Journal of Chromatography
Chromatographic Reviews
Elsevier Publishing Company, Amsterdam
Printed in The Netherlands

RNA FRACTIONATION ON MODIFIED CELLULOSES

II. DEAE-CELLULOSE

R. M. KOTHARI AND MILTON W. TAYLOR
Department of Microbiology, Indiana University, Bloomington, Ind. 47401 (U.S.A.)

(Received July 7th, 1972)

CONTENTS

1. INTRODUCTION

The successful chromatographic separations of purines and pyrimidines, nucleosides and nucleotides using synthetic ion exchangers have stimulated the research on the applicability of chromatography to nucleic acid fractionation. It is conceivable that chromatographic methods could also be useful in establishing the heterogeneity of DNAs and RNAs. Earlier attempts to use cellulose sheets and columns[1,2] were just sufficient to indicate that RNA and DNA contain a heterogeneous population differing in molecular weight, base composition, and possibly the sequential arrangement of nucleotide residues and tertiary structure; however, no subfractionation seemed possible on cellulose columns. With the introduction of substituted celluloses (by substituting different ionizable groups on hydroxyl groups of pure cellulose), it became customary to use substituted cellulose columns for the fractionation of DNAs, RNAs, and other macromolecules of biological importance. An account of applications of ECTEOLA-, ECTHAM-, amino-ethyl-, nucleic acid- and nitro-celluloses has been given earlier[3]. The present review paper attempts to give an account of the separations achieved and their biological significance, and discusses operational advantages, limitations and the scope of diethylaminoethyl-(DEAE-) cellulose.

2. DEAE-CELLULOSE

DEAE-cellulose was first prepared[4] by chemically attaching diethylaminoethyl-(DEAE-) groups to pure cellulose particles.

(A) tRNA fractionation

(a) Yeast

DEAE-cellulose was employed for the separation of tRNAs from low-molecular-weight polynucleotides and rRNA[5] and for the broad resolution of tRNA from DNA and nucleoproteins[6,7]. Adsorption at a low salt concentration and stepwise elution with 1.0 *M* sodium chloride gave enriched tRNA preparations. Although no subfractionation into specific tRNAs was noticed, 65 g of pure tRNA was obtained from 100 pounds of fresh-pressed cakes of baker's yeast[8].

In another study, DEAE-cellulose has been used in the partial purification of yeast tRNA isolated by different methods of deproteinization[9,10]. Decrease in acceptor activity after DEAE-cellulose chromatography was observed as against the earlier results[11]. It was thought that the decrease in acceptor activity may be due to removal of polynucleotide fragments, smaller than tRNA, but long enough to exhibit amino acid acceptor activity[10]. However, this explanation does not hold true since we now know that polynucleotides inhibit the acceptor activity of tRNAs.

KAWADE *et al.*[12] have obtained high-degree resolution of valyl-tRNA and partial purification of glycyl-, prolyl-, and phenylalanyl-tRNA on DEAE-cellulose and DEAE-Sephadex A-25 columns by stepwise elution with an increasing molarity of sodium chloride (pH 5.0). Two interesting results borne out of these studies were that DEAE-Sephadex A-25 appeared to be superior to DEAE-cellulose in resolution and that the presence of an amino acid residue on the tRNA moiety was not a major factor in determining the elution pattern, unlike that observed with the Kieselguhr columns[13]. However, the fractionation on DEAE-cellulose, like Kieselguhr columns, appeared to be based upon the differences in the secondary structure of tRNAs[12].

Histidyl-tRNA species from yeast, *N. crassa*, and *E. coli* were separated from other tRNAs on a DEAE-cellulose column[14]; whereas profiles of yeast and *N. crassa* histidyl-tRNA were comparable irrespective of the source of aminoacyl-tRNA synthetase, profiles of *E. coli* histidyl-tRNA differed markedly as a function of the source of the aminoacylating enzyme. The author felt that either tRNA enzyme recognition site may be differing from species to species[14] or cross acylation may be reflecting the differences in profiles. In our opinion, it is more likely that some isoaccepting species were recognized by a heterologous enzyme.

Chromatographic characteristics of yeast tRNA and whole yeast RNA were compared using an increasing concentration gradient of sodium chloride[15]. It was noted that 94–97% tRNA could be collected as a single peak between 0.40–0.56 *M* sodium chloride concentration along with approx. 7% rRNA as a contamination. Remaining rRNA required 0.1 *M* sodium hydroxide for elution indicating its strong affinity for DEAE-cellulose. Similar observations[16] indicated that for rRNA and still larger nucleic acid molecules, complete fractionation on a DEAE-cellulose column under mild conditions preserving secondary structure was difficult, possibly

due to problems of aggregation especially with guanine-rich molecules. However, in the course of time, it has now become possible to fractionate rRNA yielding 16S and 23S components[17–19].

DEAE-cellulose chromatography has been extensively employed[20–25] for the enrichment of arginyl-, valyl-, and prolyl-tRNAs at pH 7.6 in the presence of 7.0 *M* urea[20] and also for the purification of seryl-, glycyl-, threonyl-, alanyl-, tyrosyl-, and phenylalanyl-tRNAs[21]. DEAE-Sephadex gel was also used for this purpose at pH 4.5 (ref. 20) and it was noted that the elution patterns on DEAE-cellulose and DEAE-Sephadex were different. However, whether the differences were due to change in pH or in basis of separation on two different columns was not clear. Further studies[22] revealed that recovery of RNAs from DEAE-cellulose columns could be improved by adsorbing the samples at high salt concentration and recovering them at comparatively low salt concentration. Resolution of glycyl-tRNA furnished[23] two discrete fractions designated as glycyl-tRNA A (70% pure) and glycyl-tRNA B (25% pure). While fraction A appeared to be homogeneous on DEAE-Sephadex gel, fraction B was subfractionated into three peaks. BERGQUIST *et al.*[26] have obtained four glycine acceptor activities which were structurally different although each of them recognized the same codon and participated in the R_{17}–*E. coli in vitro* protein synthesizing system. Recent studies[27] have confirmed that glycyl-tRNA is resolved into four fractions on DEAE-cellulose and DEAE-Sephadex columns. Three of these fractions represented distinct species of glycyl-tRNA as ascertained by triplet binding studies. By various criteria, the fourth fraction was detected to be a biologically inactive aggregate of one of the three species; it dissociated upon heating into active monomers which again aggregated upon storage.

