different functional groups has opened a new era in the chromatographic fractionation of enzymes, DNAs, and RNAs from a wide variety of sources. If the retention of any particular molecule was merely a function of its molecular weight, solubility characteristics and finite macromolecular conformation, pure cellulose would have proved to be a satisfactory adsorbent. Rather this lack of retention on pure cellulose and the appreciable retention immediately after modification indicates that retention and, further, the resolution are related not only to the macromolecular nature, but also to the groups intrinsic in the molecules concerned.

Amongst the modified celluloses, ECTEOLA-, amino-ethyl-, DEAE-, benzoylated-DEAE-, benzoylated-naphthoylated-DEAE-, benzoylated-DEAE-silicic acid-, DNA- or polynucleotide-cellulose and nitro-cellulose have been extensively used in

the fractionation of RNA and its degradation products. Certain aspects of modified cellulose column chromatography and environmental factors affecting the resolution have been reviewed[1-5] and are, therefore, not dealt with here. The present review article attempts to give an account of applications and describes the biological significance of the separations achieved, operational advantages and limitations, and the scope of modified celluloses.

## 2. UNSUBSTITUTED CELLULOSE

The feasibility of unsubstituted cellulose in the fractionation of DNA was first explored by BENDICH AND ROSENKRANZ[6]. While investigating the chromatographic characteristics of DNAs using DEAE- and ECTEOLA-cellulose columns, it was considered worthwhile to study the separation of DNAs on pure cellulose papers and to correlate the migration of DNAs with their sedimentation patterns. A similar idea, involving the use of non-ionic forces for the separation of tRNA and rRNA, was tested by BARBER[7] using Whatman CF-11 cellulose powder (a special microgranular type of pure cellulose powder used in sheets preparation, as well as for column packing). The separations obtained with both paper and column chromatographic procedures were highly encouraging. Changing the chemical activity (apparently the conformation) of nucleic acids by using buffers containing variable amounts of alcohol made it possible to obtain sharp and reproducible separations. Recovery was 100% and the rRNA obtained after chromatographic passage had the same sedimentation pattern as before chromatography. As little as 100 $\mu$g of a mixture of tRNA and rRNA could be separated using paper chromatography, while as much as 15 mg of rRNA could be purified on a 4-g cellulose column. The technique seemed quite simple, easy, and inexpensive.

Applying the above technique, milligram quantities of replicative intermediate (RI) of phage $R_{17}$ could be purified from rRNA contamination[8]. It was also noted that whereas double-stranded nucleic acids (RI and RF*) could be eluted in buffer alone, single-stranded nucleic acids (rRNA, mRNA, viral RNA and denatured DNA) could only be eluted by 15% ethanol in buffer, while native DNA eluted predominantly with 35% ethanol. By repeated chromatography[9], it was also possible to separate $^{32}$P-labeled phage $f_2$ RNA from *E. coli* RNA. The author claimed[8] that the method could be used either at trace levels or for the isolation of large amounts of RI and suggested that it might find wide application as a separation tool in studies on the mechanism of viral RNA synthesis. In the meanwhile, modified celluloses furnished more definitive separations than pure cellulose and hence the latter are not widely used now.

## 3. ECTEOLA-CELLULOSE

ECTEOLA-cellulose contains basic groups derived from triethanolamine bound to cellulose through reaction with epichlorohydrin, and acts as an anion

* RF = replicative form.

exchanger[10]. Its efficacy for the fractionation of nucleic acids was demonstrated[11] by successfully separating artificially prepared linear and cyclic thymidine oligonucleotides ($n = 10$).

*(A) RNA fractionation*

*E. coli.* Total RNA preparations isolated from *E. coli* were partially enriched for total tRNA on ECTEOLA-cellulose, using an increasing concentration gradient of sodium chloride[12] and using a linear concentration gradient of ammonium formate at pH 4.7[13,14]. The fractions obtained (tRNA and rRNA) were characterised for their physico-chemical properties, for their participation in protein synthesis[12], or for their acceptor activity[13,14]. While it was noted that leucyl- and valyl-tRNAs were contaminated with isoleucyl- and methionyl-acceptor activity, the specific activity of valyl-tRNA was increased sevenfold over the original preparation[14].

