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RNA FRACTIONATION ON KIESELGUHR COLUMNS

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I. INTRODUCTION

A number of chromatographic methods are currently available for the fractionation of RNAs. These methods include calcium phosphate and hydroxyapatite gels; substituted celluloses such as DEAE-, ECTEOLA-, aminoethyl-, phospho-, benzoylated DEAE-, benzoylated DEAE-silicic acid-cellulose, etc.; starch, agar, and polyacrylamide gels; DEAE-Sephadex and its modified forms; reversed-phase columns; and Kieselguhr columns with their various modifications. A homogeneous entity of RNA with respect to a single biological activity can be obtained by sequential chromatography on a variety of these columns.

One of the first column chromatographic methods that furnished a reproducible fractionation of RNA used methylated bovine serum albumin-Kieselguhr (MAK); it was first introduced for DNA fractionation[1] and subsequently adapted[2] for RNA fractionation. It appears to resolve different species of RNAs on the basis of their configuration and base composition. MAK column chromatography has proved to be particularly useful in the detection of different isoaccepting species of tRNA[3–5], of alterations in tRNAs in normal and neoplastic tissues as well as new species of tRNA synthesized during virus infection[6,7].

With the increasing use of the MAK column as a tool in the analysis of a number of biochemical problems, it was thought worthwhile to give a consolidated account

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of the use of this column and the biological significance of the separations achieved from a wide variety of sources. We hope that through personal experience with this column, it may be possible to give a clearcut idea of its operational advantages, limitations, and scope.

The adsorption of RNAs on proteins (such as histone, protamine, methylated serum albumin) and polyamino acids (poly-L-lysine, poly-L-arginine and poly-L-ornithine) immobilized on Kieselguhr (also technically known as Celite and Hyflo-Super-Cel), and silicic acid has been used by many workers for the separation of different types of RNAs and, also, for subfractionation of different species of RNA from a mixture of similarly metabolically active entities. Of these adsorbents a Kieselguhr (MAK) column, impregnated with bovine serum albumin which was made less acidic by methylation, has been used most extensively.

II. HISTONE–KIESELGUHR COLUMNS

BROWN AND WATSON[8] adsorbed nucleic acids on Kieselguhr impregnated with histone and noted that, whereas native DNA was adsorbed onto the column, denatured DNA and RNA were not. Stepwise elution furnished discrete subfractionation of calf thymus DNA, and the position of elution seemed to correlate with the A–T content of the DNA. It is assumed that these fractions reflected genetic heterogeneity. The dissociation of the salt linkage (between the histone of the column and the loaded DNA) with increasing concentration of sodium chloride appeared to be the basis of DNA fractionation on this type of column. These results prompted the search for alternative column chromatographic methods for the fractionation of RNAs.

III. METHYLATED BOVINE SERUM ALBUMIN–KIESELGUHR COLUMNS

A column of Celite coated with methylated bovine serum albumin was introduced as an anion exchanger by LERMAN[1] for the fractionation of the "transforming principle" of *Pneumococcal* DNA into reproducible fractions exhibiting significant differences in their ability to transform recipient cells to streptomycin resistance. The form in which the methylated serum albumin–Kieselguhr (MAK) column is now used most widely is that of MANDELL AND HERSHEY[2], who developed it to permit the separation of DNA and RNA on the basis of their molecular size. They used this column for the resolution and identification of several natural and artificial mixtures of nucleic acids; in particular, the bacteriophage DNAs. The column consists of three layers: (a) the uppermost layer is composed entirely of Kieselguhr and serves as a mechanical barrier to the working portions of the column; (b) the middle layer is composed of methylated serum albumin with Kieselguhr; it functions as an initial adsorbent, effects the separation, sharpens the bands, and allows the resolved material to pass quickly on to the next layer because of the low content of net positive charges on methylated serum albumin; and (c) the lower layer is composed of Kieselguhr with a lower percentage (by comparison with the middle layer) of methylated serum albumin, and the resolved species of nucleic acids are eluted from this layer.