Multiple tRNA species for individual amino acids (isoaccepting tRNAs) have been purified from yeast as well as from *E. coli* using DEAE-cellulose chromatography in conjunction with DEAE-Sephadex A-50 and counter-current distribution (CCD)[24]. The isolated isoaccepting tRNA species were tested for their triplet stimulated binding to ribosomes, which revealed that while strict specificity of all the species for the first letter of the codon is observed, each species is capable of recognizing multiple codons differing in the third letter, evidence consistent with the "wobble" hypothesis[23,24].

Fractionation of yeast tRNA using an urea–sodium chloride gradient furnished[28] two species of valyl-tRNA which were not noticed earlier[20]. The first peak represented 70–75% of the total valyl-tRNA while the second peak corresponded to the remaining 25–30% valyl-tRNA. The separation seemed critically dependent on the ratio of the column length to the diameter, and suffered considerably by increasing only the diameter of the column without proportional increase in its length. The possibility that both acceptor activities differed in conformation only and that fraction I could change to fraction II was ruled out by the following experiment: complex formation with valyl-tRNA synthetase revealed that only the minor tRNA fraction could form a stable complex with the enzyme while the major could not, which may be either due to the existence of two isoenzymes, or else due to two conformations of the same enzyme with different abilities to form a stable or unstable complex.

As reported earlier[12], BAGULEY *et al.*[29] also observed that DEAE-Sephadex furnished better resolution and better flow-rate than DEAE-cellulose. Both valyl- and lysyl-tRNAs, each of which were poorly resolved on a heated DEAE-cellulose

column, were fractionated into multiple peaks by a decreasing temperature gradient. The order of elution of the various tRNA species, however, differed from that observed earlier[30]. These observations led them to hypothesize that fractionation on a DEAE-cellulose column did not depend upon G-C content alone, but upon the relative distribution of G-C- and A-U-rich regions of individual tRNA species. The authors[29] also felt that the order of elution and degree of resolution was probably a function of temperature and electrolyte as well as tRNA concentration.

Through the above studies, BAGULEY *et al.*[30] have developed a simple procedure utilizing a DEAE-cellulose column and gradient elution at elevated temperatures for the large-scale fractionation of total tRNAs by taking advantage of the differences in temperature of melting (T_m) of various tRNA species. With appropriate choice of temperature and salt gradient, as standardized from the pilot runs, the melting process and subsequent elution of tRNA entities could be controlled such that certain species of tRNA were retarded more than the others, thus effecting fractionation. They noted that commercially available DEAE-cellulose in standardized grades was suitable for adsorption and fractionation of tRNAs[30], and developed a procedure that would be applicable for fractionation of tRNA from a wide range of sources. It was suggested that the elution from this column held at elevated temperature (temperature elevation sufficient to cause transient differences in the secondary structure, but quite below that causing damage to biological expression) may prove to be a useful tool for the fractionation of rRNAs where elution may be a function of the secondary structure, which is more sensitive to variations in temperature.

Comparison of the elution profiles of yeast and *E. coli* tRNAs on a DEAE-cellulose column showed considerable differences and the procedure appeared to be sound for the purification of even minor species of tRNA[31]. The number of yeast tRNA species accepting a particular amino acid differed from that found in *E. coli*. Even the valyl-tRNA from brewer's and baker's yeast showed different profiles[31]. A decreasing temperature gradient (75 to 30°) and an increasing concentration gradient of sodium chloride gave good resolution on a DEAE-cellulose column[32]. However, a decreasing temperature gradient at a constant electrolyte concentration did not bring out clear separation. The principle underlying this separation is a notable feature. Rise in temperature brings about a gradual loss of secondary structure of tRNAs. The reacting groups of tRNAs favour a conformation which will enhance exchange with DEAE-cellulose, thus accomplishing moderate binding. By gradually decreasing the temperature and simultaneously increasing the salt concentration, certain tRNA species (depending upon T_m) are preferentially dissociated, resulting in selective elution. By this method, alanyl-, tyrosyl- and two isoaccepting species of valyl-tRNA were separated[32]. The changes in the conformation, favouring adsorption and subsequent desorption could be accomplished by also changing the pH. Thus, variations in T_m (ref. 33) or variations in pH, urea or salt concentration[34–36] have been successfully employed for the fractionation of tRNAs on a DEAE-cellulose column.

Correlation of a chemical structure with the biological expression was emphasized long ago[37]. Modification of tRNA by different chemical reactions and studying the modified tRNA was a promising approach to examine the role of its secondary structure in acceptor activity and enzyme recognition. Studying the function of separated tRNA fragments in initial phases of protein biosynthesis appeared to be

an alternate approach in the elucidation of the role of different sites of tRNA structure.

DEAE-cellulose chromatography has revealed that no damage was caused to yeast tRNA molecules even after prolonged formaldehyde treatment[38] or that no degradation of alanyl-tRNA occurred following treatment with nitrous acid for a period of 10 h (ref. 39). It was noted that native conformation of tRNA was fixed by intramolecular transverse methylene bridges in the presence of Mg^{2+} and the number of such bridges was reduced in the absence of Mg^{2+} (ref. 38). Nitrous acid-treated tRNA was active even if anticodon or dihydrouracil loop was 100% deaminated, but it lost activity if deaminated at the 3'-terminal cytidylic acid residues[39]. Due to deamination at anticodon site, IGC was changed to IGU with subsequent change in the amino acid acceptor activity (due to codon specificity) from alanine to threonine.

Similarly, studies have been performed[40–42] to test whether cleaved (half) molecules of tRNA still retained some biological activity. Alanyl-, valyl-, and phenylalanyl-tRNAs were specifically cleaved to give half molecules and these separated into the 3'- and the 5'-halves on a DEAE-cellulose column at pH 3.3 and at relatively high temperature (55–60°) using 7.0 *M* urea in the eluting medium. It was found that neither of the halves alone had acceptor activity, and that a reconstituted tRNA molecule exhibited the acceptor activity. Reconstitution was a function of Mg^{2+}. In the case of valyl-tRNA, cleavage in the anticodon site did not lead to a loss of acceptor function, but inactivated its transfer function, probably as a result of an inability to bind to the codon on ribosomes. This suggested that the anticodon site of tRNA did not participate in a specific interaction with the aminoacyl-tRNA synthetase complex. The phenylalanyl-tRNA halves (from yeast and wheat germ) were cross-combined and were tested for their phenylalanine acceptor activity using phenylalanyl-tRNA synthetase from yeast. Charging was possible, indicating certain (the other half was new due to cross combination) base pairs could be dispensed with for synthetase recognition.