In another study, total nucleic acids from *E. coli* B, *B. megaterium* and *Micrococcus pyogenes* were separated[15] apparently into two fractions corresponding to particulate ribonucleoprotein and DNA; however, rechromatography of fraction I (ribonucleoprotein) revealed that it was possibly contaminated with tRNA (a fact subsequently confirmed by assaying for acceptor activity). Upon treatment with versene, the ribonucleoprotein fraction could be subfractionated into three peaks, possibly on the basis of their relative nucleic acid to protein content.

*Yeast.* Total RNA preparation from yeast was chromatographed on ECTEOLA-cellulose[16,17]; while tRNA could be eluted easily, elution of rRNA met with many difficulties, possibly due to its high affinity (firm binding) towards ECTEOLA-cellulose[17]. It was further noted that the tRNA fraction eluted as above was homogeneous with respect to electrophoretic mobility and sedimentation analysis. In subsequent studies[18–20], it was observed that whereas tRNA could be eluted at neutral pH by 1.0 *M* sodium chloride, rRNA was only elutable at pH 9.5; the tRNA fraction represented 15–20% and the rRNA fraction 80–85% of the total RNA[19]. Recently, valyl-tRNA has been isolated from yeast tRNA using ECTEOLA-cellulose in conjunction with DEAE-Sephadex and hydroxyapatite[21].

In contrast, at high temperatures, tRNA was more tenaciously retained on the column, possibly due to unfolding of the secondary structure, while rRNA could be eluted easily. In fact, high-molecular-weight rRNA could not be retained at elevated temperature[20]. It was interesting to note that while rRNA was 90% retainable on the column when prepared by the sodium dodecyl sulfate (SDS) method, it was only 50% retainable when prepared by the phenol method. Similarly a number of rRNA preparations from *E. coli* showed variable (8–56%) retainability. However, all rRNAs, irrespective of source or method of isolation, were eluted in a partially degraded state by 0.1 *M* sodium chloride in 0.1 *M* ammonium hydroxide. The authors claimed that the pre-existence of breaks in the polynucleotide chain or that passage through ECTEOLA-cellulose itself was the basic cause of degradation[20]; however, degradation due to alkaline conditions (pH 9.5) or due to ribonuclease cannot be ruled out, since it is now known that ribonucleoprotein (RNP) particles or phenol deproteinised RNA contain latent RNAase activity.

A highly polymeric preparation of Pneumococcal RNA exhibited heterogeneity on ECTEOLA-cellulose[22].

*Phage and virus.* ECTEOLA-cellulose has been employed for the purification of phages $T_1$ and $T_2$[23,24] and also of polio and coxsackie viruses from the tissue culture fluid of infected monkey kidney cells[25]. For the latter purpose, a DEAE-cellulose column was also used. By selective adsorption at low salt concentration and subsequent use of a gradient of increasing salt concentration, $T_2$ nucleoprotein (the fraction possessing activity containing both protein and nucleic acid) was eluted as a single sharp homogeneous peak, while an osmotically shocked preparation gave a diffused elution pattern[24]. Chromatographic behaviours of the concentrates of Q fever rickettsiae from chicken embryo yolk sacs and of Colorado tick fever virus from homogenates of the brains of infected suckling mice were also examined[25]. The behaviour of these viruses depended more upon the chemical nature of their surfaces than upon their particle size.

Chromatographic resolution of purified TMV-RNA[26–28] gave six fractions (four major and two minor peaks), and in some cases seven to eight fractions upon elution with linear sodium chloride gradients; however, these separations are probably due to degraded preparations of RNA since we now know that TMV-RNA should yield one single peak.