With the MAK column prepared as above, MANDELL AND HERSHEY[2] separated

mixtures of nucleic acids from *E. coli* and phages T_2 and T_4 with a gradient of increasing concentrations of electrolytes. They observed that the order of elution was tRNA, bacterial DNA, phage DNA, and microsomal RNA. This is a general order of elution and has now been confirmed by many workers in many systems with rRNA being subfractionated into 16S and 23S RNA[9-11]. MANDELL AND HERSHEY[8] did note a variability in the elution profiles depending on the amount of nucleic acid adsorbed onto the column.

A. tRNA fractionation

(1) Bacteria

SUEOKA AND YAMANE[12] have employed a MAK column for fractionating acylated tRNAs and have shown that the column is capable of resolving aminoacyl-tRNAs by furnishing characteristic profiles for each of the 16 aminoacyl-tRNAs studied. The chromatographic elution pattern revealed isoaccepting species of several amino acid specific tRNAs, and this finding was subsequently confirmed[13]. This was perhaps the first independent study that confirmed the idea of a degenerate genetic code. They further demonstrated that the relative amounts of each tRNA species appeared to be constant under different growth conditions. Although this observation may be true for *E. coli*, it does not extend to other systems. In fact, studies on tRNA at different stages of *B. subtilis* growth have shown that the profiles are a function of growth conditions[14].

That the MAK column resolves isoaccepting species of tRNA was substantiated by THIEBE AND ZACHAU[15] who purified two species of phenylalanyl- and three species of valyl-tRNAs obtained by counter-current distribution (CCD). It was noted that these species could not be further resolved on a MAK column, possibly implying that they are homogeneous. These species of tRNA were also detected in unfractionated tRNA on the MAK column without undergoing CCD, indicating their presence as a reality and not an artifact of the fractionation procedure. Although the bases of MAK column chromatography and of CCD are different, identical data were obtained by both methods, indicating the soundness of the MAK column procedure.

MAK column chromatography has been effectively used in the study of fully methylated and methyl-deficient *E. coli* tRNAs[16,17]. Different separation profiles were obtained for normal species and their unacylated counterparts, as well as for fully methylated and methyl-deficient species; that is, four peaks of phenylalanyl-tRNA were resolved using acylated-methyl-deficient tRNA, in contrast to two peaks in fully methylated *E. coli*. The coding properties of these fractions responded differently to different nucleotide triplets, indicating that fractionation on the MAK column also has some biological basis. Differences in the elution patterns of normal and methyl-deficient *E. coli* leucyl-tRNA have also been noted [17].

E. coli leucyl-tRNA species charged by multiple forms of leucyl-tRNA synthetase furnished surprisingly different profiles[18]. The latter enzyme was obtained in three fractions from *E. coli* extracts using hydroxyapatite and DEAE-cellulose chromatography. It appeared that there are different enzyme forms (possibly a mixture of monomers) capable of charging isoaccepting species to different extents under identical sets of experimental conditions.

The MAK column has also been used for the chromatographic separation of

in vivo and *in vitro* acylated *E. coli* tRNAs specific for leucine, isoleucine, and phenylalanine. Although leucyl-tRNA gave identical elution profiles irrespective of *in vivo* or *in vitro* aminoacylation, the relative amounts of peak I and peak II of phenylalanyl- and of isoleucyl-tRNA varied under different physiological conditions[19]. Such differences in behavior may have some correlation with their regulatory role in protein biosynthesis.

That the MAK column resolves different species of tRNAs on the basis of secondary structure was also shown by LOWRIE AND BERGQUIST[20] who fractionated 5-fluorouracil (5-FU) substituted tRNA (FU-tRNA) of *E. coli* from unsubstituted tRNA. This resulted in a change in the secondary structure of FU-tRNA (up to 100% replacement of uracil by 5-FU), which was reflected in the column behavior as well as in the different thermal denaturation profiles. It was interesting to note that FU-tRNA was capable of accepting all amino acids tested and could transfer phenylalanine into polyphenylalanine and lysine into polylysine in a polynucleotide stimulated system.