(b) E. coli

DEAE- and ECTEOLA-cellulose paper sheets were explored[43] for their use in the separation of tRNAs. It was noted that the non-electrostatic forces played an important role in the adsorption of RNA and whereas salt alone was ineffective, urea combined with salt was effective for the recovery of tRNA. Use of paper sheets did not seem feasible as they handled small amount of RNA and had several operational limitations. Using a DEAE-cellulose column for separating tRNAs, DNA, ribosomes as well as their possible precursors from *E. coli* and *B. subtilis*, it was noted that Mg^{2+} concentration tremendously influenced the chromatographic behaviour, particularly of rRNAs[44,45]. Large-scale purification and partial fractionation of tRNAs (as much as 300–400 g of tRNAs from 77 kg of *E. coli*) has been achieved by DEAE-cellulose chromatography at room temperature using a salt concentration gradient elution[46] as against a batchwise procedure[8]. The partially fractionated tRNAs appeared in three peaks and it was proposed that these peaks could be further subfractionated by reversed-phase column (RPC) chromatography. If this materializes, one should be able to get ample samples of pure isoaccepting species of particular tRNA for further studies. Moreover, the results obtained in different laboratories

will then be comparable. They recommended the removal of DEAE-cellulose packing and repouring it in the column after each chromatographic run (to minimize channeling) and its regeneration through eleven steps after every third or fourth chromatographic run[46]. The authors[46] claimed that this procedure could be applicable, without any alterations, to separate all tRNAs regardless of their source.

A DEAE-cellulose column has been employed in the purification of *E. coli* tRNAs[47] and in the isolation of histidyl- and tyrosyl-tRNAs from a mixture of tRNAs[14,48,49]. A DEAE-cellulose column has been also used for the chromatographic purification of tRNAs from the whole cells and from the supernatant fraction of *E. coli* wild strain[50], and also from different amino acids- and purine-requiring *E. coli* mutants[51,52].

As indicated earlier, modification or cleavage of tRNA and studies on the modified or cleaved RNA entities has helped in a partial understanding of the secondary structure and its role in the biological functions of different regions of tRNA. Accordingly, a mixture of *E. coli* normal and 5-fluorouracil (FU)-containing tRNA has been resolved by a concave upward gradient of sodium chloride with 90% recovery (ref. 53). It was noted that FU could substitute pseudouridine and ribothymidine in addition to uridine. In spite of such a heavy substitution (up to 80%), tRNAs still exhibited the following pattern of acceptor activity: in some cases the activity remained the same as before the substitution, while in the other cases either it increased or decreased. Whether the differences in acceptor activity before and after substitution were due to differences in primary structure (as a result of substitution) was not clear. The separation may be due to greater negative charges associated with FU-tRNA. The procedure permitted separation of relatively large amounts of tRNA (15 mg of FU-tRNA could be chromatographed at one time on a 1 × 48-cm column) and could still be further scaled up.

While studying the mechanism of incorporation of selenium into proteins in *E. coli*, it was found that methionine could be replaced by selenomethionine. This was subsequently confirmed when [^{14}C]methionyl- and [^{75}Se]selenomethionyl-tRNA gave virtually overlapping elution profiles, indicating methionine and selenomethionine were attached to the same tRNA species[54].

In order to examine the role of different regions of tRNA, other than the anticodon loop, in reference to synthetase recognition, and ribosomal binding sites, tRNA was specifically cleaved into halves. In all cases, a DEAE-cellulose column proved of immense aid in separating the halves without either contamination or degradation. None of the halves of valyl-tRNA alone exhibited acceptor activity; but the activity was fully restored when both fragments were combined and renatured in the presence of either Mg^{2+} or Mn^{2+} or even Ca^{2+} (ref. 55). It was found that restoration of acceptor activity was a function of time and temperature, also, in addition to the requirement of a divalent cation[42], which allowed renaturation and subsequent attainment of original conformation[55]. It was interesting that an increase of Na^+ up to 0.08 *M* also restored activity even in the absence of divalent cations. Similar studies have been performed[56–58] on tyrosyl- and formyl-methionyl-tRNAs. The cleavage in the S region did not destroy tyrosine acceptor activity, but to some extent affected the ability of reconstituted tyrosyl-tRNA to form a triple complex with ribosome and mRNA[56,58]. In case of formyl-methionyl-tRNA, the cleavage of the dihydrouridine sequence in the D loop resulted in the loss of the acceptor activity

of DEAE-cellulose separated 5'- and 3'-halves (ref. 57). However, the activity was restored back when both halves were simply mixed. Surprisingly, the preincubation of two halves under a finite set of renaturation conditions[57] was not necessary to restore the activity.

(c) B. subtilis

Total tRNAs from *B. subtilis* were separated from rRNA (ref. 59), and from DNA and polynucleotides[44] using a DEAE-cellulose column. While tRNAs appeared as a sharp peak at 0.5 *M* sodium chloride concentration, rRNA appeared between 0.8–1.0 *M* sodium chloride. Recently, a large-scale preparation and fractionation of *B. subtilis* tRNAs on DEAE-cellulose has been achieved[46].

(d) Viral RNA

Amongst many techniques used, DEAE-cellulose column was found to be very useful in comparing the elution patterns of low-molecular-weight phage RNA and tRNA from infected and uninfected *E. coli* cells[60]. It was noted that only 43% of the low-molecular-weight viral RNA was recovered in the tRNA region, while the remaining RNA was so tightly bound to the column that its removal was only possible using alkali. The resolution was neither clear nor efficient. In another study, a sodium chloride concentration gradient (0.4–1.0 *M*) in Tris buffer (pH 7.0) furnished profiles, which significantly differed in the infected and uninfected *E. coli*. It was also observed that the elution pattern of *E. coli* tRNA was comparatively sharper than that of phage RNA. Similar studies on RNAs from uninfected and Herpes virus infected baby hamster kidney (BHK) cells showed that while certain peaks present in uninfected RNA were practically negligible in the infected RNA, more than one new arginyl-tRNA specified by Herpes virus was detected, which was absent in the RNA from uninfected cells[61]. The major arginyl-tRNA peak was further subjected to methylated bovine serum albumin on Kieselguhr (MAK) column chromatography. Arginyl-tRNA species from uninfected and 6.5-h infected cells, obtained by MAK column chromatography, were analyzed for their oligonucleotides (obtained by RNAase T_1 treatment) on DEAE-cellulose. The separation pattern of oligonucleotides was markedly different[61], indicating genuine differences in the primary structure of arginyl-tRNA before and after infection.