*Mammals.* RNA preparations from calf, rat liver and muscle, could be adsorbed on ECTEOLA-cellulose at neutral phosphate buffer (0.01 *M*) and almost stoichiometrically eluted by increasing concentrations of sodium chloride[26,29]. It was revealed that the eluted fractions (tRNA and rRNA) were still heterogeneous with respect to sedimentation analysis and that the elution profiles significantly differed for RNAs from different sources, as well as for RNAs from the same source but isolated by different methods. However, Bosch *et al.*[30] have shown that rat liver RNA isolated by two methods yielded essentially identical elution patterns. They[30] obtained four fractions, $F_1$–$F_4$: $F_1$ was non-retainable on ECTEOLA-cellulose, $F_2$ and $F_3$ could be eluted by a gradient of sodium chloride, and $F_4$ required 1.0 *M* sodium hydroxide for elution. $F_2$ was dialysable and possibly represented oligonucleotides, while $F_3$ and $F_4$ corresponded to tRNA and rRNA. Subfractionation of the rRNA yielded three fractions, of which I and II were high-molecular-weight entities, while III possessed a low molecular weight and was possibly attached to rRNA by covalent linkages. Rechromatography was helpful in further enrichment of each fraction as 80% or more of each fraction of RNA could be eluted at its original position on the chromatogram[31]. DEAE-cellulose was also used for this purpose.

Studies on the elution profiles of ribonucleoproteins and RNAs of rat liver and Novikoff hepatoma revealed that tRNA profiles from both these sources were identical on the basis of the following criteria: place of elution on the chromatogram, similarity in base composition of the fractions eluted, and acceptor ability. However, the major rRNA fraction found in hepatoma was absent in rat liver, while the major rRNA fraction in rat liver was considerably reduced in hepatoma[32,33]. The chromatographic elution pattern of the nuclear extracts, possibly of DNA, from these sources, also differed quite significantly. While studying the profiles of DNA from different normal and neoplastic tissues[34], it was found that the presence of RNA had no effect on the profiles of DNA, implying that it did not compete with DNA for adsorption sites on ECTEOLA-cellulose, and a large part of the RNA was spread all over the chromatogram. The small amount of RNA which was firmly bound to the column could be eluted with 0.1 *M* ammonia in 2.0 *M* sodium chloride.

By using a variety of ECTEOLA-cellulose preparations, differing in their relative densities of binding sites, better resolution of both tRNAs and rRNAs was achieved[35]. Inaccessibility of ECTEOLA-cellulose for larger molecules was exploited as a method for isolation of high-molecular-weight RNA free from tRNA. REINER *et al.*[36] noted that the complete elution of rat liver RNA could not be achieved by continuously increasing the ionic strength, by increasing the pH within tolerable limits at intermediate ionic strength, or without resorting to the use of alkali. A simultaneous gradient of pH and ionic strength employing lithium chloride did succeed in resolving tRNA and rRNA though the sedimentation curve seemed to reflect partial degradation and led the authors[36] to conclude that the differences in profiles might reflect depolymerization.

*(B) Basis of resolution*

The nature of the binding between ECTEOLA-cellulose and a polynucleotide chain is still unknown. It seems likely that separation of RNAs is on the basis of molecular size and base composition, particularly upon the relative adenine content[33]. It is also quite possible that the ratio of protein to nucleic acid (in the case of RNP) and relative arrangement of G-C-and A-U-rich regions of the polynucleotide chain (in case of RNA) may determine the elution pattern on ECTEOLA-cellulose.

*(C) Limitations of ECTEOLA-cellulose*

ECTEOLA-cellulose was chosen for RNA fractionation since the relatively few charges and weak binding forces it contains facilitate easy elution of nucleic acids. It has become clear that uneven distribution of charges during the modification of cellulose is an inherent defect which gives rise to heterogeneity in binding sites, as revealed from the dissociation curves[10]. ECTEOLA-cellulose, therefore, does not offer any positive advantage over DEAE-cellulose. Furthermore, peaks obtained with DEAE-cellulose are much sharper than with ECTEOLA-cellulose[37].

Routine experiments with ECTEOLA-cellulose are not possible due to the extensive time required for elution[38]. BENDICH *et al.*[39] noted that three weeks were required to complete the separation of nucleic acids and their attempts to shorten this period by increasing the flow-rate reduced the resolving power and resulted in an overlapping of the peaks. Moreover, the resolving power varied from batch to batch of ECTEOLA-cellulose.