Analysis of aminoacyl-tRNAs of spore, sporulating and vegetative cells of *B. subtilis* on the MAK column have shown significant differences in the elution profiles of a number of aminoacyl-tRNAs[21-26]. These included variations in the ratio of the two valyl-tRNAs, increased lysine acceptor activity, an extra peak of seryl-tRNA, and alterations in the pattern of tyrosyl-tRNA. However, the relationship of these changes to different growth stages studied is in doubt, since many of them also occur in response to changes in the composition of growth media[23,24,27]. At one stage, DOI *et al.*[24] felt that the lack of sensitivity of the MAK column procedure may have precluded any observation of minor changes with other aminoacyl-tRNAs. The validity of this statement requires additional evidence; otherwise, it seems that differences were expected, but were not exhibited on the chromatogram.

The chromatographic profiles of *B. subtilis* lysyl-tRNA in the oxidized and non-oxidized (natural) state were studied, and the differences in their profiles were noted[28-30]. Oxidation of tRNA prior to aminoacylation resulted in either no change or partial inactivation or complete loss of acceptor activity, possibly depending on the degree of oxidation. Binding efficiency of lysyl-tRNA to poly-A-ribosome complex was also reversibly altered by iodine oxidation and thiosulfate reduction. It is suggested[28] that the possible presence of thiobases in tRNA is at the root of this altered behavior and may have a role in controlling the rate of protein synthesis at the translational level. DOI AND GOEHLER[28] claim that their results have for the first time illustrated the requirement of a finite conformation of tRNA for efficient binding to poly-A-ribosome complex, the changes in conformation being derived from optical rotatory dispersion (ORD) studies. tRNAs from *B. subtilis* cells in log and stationary phases and from spores were also compared for such type of studies. These observations indicated conclusively that oxidation of tRNA resulted in the loss of original conformation and that the altered conformation appeared on the chromatogram as an independent entity.

The MAK column has been used[31], also, for the separation of methionyl-tRNA from N-formyl-methionyl-tRNA in *B. subtilis*, where no differences could be detected between N-formyl-methionyl-tRNA from vegetating cells and spores.

(2) Phage

The MAK column has been used in phage infected cells for two purposes primarily: (a) to isolate and characterize the phage specific message (refs. 32–34, see under mRNA), and (b) to screen for possible changes in tRNA profiles in phage infected cells[7,35–37]. For example, the chromatographic behavior of aminoacyl-tRNAs of *E. coli* after infection with bacteriophage T_2 revealed that out of the 17 aminoacyl-tRNAs examined, the leucyl-tRNA profile differed before and after phage infection; and the alteration appeared to be the result of an early event during phage infection. Further studies[36] revealed that phage T_4 and T_6 infection also brought about similar changes in the leucyl-tRNA pattern, whereas no such modification was observed after T_1, T_3, T_5, or T_7 infection or during induction of λ prophage. In further studies, MAK columns were used extensively to examine the elution pattern of tRNA from *E. coli* B before and after different intervals of phage T_2 infection, of leucyl-tRNA prepared by two different methods, and of leucyl-tRNA at different growth stages of *E. coli*[7]. Similar studies were also carried out by NIRENBERG *et al.*[37]. In some cases, codon responses of the fractionated samples were studied for their binding ability to ribosome in response to a polynucleotide template and were found to be different upon phage infection. This indicated that fractionation on the MAK column was biologically meaningful.