Biologically active RNA preparations from bacteriophages MS_2 and $Q\beta$ have been purified on a DEAE-cellulose column[62]. The recovery of MS_2 RNA could be improved by adsorbing at 0.3 *M* rather than at 0.1 *M* electrolyte concentration[63]. It was found that RNA thus purified was devoid of RNAase activity and contaminating phage, thus providing suitable material for reconstitution of infective particles of phage MS_2 and $Q\beta$. The author[62] claims that a spherical conformation has a much smaller net negative charge on the surface than in an extended form of single-stranded high-molecular-weight RNA. This presumably has permitted the chromatographic separation of RNA from phages $Q\beta$ and MS_2 on a DEAE-cellulose column, which has been customarily used for separating low-molecular-weight RNA. In further studies, altered response of *E. coli* prolyl-tRNA, after phage $Q\beta$ infection, to polycytidylic (poly C) acid was studied with the aid of DEAE-cellulose chromatography[64]. The significance of this alteration after infection is not yet known.

A procedure for the purification of ^{32}P-labeled phage R_{17} RNA of high-specific

activity has been worked out[65], which involves DEAE-cellulose chromatography of clarified phage lysate. Phage RNA was preferentially adsorbed on the column, while low-molecular-weight impurities and inorganic ^{32}P (representing more than 60% of the total radioactivity) came in the effluate without retention. Elution of the column yielded intact phage R_{17} RNA with minimum radiation damage.

(e) Plants

Total RNA preparations from dividing, elongating, and maturing pea seedling roots have been resolved into metabolically distinct species using differential solubilization, gel electrophoresis and DEAE-cellulose column chromatography[66]. 4S RNA was purified by the latter technique. The ratios of one species of RNA with respect to the other at the above developmental stages were studied. Leucyl-tRNA from soya bean cotyledons has been purified on a DEAE-cellulose column; the purified species was resolved into six isoaccepting species by Freon column chromatography[67].

(f) Animals

Total RNA preparation from Brachiopod Lingula has been fractionated into five peaks on DEAE-cellulose and Sephadex G-100 gel columns[68]. The fractionated species corresponded with 5S RNA, 4S RNA, which contained some minor bases and possessed amino acid acceptor activity, a polynucleotide composed of about fifty nucleotide residues (possibly a degradation product), RNA with a molecular weight of about $1 \cdot 10^5$, and a high-molecular-weight RNA.

Partial purification of rat liver leucyl-tRNA has been accomplished by chromatographing the total tRNA preparation on a DEAE-cellulose column and eluting with lithium chloride buffer at pH 4.9 (ref. 11). Retention at low salt concentration and almost stoichiometric (90%) elution at high salt concentration gave reproducible profiles. By this method, a large quantity of tRNA could be fractionated in a short period without loss of acceptor activity. Upon rechromatography, esterified form of leucyl-tRNA could be separated from the bulk of the non-esterified counterpart. However, KAWADE *et al.*[12] have noticed that the presence of amino acid residue on tRNA does not alter the elution profiles on DEAE-cellulose. Recently, a DEAE-cellulose column has been employed for the separation of isoaccepting species of tRNAs from different mammalian sources[69].

Non-labeled rat liver tRNA and a labeled protected polynucleotide (a core of rRNA resistant to RNAase action) moiety were separated using a sodium chloride gradient (pH 7.4) with or without 6.0 *M* urea, yielding sharp profiles[70].

Attempts have been made to fractionate and study chromatographic and biological characteristics (*viz.* elution profile and hybridization ability) of chromatin-bound RNA (cRNA) from calf thymus[71], and cRNA as well as cRNA–protein complex from rat ascites tumour[72]. It was surprising that associated protein had no influence on the elution profiles; also, the salt concentration requirement for elution of cRNA was the same regardless of the presence or absence of the associated protein.

In vivo chemical modifications of tRNA due to thiolation, methylation, rearrangements necessary for pseudouridine formation, etc. have been known to occur, after polynucleotide assembly was completed. HANKINS AND FARKAS[73] have discovered a new type of modification of tRNA from rabbit reticulocyte system, namely, guanylation, *i.e.* incorporation of GMP after completion of tRNA synthesis.

DEAE-cellulose chromatography was routinely followed to separate guanylated tRNA species. The column was washed with 0.4 *M* ammonium formate to remove all low-molecular-weight species including labeled GTP, while tRNA remained bound onto the column. It was subsequently eluted by 1.0 *M* saline to rule out any possibility of occlusion of GTP in tRNA. The column could also be used to assess to what extent guanylated tRNA was contaminated with other low-molecular-weight impurities, if any.

Use of a DEAE-cellulose column in conjunction with Sephadex G-200 in the presence of sodium dodecyl sulfate (SDS) has been reported for the purification of peptidyl-tRNA from rabbit reticulocyte ribosomes[74]. The procedure was extended for the isolation of peptidyl-tRNA from rat liver and from *E. coli* ribosomes[75]. The authors[74] claimed that their procedure may find general applicability for the fractionation of peptidyl-tRNAs from a number of sources.

(B) rRNA fractionation

(a) E. coli

Several workers[76–80] have analysed the process of ribosome formation by labeling *E. coli* cells with [^{14}C]uracil in a state of steady growth for various periods and by detecting labeled components resolved on a DEAE-cellulose column and by sucrose density gradient centrifugation. It was noted that radioactivity appeared first in 14S (eosome) particles, then in 30S and 43S (neosome) particles and finally in 50S and 30S subunits.

FURANO[17] and FURANO AND HOLLIS[18] have developed an effective method for the purification of biologically active ribosomes, employing a DEAE-cellulose column. Elution with a gradient of sodium chloride yielded ribosomes as a single sharp peak between 0.3–0.4 *M* sodium chloride. Ribosomes thus isolated, however, differed in their composition and physico-chemical properties (*viz.* response to pH, Mg^{2+}, etc.) from the ribosomes isolated by the conventional centrifugation procedure. DEAE-ribosomes (isolated on a DEAE-cellulose column) had 70% RNA and contained about 30% less protein than ribosomes obtained by repeated centrifugation, although sedimentation values and electrophoretic mobilities of ribosomes isolated by both procedures were by and large identical; both were able to bind phenylalanyl-tRNA in the presence of polyuridylic acid (poly U). Similar studies on the purification of ribosomes using a DEAE-cellulose column led STANLEY *et al.*[81] to the discovery of the initiator required for protein synthesis. It was observed that whereas synthetic messenger (poly U, poly A) could promote polypeptide synthesis in a cell-free system derived from *E. coli* equally well with crude or purified ribosomes, natural messenger (phage MS_2 RNA) could not do so with purified ribosomes. Phage MS_2 RNA stimulated polypeptide synthesis with crude ribosomes only. This led the authors to suspect that purification of ribosomes had resulted in the loss of a factor designated as "initiator", which was later on found to be formyl-methionyl-tRNA.