Following the adsorption of high-molecular-weight RNA (rRNA) on an ECTEOLA-cellulose column, neither is complete recovery obtained under mild eluting conditions, nor is rRNA subfractionated into two main components. Application of high pH results primarily in the alteration of secondary structure and subsequent degradation. Therefore, the eluted RNA fraction seldom represented intact nucleic acid molecules (the S values of the fractions ranged from 1–6 (ref. 40)). The chances of degradation are greater with high "N" content of ECTEOLA-cellulose (although it favours high capacity), as the alkali requirement for elution increases with "N" content[41]. Consequently, the utility of ECTEOLA-cellulose is limited to the fractionation of small polynucleotides and tRNA[1,3].

## 4. ECTHAM-CELLULOSE

Fractionation of rRNA has always met with difficulties of either poor retention on the adsorbent or such firm binding that elution was not possible under mild conditions. The experiences with ECTEOLA-cellulose[40] or with DEAE-cellulose[42] were not encouraging in this regard, and although the methylated albumin on Kieselguhr (MAK) column could sharply resolve rRNA from microbial sources, it could not do so neatly for rRNA from mammalian origins[43]. PETERSON AND KUFF[44] successfully circumvented these problems by introducing a modified cellulose column, similar to the ECTEOLA-cellulose column. The newly introduced adsorbent, ECTHAM-cellulose (the name is derived from epichlorohydrin and Tris buffer, the agents used to modify the cellulose powder), has an affinity for mammalian ribosomes sufficient to allow retention, but low enough to permit easy elution in an undissociated form under mild conditions using dilute salt solutions. The procedure afforded fractionation of ribosomes free from the microsomal membranes to which they had been attached.

Ribosomes isolated from rat liver and mouse plasma cell tumours[44] as well as from rabbit reticulocytes[45] have been fractionated, while maintaining their capacity to incorporate amino acids in a cell free system. Elution with a sodium chloride gradient yielded two major peaks. Although each of the rabbit reticulocyte ribosome peaks could be successfully rechromatographed as a sharp single peak at the original position of elution, rechromatography of rat liver ribosomes after storage at o° was not successful as the first peak shifted towards the second peak. It was also noted that $Mg^{2+}$ concentration had a tremendous influence upon the chromatographic characteristics of ribosomes. Although this observation implies that a finite three-dimensional macromolecular conformation may have a role in the fractionation of ribosomes on ECTHAM-cellulose and circumstantial data do indicate that separation may be dependent upon the sedimentation value and the protein/RNA ratio, the basis of separation is not yet understood and whether the elution properties reflect ribosomal surface properties, physical state or conformation still remains to be determined.

ECTHAM-cellulose columns provide a rapid column procedure of obtaining pure and functionally intact ribosomes with a single chromatographic run. This method gives reproducible results and would seem to have a potential applicability for the fractionation of tRNA if suitable gradients were applied.

## 5. CM-CELLULOSE

Cellulose modified by chemically substituting carboxy-methyl groups (CM-cellulose) was examined for its ability to fractionate nucleic acids[39], and it was found to have no affinity for nucleic acids. However, it has been used for the fractionation of oligonucleotides resulting from RNAase $T_1$ digests of yeast RNA[46] and extensively employed in the resolution of proteins associated with nucleic acids[47]. The separation profiles[46] obtained with CM-cellulose were comparable with those on DEAE-cellulose. Basically, there are no reports regarding the applicability of CM-cellulose in the fractionation of RNAs.

## 6. AMINO-ETHYL-CELLULOSE

ZAMECNIK *et al.*[48] proposed an alternate approach to the problem of RNA fractionation by introducing a new column procedure and the chemical modification of tRNA prior to chromatographic separation. They loaded a dialdehyde complex of tRNA on an amino-ethyl-cellulose (AE-cellulose) column, where it was retarded by the formation of a Schiff's base with the amino group of the modified cellulose and was then rapidly eluted from the column. Complete success in separation depended upon a rapid elution rate, otherwise the complex formed was destroyed by a $\beta$-elimination reaction due to the conditions prevailing on the column. Leucyl-tRNA isolated by this method was 70–80% pure, but the painfully slow flow-rate of 4 ml/h discouraged its further use[48]. ZUBAY[49] improved the above procedure and obtained leucyl-tRNA of high yield and high purity.