Additional evidence has been afforded that the MAK column resolves isoaccepting species and can correlate secondary structure to biological significance[38]. The triplet binding response to ribosome[37,38] of the MAK column fractionated-leucyl-tRNA demonstrated the presence of 4 or 5 species of leucyl-tRNA in uninfected *E. coli*. After phage T_2 infection, however, quantitative variations were observed in the elution profiles, although the basic pattern of elution was more or less the same. It is believed that the differences in the percentage distribution in different fractions of leucyl-tRNA before and after phage infection may have some correlation with the need for phage specific protein synthesis and are transcribed from phage DNA. The codon assignments for 4 out of 5 leucyl-tRNA fractions obtained on both a reversed phase column (RPC) and a MAK column have been established[5,30].

(3) Yeast

MAK column chromatography has been utilized[40] for the partial fractionation of yeast seryl-tRNA into two, and possibly three fractions. These three fractions and those obtained by the CCD technique appear to be identical. Although glycyl-tRNAs from brewer's yeast have been fractionated on DEAE-Sephadex, these species were not separable on the MAK column[41].

(4) Animals

(a) Sea urchin. MAK column chromatography has been found to be a useful tool in the analysis of the sequence of molecular events that take place during the early stages of sea urchin development[42–46]. In the unfertilized sea urchin eggs, RNA and protein synthesis are halted. Upon fertilization, mRNA appears to be activated; and the pre-existing ribosomes and tRNA are utilized for protein synthesis. The nucleic acids of sea urchin at different developmental stages are broadly distributed as usual into three peaks representing tRNA, rRNA and DNA[42]. It was claimed that

methylation of nucleic acids is not initiated until postgastrula formation. It has been further shown[43] that no new tRNA is synthesized prior to gastrulation suggesting that any new species found[44,45] must be the result of enzymatic modification rather than *de novo* synthesis. However, mRNA, unlike other species of RNA, is synthesized during the blastula stage; and a new species of lysyl-tRNA appears during the first 1.5 h after fertilization[44].

A more detailed examination of the tRNAs of the blastula stage of sea urchin embryos[45] showed that new or altered species of lysyl-, leucyl-, and seryl-tRNAs were detectable when compared to the profiles obtained with tRNA from unfertilized eggs. Other tRNAs examined showed no differences, suggesting that these modifications were specific. Some differences, of course, may exist in the tRNAs not studied. The major peaks on the chromatogram could possibly be further subfractionated by more effective gradients and may furnish multiple species of tRNA. Recently evidence has been cited[40] that such alterations in tRNAs occur concomitantly with a burst of tRNA methylase activity in the sea urchin eggs.

(b) Insects. The MAK column has been used in the purification and fractionation of tRNA from two kinds of silk worms *Bombyx mori* and *Philosamia cynthia ricini*[47,48]. The elution pattern obtained[47] for aspartyl- and seryl-tRNA of silk gland was quite different from that of *E. coli*, while glycyl-, alanyl-, and tyrosyl-tRNAs corresponded in elution characteristics with the corresponding fractions of *E. coli* which served as a reference sample for comparison. It was further noted[48] that the silk gland contained large amounts of glycyl-tRNA. This, of course, correlates with the large content of glycine in silk fibroin.

(c) Amphibians. To get more information on the regulation of hemoglobin synthesis during metamorphosis, tRNAs from blood cells of larvae and adult *Rana catesbeiana* were fractionated on a MAK column[49]. Striking differences between the larval and adult organisms were noted with respect to the profiles of arginyl- and methionyl-tRNAs. It is interesting to note that methionyl-tRNA, present in adults, is practically missing in the larval stage. Similar studies on the analysis of RNA synthesized during the different stages of amphibian embryogenesis are also reported by BROWN AND LITTNA[50,51]. These studies revealed the appearance of certain species of RNA at a specific stage of development with comparative repression at another stage, indicating a possible role in initiation of new classes of protein synthesis only at a certain stage of metamorphosis.

(d) Mammals. ELLEM AND SHERIDAN[52] found that drastic conditions of elution were necessary to recover total L-cells RNA. As much as 30% of the total RNA could not be eluted, and the use of high temperature for the elution resulted in changes in the properties of the tRNA. Similar results were also obtained in the case of HeLa cell (human cancer line) RNA fractionation[53].