Low-molecular-weight rRNA isolated from *E. coli* cells grown in the presence of 5-fluorouracil (FU) consisted of a mixture of normal and FU-containing 5S RNA. Although Sephadex G-100 gel chromatographed this mixture as a single homogeneous peak, it was separated on a DEAE-cellulose column into two fractions by a concave

upward gradient of sodium chloride[82]. It is worth noting that rechromatography of these fractions did not reveal the presence of any contaminant, with more than 87% recovery. The replacement of uracil by FU was highly specific in rRNA without substituting other bases, unlike seen in case of tRNA[53].

COMB *et al.*[83–85] have achieved sharp separations of ^{32}P-labeled 5S RNA (ref. 83) and tRNA (ref. 84) following GOLDTHWAIT AND KERR's procedure[86] from *B. emersonii*. High salt concentration was used for the elution of the only random coil forms of RNA present at 80° (ref. 85). At room temperature, no separation was possible as the random coil formation takes place only at high temperature and it seems that random coils have less affinity for DEAE-cellulose, which facilitates easy elution. Recently, MONIER[87] has used DEAE-cellulose for large-scale purification of 5S RNA free from traces of proteins, polysaccharides, tRNA, and high-molecular-weight rRNA.

RNA isolated from glucose and Mg^{2+}-deprived (for 70 h, at 37°) culture of *E. coli* cells was subjected to DEAE-cellulose chromatography. Elution pattern revealed that only 5% of the normal content of cellular ribosomes existed, and in spite of such a heavy depletion of rRNA level, synthesis of mRNA remained unaffected[88].

Fractionation of rRNA and radioactive fragments of phage f_2 RNA was achieved on DEAE-cellulose, as well as by (5–20%) sucrose density gradient centrifugation[89]. Using an increasing concentration gradient of sodium chloride, the labeled fraction came out quite early, followed by 4S RNA and tRNA whereas the eluates with 1.0 *M* ammonium hydroxide (used after the gradient was over) corresponded to 16S and 23S rRNA. No other small fragments were detected and sharpness of the peaks seemed to give considerably homogeneous entities. It was probable that ammonium hydroxide had induced partial degradation of 16S and 23S rRNA. In further studies, RNA sequences transcribed *in vitro* on phage φ 80 DNA by *E. coli* RNA polymerase were also characterized[90]. A DEAE-cellulose column could sharply resolve the 6S RNA peak from the contaminating tRNA (obtained by sedimentation profiles) into two components[91]. It was noted that when RNA was synthesized in the presence of the ρ factor, normally present 26S and 37S peaks disappeared, while two major peaks emerged in the 6S and 13S regions. 4S RNA species was present at the front shoulder of the 6S peak.

(b) Mammals

Fractionation of membrane-bound RNA of the smooth endoplasmic reticulum revealed that this high-molecular-weight RNA was partially contaminated with tRNA[92]. It was felt that the particular fraction was not rRNA, but may be related to the stable cytoplasmic mRNA. mRNA, 18S and 28S rRNA from rat liver have been resolved by eluting with high concentration of triethylammonium acetate in aqueous isopropanol[80]. Triethylammonium acetate proved to be a highly specific eluting agent since its chloride form was ineffective as an eluting agent, most probably due to its limited solubility in aqueous isopropanol. It appeared that the molarity at which different species of RNA could be eluted was determined by the molecular weight and partially by the adenine content of RNA. However, either the latter suggestion contradicted the earlier findings on tRNA separation[29,30], or it could be that bases of separation of tRNA were different from those of rRNA. That elution

was a function of molecular weight was substantiated by the observation wherein *E. coli* rRNA was eluted earlier than rat liver rRNA.

Dog pancreatic and liver ribosomes have been fractionated[19] on DEAE-cellulose by a potassium chloride gradient into two components, 38S and 57S. The latter component was to some extent contaminated by 38S particles. Moderate to low ionic strength, pH, and zero Mg^{2+} concentration determined the dissociation and subsequent elution of ribosomes into subunits with 70–80% recovery. It is worth noting that ribosomes fractionated at 0° to 2° exhibited significant ability to incorporate phenylalanine while those fractionated between 4° to 6° exhibited very poor ability to incorporate phenylalanine.

One of the bottlenecks in the slow progress in the study of mammalian rRNA and ribosomes (in comparison to tRNAs) was largely due to difficulties in either fractionating rRNA or purifying active ribosomes. However, the above studies have proved that rRNA could be fractionated on a DEAE-cellulose column either by a temperature gradient[30] or by change in the chemical nature of the eluting agent[80] or by controlling the concentration of Mg^{2+}, which tremendously influenced the chromatographic characteristics of rRNA as well as ribosomes.

(C) Basis of separation

At this state, it may be worthwhile to compare the basis of fractionation on a DEAE-cellulose column as suggested by STAEHELIN *et al.*[93] and by TOMLINSON AND TENER.[94,95].

According to STAEHELIN *et al.*[93], oligonucleotides could be adsorbed and eluted at pH 8.6. At this pH, –OH groups of the bases showed different charges of dissociation and therefore provided an additional basis of chromatographic discrimination between individual oligonucleotides. A mixture of oligonucleotides differing in size and inseparable at pH 8.6 could be rendered separable at pH 7.0 when additional charges were made available due to dissociation of –OH groups. The elution pattern was dependent on the bases and their sequential arrangement in the oligonucleotide chain, in the following ways: (1) adenine containing oligonucleotides emerged before guanine containing ones of the same size; (2) with identical purine composition, cytosine-rich oligonucleotides emerged before uracil-rich ones; (3) with overall similar base composition, the isomer in which guanine was farther from the pyrimidine end, emerged earlier from its analogue.

According to TOMLINSON AND TENER[94,95], increase in chain-length was followed by firm retention and the vitiating influence of secondary interactions through H-bond formation (particularly in the fractionation of polynucleotides during desorption) could be overcome by the use of a gradient of urea in the eluting medium. Formaldehyde, glycol, and, in general, any chemical entity capable of disrupting H-bonds, exerted similar beneficiary effect. The separation of oligonucleotides, therefore, depended on the total number of ionizable phosphate groups present at the pH of adsorption and was almost independent of base composition. As the total number of phosphate groups was directly related with the chain-length (molecular size or weight), fractionation occurred to some extent according to the net charge and degree of polymerization.