Using an ammonium bicarbonate buffer for elution[37], the separation of oligonucleotides (RNAase digest) on AE-cellulose gave profiles which were comparable to those on DEAE- and ECTEOLA-cellulose. However, the peaks obtained with DEAE-cellulose were much sharper than with the other two[37].

Amino-ethyl celluloses has not been used for quite a while probably because the procedure was difficult, had certain operational drawbacks, required complete familiarity before handling for routine fractionation purposes, and seemed to have no positive advantage over the other modified celluloses. YOLLES[50] studied the binding of oxidized ribonucleosides and RNA to a cellulose derivative, *p*-hydrazinobenzyl-cellulose. The nucleosides or RNA, thus attached to the cellulose, could be recovered by elution with benzaldehyde. However, the procedure had several operational drawbacks and hence could not be pursued. For meaningful studies, keeping the intact structure of RNA is highly essential, and the methods used to release the oxidized RNA or polynucleotides bound to AE-cellulose were very drastic, *viz.*, use of hydrochloric acid as an eluting agent[51,52].

Recently, GILHAM and associates[53–55] have worked out conditions for efficient binding to, and easy elution from AE-cellulose, under which the primary structure of RNA remains unaltered. Terminal polynucleotides of RNAs from bacteriophages $f_2$ and $Q\beta$[53,54,56,57], from viral and cellular rRNA[58], and from RNAase digests of $R_{17}$[59] were separated. Primarily, the method is not useful for the fractionation of RNAs, but is employed for the analysis of the primary structure of RNA. It involves the interaction between the nucleic acid entity and insoluble polymers like AE-cellulose or even CM-cellulose. The method has several advantages, *viz.*, it is quick and reproducible, while giving almost quantitative recovery of a product of high purity[54].

## 7. NUCLEIC ACID-CELLULOSE

The ease with which different modified celluloses could be prepared (by substituting different functional groups) stimulated the idea of introducing different mononucleosides[41], synthetic oligo- and polynucleotides[60–63], and naturally occurring nucleic acids[64,65] as modifiers. Cellulose modified by chemically immobilizing nucleic acid molecules through covalent linkages has proved to be an ideal adsorbent to study

interaction specificity in base sequence, to isolate complementary nucleic acid molecules[41,55,60-66], and also to specifically fractionate nucleic acid metabolizing enzymes[67-72]. The last use stems from the classical work of purifying amylases on starch gels, thus exploiting the affinity of the enzyme to form a complex with the specific substrate.

Studies using cellulose modified by covalently attaching adenosine, guanosine, cytidine and uridine set down the basis of affinity chromatography[41]. It was observed that (a) uridine- and particularly cytidine-celluloses bound rRNA so tenaciously that elution was possible only with alkali; (b) on adenosine-cellulose, more than 50% of rRNA was easily elutable by sodium chloride gradient and the remaining rRNA required ammonia or sodium hydroxide for elution; and (c) guanosine cellulose exhibited less affinity for rRNA and the latter could be eluted with buffers of low ionic strength. Upon rechromatography, rRNA elutable from adenosine-cellulose by the sodium chloride gradient appeared as a sharp homogeneous peak, demonstrating the soundness and reproducibility of the technique. The major drawback with these columns was that different batches of the adsorbents yielded different elution patterns.