The tRNAs of a large number of different tissues or organs from a variety of animal species were screened by comparing the chromatographic profiles of ^{14}C- and ^{3}H-labeled aminoacyl-tRNAs on MAK columns[3]. In general, no differences were detected among different tissues from the same animal species, except for a peak of seryl-tRNA present in the kidney but absent in the liver. Similar studies were also done with a number of cell lines, including HeLa, MDBK (bovine kidney), adenovirus transformed hamster cells, and chick fibroblasts. In general, no differences were detected; however, when tRNA isolated from an Ehrlich ascites tumor (EAT) cell

was compared to that of mouse liver tRNA, differences in profiles were found for glycyl-, phenylalanyl-, seryl-, and tyrosyl-tRNAs. For example, the tyrosyl-tRNA of a large number of cell lines fell into three possible classes: containing peak I alone, peak II alone, or both peaks I and II. A number of tumor cells (*e.g.*, HeLa and viral transformed cells) contained both species.

It would be interesting at this stage to hypothesize that the presence of both peaks in tumor cells, but only one or the other in normal cell lines might indicate a change in tRNA profiles with differentiation. HOLLAND *et al.*[54] tested such a hypothesis by following the MAK column chromatographic profiles of tyrosyl-tRNAs during the selection of fibroblastic cells from mouse and chick embryos. Whereas two species of tyrosyl-tRNA were present in the embryos, the fibroblastic type (type I) became predominant with continuous culture and selection. The same fibroblastic type of tyrosyl-tRNA appeared to be present in both avian and mammalian sources. This type of difference, whether it is due to enzymatic modification or differential gene transcription[11], obviously was a result of differentiation.

In further studies using MAK column chromatography, detectable differences among tRNAs from mammalian, avian, and bacterial systems were reported[55]. In many cases these differences were accompanied by a loss of acceptor activity between the heterologous tRNAs and acylating enzymes. Further studies demonstrated[56] that phenylalanyl-tRNA from EAT cells, which differs from liver phenylalanyl-tRNA in its elution profile on a MAK column, does not have an altered ability to respond to poly-U in an *in vitro* protein synthesizing system from *E. coli*.

Aminoacyl-tRNAs from rat liver and from different plasmocytomas were compared[57] by MAK column chromatography. tRNA specific for leucine and threonine furnished differences in profiles, certain peaks being present in normal tissue which were missing from neoplastic tissue. The authors hold the opinion that the differences observed might be related to the malignant nature of the systems studied.

Mitochondrial and cytoplasmic aminoacyl-tRNAs of rat liver have also been compared[58,59] using MAK column chromatography. Specific mitochondrial species of leucyl-, tyrosyl-, aspartyl-, valyl-, and seryl-tRNAs were detectable. It is not clear whether mitochondrial tRNAs are synthesized exclusively in the mitochondria. Sufficient care has been exercised by BUCK AND NASS[59] to ensure that the differences observed were not artifacts of isolation or acylation. It is, however, difficult to understand their observation that denatured tRNAs furnished profiles similar to native tRNAs since the MAK column resolves on the basis of secondary structure[60,61]. Differences in chromatographic profiles of leucyl-tRNA from mitochondrial and extramitochondrial (cytoplasmic) fractions were also noted in *Tetrahymena pyriformis*[62].

(5) Plants

MAK columns have been extensively used to study the changes in the pattern of RNA synthesis in peanut cotyledons during germination[63] and in the development of wheat embryo[61,64]. RNA was eluted into 6 fractions: tRNA I, tRNA II, DNA–RNA, light rRNA, heavy rRNA, and mRNA; and it was observed that the chromatographic profiles of 2-, 7-, and 14-day-old peanut cotyledons showed more mRNA synthesis as the plant seedling grows older up to a particular period. These changes in the pattern of nucleic acid synthesis are believed to have some correlation

with the appearance of particular enzymes in higher concentrations at a definite period of growth.