The above bases of separation were proposed primarily with respect to the

behaviour of oligonucleotides or synthetic polynucleotides. With extensive use of a DEAE-cellulose column in RNA fractionation, ideas regarding the basis of separation require to be revised. It must be admitted that the overall base composition possible under artificial conditions (as in synthetic polynucleotides) may not exert similar effects as the natural polymers. It now looks certain that resolution on a DEAE-cellulose column is a function of molecular size (therefore net charge and degree of polymerization) and also of the relative distribution of G-C- or A-U-rich regions in the molecule (therefore conformation). Three-dimensional macromolecular conformation cannot be oversighted in view of the obligatory requirement of a random coil form (high temperature) necessary for elution.

(D) Advantages of DEAE-cellulose

A DEAE-cellulose column is a rapid and easy tool for broad fractionation of different species of RNAs and subfractionation of tRNA into isoacceptor species. It has also proved satisfactory in the fractionation and rechromatography of ribonuclease catalysed degradation products of natural and synthetic polymers. It is easy to operate, gives good flow-rates, quantitative recovery and reproducible elution profiles. Technically, it is not complicated as CCD; and unlike MAK columns, it does show ability in the fractionation of mammalian rRNA and ribosomes preserving their biological activity. If a broad outlook of existing methods of fractionation is taken, it indicates that DEAE-cellulose has appreciably higher capacity than ECTEOLA-cellulose, MAK columns, and hydroxyapatite gels and therefore can handle large quantity of material in a single chromatographic run.

Since the modification of the molecular structure of the cellulose normally results in increased swelling of the ion exchangers, they are now cross-linked prior to substitution which has restricted the swelling and increased the dimensional stability. Also the substitution of charged groups during modification is uniform. The newly introduced DEAE-cellulose offers a dense and uniform packing without affecting the flow-rate and is equally efficient in either column or batchwise operations. It is therefore claimed[96] that newly introduced modified celluloses have ability to furnish superior resolution of high-molecular-weight polyelectrolytes.

(E) Limitations of DEAE-cellulose

It has been noticed[44] that exchange capacity varied with variations in the batch of DEAE-cellulose. Although it is true that capacity of an exchanger depends upon the pH and ionic strength of the buffer used, variations in the capacity with variation in the batch of DEAE-cellulose are not negligible. This is most probably due to heterogeneous distribution of ion-exchange sites affording poor equilibration rate subsequently leading to low capacity. Variations in capacity lead to variations in reproducibility (if the column was operated approaching the capacity) posing difficulties in interpretation.

Several batches of DEAE-cellulose, other than the one supplied by California Corporation were found unsatisfactory[44]. It was further noted that only mesh size between 230–335 was satisfactory. Similar observations were also made by TAKEDA[14]. He noted that the elution pattern of yeast tRNA under identical experimental con-

ditions differed as a function of DEAE-cellulose, whether supplied by Whatman Paper Company or by Brown Company.

While comparing the degree of purification of tRNAs, it was found that resolution on DEAE-Sephadex A-25 was superior to DEAE-cellulose[12]; it has higher capacity than DEAE-cellulose and is better for making long and narrow columns[97]. However, it must be mentioned here that gel filtration properties of DEAE-Sephadex may be either an advantage[95] or a disadvantage[98] depending upon the mixture of components to be resolved. Although DEAE-cellulose is superior (to other less commonly used modified celluloses[3,8,99]), intrinsically it gives better separations only at the beginning of the chromatogram than during its latter period. This may possibly be due to its high affinity towards guanine-rich polynucleotide molecules.

In addition to the extensive procedure of regeneration[46], DEAE-cellulose requires degassing after equilibration, just before packing the column. This has to be very scrupulously avoided while dealing with the carbonate and bicarbonate ions in buffers. These ions are tenaciously held on the exchanger; their removal by degassing, after equilibration, upsets the equilibrium and the column requires reequilibration before operation. Incidentally, this difficulty is not realized in RNA fractionation as carbonate and bicarbonate buffers are not used.

Modification and cross-linking of the DEAE-cellulose (or celluloses in general) under less rigidly controlled manufacturing conditions, greatly affected the charge distribution which subsequently affected the ion-exchange separations. This was especially more critical when contact time was extremely short and rapidity of the procedure had to be observed to retain the biological expression of the molecule after separation; *viz.* the procedure developed by ZAMECNIK *et al.*[100] required rapid elution of tRNA. It was also found[86] that *E. coli* rRNA was degraded during elution.

One of the drawbacks of the microgranular series of ion exchangers, inclusive of DEAE-cellulose, is that the use of 0.5 *M* acid or alkali or oxidizing agent leads to irreversible changes in the matrix of cellulose making them less reliable for further use. Even DEAE-cellulose treated with 1.0 *M* sodium chloride gave artifacts during purification of RNA from wheat leaves[101].

3. SUMMARY

As RNAs are negatively charged polyelectrolytes and behave as anions in neutral and weakly acidic solutions, DEAE-cellulose as an anion exchanger has proved to be useful in resolving tRNAs from a wide variety of sources. In addition to its significant employment in purifying individual tRNA species from the mixture of tRNAs, it has also afforded definitive subfractionation of isoacceptor species of several tRNAs by using either temperature and/or salt gradient or by changing the pH. It has also proved successful in the fractionation of functionally intact rRNA and ribosomes, even of mammalian origin, a task which could not be satisfactorily accomplished with Kieselguhr columns. This fractionation could be achieved on a DEAE-cellulose column either by controlling the concentration of Mg^{2+} or by changing the chemical nature of the eluting agent. A DEAE-cellulose column has been advantageously employed for studying the chromatographic behaviour of either chemically modified or enzymatically cleaved tRNA species. Such studies

have been found of immense importance in our understanding with regard to the necessity of different "regions" of tRNA structure in its biological expression. In all these purifications and subfractionations on a DEAE-cellulose column, molecular size and relative macromolecular conformation play a significant role. With regard to its ease and rapidity of operation, chromatographic reproducibility and other operational advantages, it stands far superior to other modified celluloses described earlier.