Polynucleotide-cellulose columns[55,60-63,66,73,74] developed as a logical extension of the above approach were used to fractionate oligo- and polynucleotides and homopolymers which were capable of forming H-bonds with the oligonucleotides immobilized on the cellulose. Two methods have been generally employed to prepare polynucleotide-cellulose columns: (a) condensation of synthetic oligo- or polynucleotides on cellulose in anhydrous conditions[62] and (b) covalent binding of nucleosides and synthetic or natural polynucleotides to cellulose in aqueous conditions[73]. However, recently ALBERTS AND HERRICK[70] used a substantially simplified process to prepare a column consisting of phage $R_{17}$ RNA-cellulose. The principle features of polynucleotide-cellulose columns are annealing and the formation of a specific hybrid under conditions favourable to renaturation (annealing at low ionic strength and low temperature). Since the different adsorbate molecules (hybrids formed due to complementarity in base sequence) have different affinity coefficients (this term is coined on the basis of partition coefficient in partition chromatography), they were fractionally dissociated from the column by employing temperature[60] and/or salt gradients[66].

ADLER AND RICH[61] modified the above procedure to some extent by attaching polyribonucleotides to phosphocellulose. The newly formed phosphocellulose-polyribonucleotide column could be used for the specific removal of complementary polydeoxyribonucleotide molecules. Polyadenylic acid (poly A), polyuridylic acid (poly U), polycytidylic acid (poly C), phage $T_4$ DNA, and viral RNA were also immobilized on cellulose in a similar way to serve similar purposes. For instance, a phosphocellulose-phage $T_4$-DNA column was used for the separation of phage $T_4$-DNA-specific RNA from a mixture of nucleic acids. Phosphocellulose columns without polynucleotides have been, however, employed for the fractionation of ribosomal proteins from *E. coli*[75] and also for the separation of alkaline digests of nuclear RNA from Chinese hamster ovary cells using an ammonium formate (pH 3.85) gradient (elution of the nucleosides was in the order of uridine, guanosine, adenosine and cytidine[70]).

Simultaneously with ADLER AND RICH[61], BAUTZ AND HALL[64] achieved the quantitative isolation of phage $T_4$-specific RNA, using a technique based upon RNA–DNA hybrid formation. Their method allowed the enrichment of RNA apparently corresponding to the rII region of the phage $T_4$ genome. For this purpose, phage $T_4$

DNA was arrested on acetylated phosphocellulose by a procedure similar to that used in the preparation of synthetic polynucleotides[77]. Covalent bond formation between –OH groups of phosphocellulose and DNA was thought to be the mechanism in obtaining the column. Optimal hybrid formation was achieved by incubation at 55° for 12–15 h in 0.3 *M* sodium chloride. At 65°, the DNA–RNA complex could be eluted by 1.5 *M* sodium chloride. RNA eluting out of the column showed a low density but whether it was due to a changed conformation, due to attachment of a small fraction of DNA, or due to some other factor(s) was not known. mRNA specific for phage $T_2$ DNA was also purified on the same basis[65]. HALL AND SPIEGELMAN[78] showed that the complementarity was solely responsible for hybrid formation and that the overall base composition had no role. Furthermore, fractionation also seemed to be directly based upon complementarity in base sequence and not upon base composition. Although the role of molecular weight and conformation was not emphasized, it is also of prime significance in annealing (hybridization).

EDMONDS and associates[66,74,79–82] have used polythymidylic acid (poly T-cellulose) columns extensively to study the interaction specificity and to isolate poly A from calf thymus nuclei[66], from Ehrlich ascites tumour cells growing either in mice or during short incubations *in vitro*[79], from HeLa cells[80], from *E. coli*, and from *S. cerevisiae*[81]. Since H-bond formation between complementary polynucleotides is dependent upon ionic strength of the buffer rather than on temperature, continuous gradient elution was employed to release poly A from poly T-cellulose columns. These studies unequivocally proved that poly A is of nuclear origin and that it is not a depolymerization product of nuclear genetic material. It could possibly be a separate intact entity endowed with a specific biological role. Its size (10S), its natural occurrence in the nucleus[66,79,80], and its association with mRNA[82] indicated that it may have a messenger-like role or be related to mRNA. Isolation of poly A from different sources[60,63,74] on temperature-controlled poly T-cellulose columns showed that although this column is highly effective in resolving short AMP-oligomers by progressive thermal denaturation, resolution of oligomers larger than ten to twelve monomers was difficult as the $T_m$ approached its limit.