The elution patterns of wheat embryo tRNAs specific for phenylalanine, glycine, threonine, leucine, serine, and proline were similar; but for valine, histidine, glutamic acid, arginine, and lysine were different with the development of the plant seedling[61,64]. Possibly, these changes are due to profoundly altered physiological processes in an organism during development. Furthermore, in view of the role of tRNAs in translation processes, changes in percentage distribution of tRNA may be necessary in the events of differentiation.

B. Viral RNA fractionation

(1) Herpes virus

Total tRNAs isolated from Herpes simplex virus infected and uninfected baby hamster kidney (BHK) cells have been chromatographed on the MAK column[65]. The differences in the elution pattern were significant in some cases, but marginal in others. The appearance of an extra fraction of arginyl-tRNA after infection, under identical experimental conditions was, reported[6]. A T_1-RNase digest of arginyl-tRNA showed two peaks in the infected preparation which were missing from uninfected preparations. DNA-s-RNA hybridization studies provided evidence for the virus specific RNA molecules which had many of the attributes of tRNA. However, MORRIS *et al.*[66] were unable to confirm the appearance of a new Herpes specific arginyl-tRNA by reversed-phase chromatography in infected Hep-2 cells.

(2) Foot-and-mouth disease virus

VANDE WOUDE *et al.*[67] employed a MAK column for the enrichment of BHK tRNA activity before and after foot-and-mouth disease virus (FMDV) infection. The tRNAs from FMDV-infected cells showed uniformly low levels of RNA methylation throughout the elution pattern implying that infection inhibits methylation in all tRNA species.

(3) Oncornavirus

A low-molecular-weight RNA fraction isolated from the oncogenic virus, BAI strain A (avian myeloblastosis) by MAK column chromatography accepted amino acids and transferred them to the site of polypeptide elongation under the conditions established *in vitro* for cellular tRNA[58,60]. The behavior of this viral RNA fraction on the MAK and DEAE-cellulose columns[60] was very similar to that of tRNAs of cellular origin. Total viral RNA was separable into two fractions; 20% had low-molecular-weight and nearly half of this had tRNA activity. The distribution of tRNAs from the virus differed from that found in the infected normal chick liver cells, suggesting that functional tRNA is a part of the viral particle. In further studies, the elution profiles of the virus and of viral transformed cell tRNAs (leukemic myeloblasts) were compared by using a double-labeling technique[70]. Differences were observed for the isoaccepting species of lysyl-tRNAs.

(4) Picornavirus

The MAK column was tested for its efficacy in the fractionation of a new

species of RNA after viral infection[71]. RNA from Mengo encephalitis virus infected EAT cells exhibited clearcut differences in its chromatographic behavior as compared with that from uninfected cells. The column found further applicability in the separation of tRNA and mRNA from viral infected and uninfected bacterial sources[72,73] and for the resolution of a double-stranded replicative form of phage M12 RNA from other nucleic acids[74].

The MAK column has proven to be a rapid and reproducible method of fractionating and enriching poliovirus RNA from the host cell (either HeLa or human amnion cells) RNA[75-82] and, also, for the purification of a replicative intermediate[82-84]. It was noted that single-stranded viral RNA (ssRNA) is strongly retained, eluting after the host cell rRNA; whereas the double-stranded RNA (dsRNA) eluted much earlier[80,82]. Fungal phage dsRNA, also, appeared at the same locus on the chromatogram as that of poliovirus dsRNA[84]. The profiles also revealed that synthesis of dsRNA takes place before viral ssRNAs are formed[82]. However, recovery was only 80% (ref. 83) and was strongly dependent on the flow-rate of the salt-gradient and temperature of the column[78]. It is worth noting that viral RNA retained its infectivity after passage through the column[75,76] in contrast to the instability of the non-infective RNA fraction[76]. The instability remained even after column chromatography, making it difficult to estimate the sedimentation coefficient. This behavior led the authors to propose that the particular fraction is probably mRNA. Since the ssRNA and dsRNA have the same contour length, the molecular size and secondary structure appeared to be the basis of fractionation, as initially advocated by MANDELL AND HERSHEY[2]. dsRNA enriched by MAK chromatography was also homogeneous with respect to sedimentation analysis, proving the high resolving ability of the MAK column and again confirming the prediction[60].