REFERENCES

1 A. Bendich and H. S. Rosenkranz, *Arch. Biochem. Biophys.*, 94 (1961) 417.
2 R. Barber, *Biochim. Biophys. Acta*, 114 (1966) 422.
3 R. M. Kothari and M. W. Taylor, *J. Chromatogr.*, 73 (1972) 449.
4 H. A. Sober and E. A. Peterson, *Ion Exchangers in Organic and Biochemistry*, Interscience, New York, 1957, p. 318.
5 R. W. Holley, J. Apgar, B. P. Doctor, J. Farrow, M. A. Marini and S. H. Merrill, *J. Biol. Chem.*, 236 (1961) 200.
6 H. G. Khorana and J. P. Vizsolyi, *J. Amer. Chem. Soc.*, 83 (1961) 675.
7 J. D. Hawkins, *Biochem. J.*, 83 (1962) 33P.
8 R. W. Holley, *Biochem. Biophys. Res. Commun.*, 10 (1963) 186.
9 M. Staehelin, *Biochim. Biophys. Acta*, 49 (1961) 11, 20, 27.
10 C. B. Klee and M. Staehelin, *Biochim. Biophys. Acta*, 61 (1962) 668.
11 K. Nishiyama, T. Okamoto and I. Watanabe, *Biochim. Biophys. Acta*, 47 (1961) 193.
12 Y. Kawade, T. Okamoto and Y. Yamamoto, *Biochem. Biophys. Res. Commun.*, 10 (1963) 200.
13 R. M. Kothari, V. Shankar and M. W. Taylor, *J. Chromatogr.*, 70 (1972) 341.
14 I. Takeda, *Biochim. Biophys. Acta*, 169 (1968) 272.
15 R. Monier, M. L. Stephenson and P. C. Zamecnik, *Biochim. Biophys. Acta*, 43 (1960) 1.
16 H. Hayashi and F. Egami, *J. Biochem. (Tokyo)*, 53 (1963) 176.
17 A. V. Furano, *J. Biol. Chem.*, 241 (1966) 2237.
18 A. V. Furano and V. W. Hollis, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 26 (1967) 612.
19 S. R. Dickman and E. Bruenger, *Biochemistry*, 8 (1969) 3295.
20 J. D. Cherayil and R. M. Bock, *Biochemistry*, 5 (1965) 1174.
21 C. J. Smith and E. Herbert, *Science*, 150 (1965) 384.
22 W. M. Stanley, Jr. and R. M. Bock, *Biochemistry*, 4 (1965) 1302.
23 D. Söll, J. Cherayil, D. S. Jones, R. D. Faulkner, A. Hampel, R. M. Bock and H. G. Khorana, *Cold Spring Harbor Symp. Quant. Biol.*, 31 (1966) 51.
24 D. Söll, D. S. Jones, E. Ohtsuka, R. D. Faulkner, R. Lohrmann, H. Hayatsu, H. G. Khorana, J. D. Cherayil, A. Hampel and R. M. Bock, *J. Mol. Biol.*, 19 (1966) 556.
25 D. J. Armstrong, F. Skoog, L. H. Kirkegaard, A. E. Hampel, R. M. Bock, I. Gillam and G. M. Tener, *Proc. Nat. Acad. Sci. U.S.*, 63 (1969) 504.
26 P. L. Bergquist, D. J. W. Burns and C. A. Plinston, *Biochemistry*, 7 (1968) 1751.
27 A. Hampel, J. Cherayil and R. M. Bock, *Biochim. Biophys. Acta*, 228 (1971) 482.
28 U. Lagerkvist and L. Rymo, *J. Biol. Chem.*, 244 (1969) 2476.
29 B. C. Baguley, P. L. Bergquist and R. K. Ralph, *Biochim. Biophys. Acta*, 108 (1965) 139.
30 B. C. Baguley, P. L. Bergquist and R. K. Ralph, *Biochim. Biophys. Acta*, 95 (1965) 510.
31 P. L. Bergquist, B. C. Baguley, J. M. Robertson and R. K. Ralph, *Biochim. Biophys. Acta*, 108 (1965) 531.
32 A. D. Mirzabekov, A. I. Krutilina and A. A. Bayev, *Biochim. Biophys. Acta*, 129 (1966) 426.
33 P. L. Bergquist, B. C. Baguley and R. K. Ralph, *Methods Enzymol.*, 12 (1967) 660.
34 M. Miyazaki, M. Kawata and S. Takemura, *J. Biochem. (Tokyo)*, 61 (1966) 519, 526.
35 M. Miyazaki, M. Kawata, K. Nakazawa and S. Takemura, *J. Biochem. (Tokyo)*, 62 (1967) 161.
36 R. M. Bock and J. D. Cherayil, *Methods Enzymol.*, 12 (1967) 638.
37 A. Carrel, *Science*, 73 (1931) 297.
38 V. D. Axelrod, M. Ya. Feldman, I. I. Chuguev and A. A. Bayev, *Biochim. Biophys. Acta*, 186 (1969) 33.
39 M. S. May and R. W. Holley, *J. Mol. Biol.*, 52 (1970) 19.
40 A. D. Mirzabekov, D. Grünberger and A. A. Bayev, *Biochim. Biophys. Acta*, 166 (1968) 68.