Recently, the efficacy of oligonucleotide-cellulose columns for the selective pick-up of complementary nucleic acid molecules from a mixture of nucleic acids and its dissociation by linear temperature gradient has been tested by ASTELL AND SMITH[83]. The studies showed that entire oligonucleotides attached to the cellulose were capable of H-bonding with complementary molecules and that resolution was such that oligonucleotides differing in chain-length even by one nucleotide residue could be resolved. If the separations are reproducible, the column provides a highly sensitive tool for the fractionation of complementary nucleic acid molecules.

The idea of the polynucleotide-cellulose column was further extended in the preparation of DNA–agar columns to study interaction specificity and to separate complementary RNA or DNA molecules[84–86]. In some cases agar gel could be replaced by a versatile acrylamide gel as the matrix to immobilize synthetic polynucleotide or natural DNA[87,88].

Thus, polynucleotide-cellulose columns are sensitive tools for the study of highly specific and easily controlled interactions between complementary nucleic acids molecules. The technique has vast potential and can be applied for the isolation of purine and pyrimidine isostichs and isopliths present in nucleic acids from diverse

sources. The regeneration of such columns is very simple and the regenerated column can be used repeatedly; however, repeated use lowers the capacity of the column.

## 8. SULPHONATED CELLULOSE

Sulphonated cellulose, cellulose to which sulphonic acid ($-SO_3H$) groups are attached, has been used in protein fractionation but has not been tried as an adsorbent for the fractionation of RNAs. The most probable reason is that the high acidity of $-SO_3H$ groups may bring about apurinization, slow degradation, and possibly change the macromolecular conformation of RNAs, all of which would naturally be reflected in their chromatographic characteristics.

## 9. NITRO-CELLULOSE

NYGAARD AND HALL[89] discovered that nitro-cellulose membrane filters have adsorption characteristics which permit the separation (due to non-retainability) of free tRNA, rRNA and mRNA and native double-stranded DNA from denatured DNA and RNA-bound DNA (RNA–DNA duplex) which are retained at moderate ionic strength. However, contrary to reports[90] that denatured DNA is invariably retained, others have noticed that little, if any, denatured DNA was adsorbed on nitrocellulose (NC) filters[91]. The reason for this contradiction is not clear, but it is now certain that NC membrane filters bind single-stranded DNA, that the bound DNA is capable of forming a complex (hybrid) with homologous (having complementary base sequence) RNA[92], and that the rate of complex formation is a function of salt concentration and temperature of the system[93]. NC membrane filters have been frequently used as a rapid and convenient means of detecting RNA–DNA hybrids in homology studies. These applications will not be further discussed as this discussion is restricted to NC columns which are used as preparative tools.

NC columns were prepared at first[94] for the isolation of pulse-labeled *E. coli* RNA. The procedure employed was biphasic: it involved the interaction of *E. coli* denatured DNA with nitrocellulose and the DNA–nitrocellulose complex (DNA–NC column), thus formed, served as an adsorbent to specifically remove complementary RNA species. By loading RNA at 55° in 0.5 *M* KCl–0.01 *M* Tris-HCl buffer (pH 7.3), hybridization was permitted to take place and the hybrid formed (resistant to RNAase) was eluted either by dilute salt solution or even by distilled water. The studies revealed that in addition to salt concentration and temperature, duplex (hybrid) formation was controlled by the concentration of DNA and RNA, by the time of incubation, and by pH. Further studies indicated that complex formation was also dependent upon the number and composition of interacting nucleotides; the presence or absence of adjacent occupancy; and number, type, and location of unmatched pairs of nucleotides[95]. Oligonucleotides containing ten or more nucleotides could form RNAase-resistant complexes under optimal conditions. Even the shortest oligomers capable of forming a complex with denatured DNA did so with a high degree of species specificity[95]. All these studies concluded that the NC column has advantages over membrane filters in that comparatively large amounts of material

could be handled (improved capacity), the column was stable, and the recovery was improved[96,97].