(5) Phage

The MAK column was used[85] to examine the chromatographic profiles of RNAs isolated from coliphages, MS_2 and $Q\beta$, which differ from each other in surface charges and serological properties. The double-labeling technique revealed that MS_2 RNA-[^{32}P] always eluted between 16S and 23S *E. coli* rRNA, while $Q\beta$ RNA-[^{3}H] preceded the 16S component. The separated fractions differed significantly in their base composition.

(6) Plant virus

MATUS *et al.*[86] employed a MAK column to separate RNA from Chinese cabbage and tobacco leaves before and after turnip yellow mosaic virus (TYMV) infection. Profound differences in the profiles between the infective and cellular RNA were found; in addition, the profiles of the RNA after 10-days' infection differed from those after 30-days' infection. The observed differences were confirmed by running sucrose density gradient centrifugation simultaneously. The biological significance of the observed differences is not yet clear.

C. rRNA fractionation

(1) Bacteria

SUEOKA AND CHENG[60] used the MAK column for the partial resolution of rRNA

and tRNA from *E. coli* and found a usual and reproducible elution pattern, the result of an interplay of molecular size, hydrogen bonding (secondary structure) and base composition. In their opinion, since the MAK column mainly operates on differences in molecular size, it could possibly replace density gradient centrifugation. However, this has not been borne out.

MONIER *et al.*[87] used the MAK column for the chromatographic separation of *E. coli* 5S-precursor RNAs from 5S RNA after short periods of labeling. The precursor 5S RNA appeared to bind more strongly to the column than the mature 5S RNA, thus enabling a clearcut resolution. The column could also resolve mature 5S RNA from the 43S precursor particles[87], and also mature (functional in protein biosynthesis) ribosomes and immature (that which did not contain a full complement of protein and is incapable of protein synthesis) ribosomes of *E. coli* auxotrophs[88]. Similar studies are also reported by LAMBORG[89]. Ionic strength of the eluting agent and temperature were found to be the key factors affecting resolution. These studies thus support the earlier data[90,91] that high concentration of salt and high temperature are required for the resolution of rRNAs, particularly those of mammalian origin. The changed pattern of elution profiles due to changed structure of rRNA subsequent to reconstitution has also been demonstrated[92]. Heat denatured 16S rRNA isolated from *E. coli* combined with the 30S ribosomal protein fraction, giving a partially active 30S ribosome. The resulting change in the structure, reflected in the changed elution profiles, substantiates earlier results[90,91] that the MAK column resolves on the basis of secondary structure.

The MAK column has been employed routinely to examine the effect of different antibiotics on RNA synthesis in many systems; *viz.*, the effect of pluramycin[93], rifampicin[94], actinomycin D[95,96], and chloramphenicol[97]. Generally, all species of RNA are inhibited to an almost equal extent[93]. rRNA from chloramphenicol-treated *E. coli* had less affinity for the MAK column in comparison to its untreated counterpart, and this difference in behavior is reflected in early elution[97]. Both the 16S and 23S components of chloramphenicol-treated *E. coli* rRNA sedimented slightly faster through sucrose gradients. The difference noted may be due to variations in secondary structure, and it is suggested[97] that chloramphenicol rRNA may have a greater degree of helical structure than untreated (normal) rRNA.

It was noted that the overall suppression of nucleic acid synthesis by the plant hormone dormin could be reversed by cytokinin administration as judged from MAK column elution profiles[98].

rRNAs from different species of *Bacillus* have been resolved[99] according to the procedure laid down by YANKOFSKY AND SPIEGELMAN[100] who fractionated 4S, 16S and 23S components of rRNA from *B. megaterium* on MAK columns. 16S and 23S components of rRNA obtained by repeated MAK column chromatography[99] from different *Bacillus* species were utilized for the heterologous hybridization experiments and to examine base complementarity, if any, between DNA and RNA. Similar studies were also carried out by MARGULIES *et al.*[101].