41 N. IMURA, H. SCHWAM AND R. W. CHAMBERS, *Proc. Nat. Acad. Sci. U.S.*, 62 (1969) 1203.
42 R. THIEBE AND H. G. ZACHAU, *Biochem. Biophys. Res. Commun.*, 36 (1969) 1024.
43 R. K. JACOBSON AND S. NISHIMURA, *J. Chromatogr.*, 14 (1964) 46.
44 E. OTAKA, S. OSAWA, Y. OOTA, A. ISHIHAMA AND H. MITSUI, *Biochim. Biophys. Acta*, 55 (1962) 310.
45 R. B. ROBERTS, R. J. BRITTEN AND E. T. BOLTON, *Microsomal Particles and Protein Synthesis*, Pergamon Press, London, 1958, p. 84.
46 C. W. HANCHER, E. F. PHARES, G. D. NOVELLI AND A. D. KELMERS, *Biotechnol. Bioeng.*, 11 (1969) 1055.
47 O. CIFERRI, F. SALA AND G. POMA, *Biochim. Biophys. Acta*, 87 (1964) 508.
48 A. LANDY, J. ABELSON, H. M. GOODMAN AND J. D. SMITH, *J. Mol. Biol.*, 29 (1967) 457.
49 H. M. GOODMAN, J. N. ABELSON, A. LANDY, S. ZADRAZIL AND J. D. SMITH, *Eur. J. Biochem.*, 13 (1970) 461.
50 M. A. A. MIRZA AND M. CANNON, *Biochim. Biophys. Acta*, 149 (1967) 596.
51 M. CANNON AND E. G. RICHARDS, *Biochem. J.*, 103 (1967) 23C.
52 M. CANNON, *Biochim. Biophys. Acta*, 129 (1966) 221.
53 I. I. KAISER, *Biochemistry*, 8 (1969) 231.
54 J. L. HOFFMAN, K. P. MCCONNELL AND D. R. CARPENTER, *Biochim. Biophys. Acta*, 199 (1970) 531.
55 K. ODA, F. KIMURA, F. HARADA AND S. NISHIMURA, *Biochim. Biophys. Acta*, 179 (1969) 97.
56 T. SENO, M. KOBAYASHI AND S. NISHIMURA, *Biochim. Biophys. Acta*, 182 (1969) 280.
57 T. SENO, M. KOBAYASHI AND S. NISHIMURA, *Biochim. Biophys. Acta*, 190 (1969) 285.
58 T. SENO AND S. NISHIMURA, *Biochim. Biophys. Acta*, 228 (1971) 141.
59 J. E. M. MIDGLEY, *Biochim. Biophys. Acta*, 61 (1962) 513.
60 J. W. CARNEGIE, A. O'C. DEENEY, K. C. OLSON AND G. S. BEAUDREAU, *Biochim. Biophys. Acta*, 190 (1969) 274.
61 H. S. SHARPE, W. M. SHEPHERD AND J. HAY, *Cold Spring Harbor Symp. Quant. Biol.*, 31 (1966) 583.
62 P. P. HUNG, *Biochim. Biophys. Acta*, 186 (1969) 220.
63 J. W. SEDAT, R. B. KELLEY AND R. L. SINSHEIMER, *J. Mol. Biol.*, 26 (1967) 537.
64 P. P. HUNG AND L. R. OVERBY, *J. Biol. Chem.*, 243 (1968) 5525.
65 R. F. GESTELAND AND P. F. SPAHR, *Biochem. Biophys. Res. Commun.*, 41 (1970) 1267.
66 L. N. VANDERHOEF AND J. L. KEY, *Biochim. Biophys. Acta*, 240 (1971) 62.
67 M. B. ANDERSON AND J. H. CHERRY, *Proc. Nat. Acad. Sci. U.S.*, 62 (1969) 202.
68 N. SHIMIZU AND K. I. MIURA, *Biochim. Biophys. Acta*, 232 (1971) 271.
69 W. K. YANG AND G. D. NOVELLI, *Methods Enzymol.*, 20 (1971) 44.
70 S. PETROVIĆ AND B. BRKIĆ, *Biochim. Biophys. Acta*, 217 (1970) 95.
71 H. W. V. HEYDEN AND H. G. ZACHAU, *Biochim. Biophys. Acta*, 232 (1971) 651.
72 R. A. JACOBSON AND J. BONNER, *Arch. Biochem. Biophys.*, 146 (1971) 557.
73 W. D. HANKINS AND W. R. FARKAS, *Biochim. Biophys. Acta*, 213 (1970) 77.
74 R. C. SLABAUGH AND A. J. MORRIS, *J. Biol. Chem.*, 245 (1970) 6182.
75 R. C. SLABAUGH AND A. J. MORRIS, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 29 (1970) 468.
76 B. J. MCCARTHY AND R. J. BRITTEN, *Biophys. J.*, 2 (1962) 35.
77 R. J. BRITTEN AND B. J. MCCARTHY, *Biophys. J.*, 2 (1962) 49.
78 R. J. BRITTEN, B. J. MCCARTHY AND R. B. ROBERTS, *Biophys. J.*, 2 (1962) 57, 83.
79 J. E. M. MIDGLEY AND B. J. MCCARTHY, *Biochim. Biophys. Acta*, 61 (1962) 696.
80 J. H. PARISH, *Biochim. Biophys. Acta*, 169 (1968) 14.
81 W. M. STANLEY, JR., M. SALAS, A. J. WABHA AND S. OCHOA, *Proc. Nat. Acad. Sci. U.S.*, 56 (1966) 290.
82 I. I. KAISER, *Biochemistry*, 9 (1970) 569.
83 N. SARKAR AND D. G. COMB, *J. Mol. Biol.*, 39 (1969) 31.
84 D. G. COMB AND N. SARKAR, *J. Mol. Biol.*, 25 (1967) 317.
85 D. G. COMB AND Z. W. TOVA, *J. Mol. Biol.*, 23 (1967) 441.
86 D. A. GOLDTHWAIT AND D. S. KERR, *Biochim. Biophys. Acta*, 61 (1962) 930.
87 R. MONIER, *Procedures Nucleic Acid Res.*, 2 (1971) 618.
88 G. H. PIGOTT AND J. E. M. MIDGLEY, *Biochem. J.*, 108 (1968) 34P.
89 M. TAKANAMI, Y. YAN AND T. H. JUKES, *J. Mol. Biol.*, 12 (1965) 761.
90 M. TAKANAMI, T. OKAMOTO AND M. SUGIURA, *Cold Spring Harbor Symp. Quant. Biol.*, 35 (1970) 179.
91 T. OKAMOTO, M. SUGIURA AND M. TAKANAMI, *Biochemistry*, 9 (1970) 3533.
92 V. SHAPOT AND H. C. PITOT, *Biochim. Biophys. Acta*, 119 (1967) 37.
93 M. STAEHELIN, E. A. PETERSON AND H. A. SOBER, *Arch. Biochem. Biophys.*, 85 (1959) 289.
94 R. V. TOMLINSON AND G. M. TENER, *J. Amer. Chem. Soc.*, 84 (1962) 2644.
95 R. V. TOMLINSON AND G. M. TENER, *Biochemistry*, 2 (1963) 697, 703.

96 *Data Manual and Catalog 200, Whatman Ion-Exchange Celluloses*, 1966.
97 R. W. HOLLEY, J. APGAR, G. A. EVERETT, J. T. MADISON, S. H. MERRILL AND A. ZAMIR, *Cold Spring Harbor Symp. Quant. Biol.*, 28 (1963) 117.
98 M. STAEHELIN, *Progr. Nucl. Acid Res. Mol. Biol.*, 2 (1963) 169.
99 R. M. KOTHARI, *Chromatogr. Rev.*, 12 (1970) 127.
100 P. C. ZAMECNIK, M. L. STEPHENSON AND J. F. SCOTT, *Proc. Nat. Acad. Sci. U.S.*, 46 (1960) 811.
101 D. WANG AND D. MANCINI, *Biochim. Biophys. Acta*, 129 (1966) 231.