During further studies[98,99] it was observed that differential elution of DNA–RNA hybrids and non-hybridized single-stranded DNA could be facilitated from the column by lowering salt concentration, by increasing the pH, or attaining both effects simultaneously. The observation[98] that the molecular size of the hybridized RNA was not altered during adsorption and elution from the column, as judged from sedimentation analysis, was contradicted by RIGGSBY[97], who noted partial degradation of hybridized RNA after elution. Separations of nucleic acids from yeast and HeLa cells were possible and apparently the separations were dependent upon molecular size and not on G + C content.

RNA fragments specific to the rIIA and rIIB cistrons of phage $T_4$ genome have been isolated using [$^3$H]uracil-incorporated mRNA from *E. coli* infected with phage $T_4r^+$, and DNA from the appropriate deletion mutant. The authors[100,101] claim that with little improvement, their procedure could be successfully applied to the isolation of operon or even messengers of specific bacterial genes. Recently, RIGGSBY[97,102] has systematically adapted the NC column procedure for the isolation of milligram quantities of mRNA (from the infected host cell) specific for phage $T_2$ and for the separation of bacteriophage DNA from DNA of infected bacteria. It was noted that the NC column was capable of binding about 10% as much phage $T_2$ DNA or RNA as the amount of DNA immobilized on the column, that the recovery was 70%, and that the addition of Celite improved the flow-rate to some extent. This efficacy of the DNA–NC column was further tested for fractionation of DNA from *E. coli* and phage $T_5$ and for RNA from *E. coli* and phage $T_2$. Hybridization studies were carried out before and after fractionation on the DNA–NC column. The author[97] feels that this method is useful for the analytical separation of native and denatured DNA and that it may prove useful for the isolation of large amounts of mRNA unique to a specific organism or gene on the basis of gene specificity (sequence homology).

A microscale preparative procedure employing DNA–NC columns was attempted[103] in order to study the distribution of transcribed regions of phage $\lambda$. The technique was refined by immobilizing self-annealed and separated (by density gradient centrifugation) DNA strands on the NC column. This step has yielded 99% pure DNA; thus, the hybrid formed will be highly specific, subsequently yielding a homogeneous species of mRNA. A prehybridization step was incorporated to improve upon the sensitivity of the technique by allowing RNAase treatment and subsequent inactivation of RNAase by iodoacetate. With this refined technique, it was possible to compare the levels of phage $\lambda$-specific mRNA in non-lysogenic *E. coli*, in non-induced lysogens, and in early and late induced infective lysogens.

Using wild type *D. melanogaster* total rRNA preparations and purified 28S rRNA, elution profiles of rRNA–DNA hybrids were compared[99]. It was noted that the size of the DNA and the level of saturation reached during hybridization (degree of hybridization) influenced the elution profiles. At least a large fraction of the genes for 28S rRNA was found adjacent to the genes for 18S rRNA.

Thus, the DNA–NC column technique seems to be excellent for studying sequence homology and for isolating gene-specific mRNAs. The incorporation of protective measures to avoid RNA degradation will certainly enhance its utility.

## 10. SUMMARY

While pure cellulose has a very limited scope in the fractionation of RNAs, there is a dramatic increase in the retainability of nucleic acid molecules after modification of the cellulose by substituting with a variety of groups or molecules. Applicability of ECTEOLA-cellulose has been limited to the fractionation of tRNA, in view of its inability to subfractionate and quantitatively recover rRNA into two main components. However, the weak binding tendancy of ECTEOLA-cellulose may prove to be useful for the fractionation of isoaccepting species of tRNA. ECTHAM-cellulose has successfully supplanted ECTEOLA-cellulose for the fractionation of active ribosomes without degradation. Its potential with regard to tRNA fractionation remains to be explored. Amino-ethyl-cellulose has a very limited scope in the fractionation of tRNA; its primary use is in the analysis of the primary structure of tRNA. Nucleic acid- and nitro-cellulose columns have proved to be ideal tools for studying sequence homology and for isolating complementary nucleic acid molecules.

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