Total RNA and 5-fluorouracil (FU) containing RNA from *B. subtilis* were neatly resolved, furnishing distinct profiles[102]. FU-ribosomes, thus isolated, were subjected to sucrose density gradient centrifugation and thermal denaturation studies. Unlike the results observed in tRNA, no change was revealed in size, shape, and conformation of the two rRNAs, suggesting FU substitution has little or no

effect although FU appeared to replace uracil in RNA without selectivity for one type of RNA over another.

(2) Mammals

MAK column chromatography has been used[103] for the resolution of rat kidney tRNA where it was noted that RNA is distributed into three peaks corresponding to tRNA, 20S-rRNA, and rapidly labeled (after ^{32}P-administration) RNA. MAHLER *et al.*[104] fractionated RNA from the cerebral cortex of mature male and female rats on a MAK column as well as by sucrose density gradient centrifugation. All of the three fractions, 4S, 17S, and 28S, obtained by either technique, were comparable and were rich in rRNA. The base composition in all three fractions were markedly complementary, the differences being small, but significant. Rapidly labeled RNA from rat liver nuclei were resolved[105] into four fractions: tRNA, rRNA, and two mRNAs. One of the fractions was similar to rRNA, with a high G–C composition, while two other fractions of high molecular weight appeared to be like DNA.

Nucleic acids isolated from mouse liver and Ehrlich ascites tumor (EAT) cells, treated with various concentrations (0.004 to 1.6%) of formaldehyde for 1 min at 22°, were separable[106]. Decreased recovery from the column was found to be dependent on the formaldehyde concentration and also on the structure of treated nucleic acid as judged from spectrophotometric measurements. rRNA was most sensitive to formaldehyde, while tRNA was least sensitive (DNA was intermediate). Formaldehyde formed a stable complex with RNA, and the resulting complex had more affinity for the MAK column. This resulted in delayed elution and alteration in the elution profiles.

LINGREL[11] has characterized different types of RNA synthesized in rabbit bone marrow preparations on a MAK column. Total nucleic acid preparations from KB cells and from rat liver gave the usual elution profiles[10]. However, a new fraction of RNA was detected in both the preparations, showing a number of properties identical to 5S rRNA of bacterial systems. Further studies on the analysis of the 5S RNA fraction obtained by MAK column chromatography[107] suggested that it may not be either a precursor of tRNA or a degradation product of rRNA. Furthermore, mathematical analysis and chemical evidence supported this conclusion; and GALIBERT *et al.*[107] feel that 5S RNA may be considered a new species of rRNA.

MAK column chromatography has been employed by YOSHIKAWA *et al.*[108] to fractionate RNA isolated from cultured FL cells (an established line from human amnion). They observed the usual three peaks, corresponding to tRNA, rRNA, and mRNA. It was noted[98] that resolution could be improved by operating the column at 30–35°. Actinomycin D chase experiments have furnished additional information about the metabolic state of two fractions: 40 and 50S. The assumption[98] that one of the rapidly labeled fractions having the sedimentation coefficient of 50S is not an aggregate of smaller molecules, but represents a single mRNA molecule corresponding to an entire operon comprised of many cistrons, seems to be far-fetched. FUKADA *et al.*[98] seem to contradict their statements as regards size and stability of mRNA.

The use of the MAK column in the chromatographic separation of HeLa cell rRNA, its precursor (QRNA), and a rapidly labeled high-molecular-weight DNA-like RNA (Q_2RNA) afforded very poor resolution[109]. The rate of biosynthesis of different species of nucleic acids in HeLa cells (S_3 strain) having different states of

activity were compared by using a double-labeling technique[110]. The profiles differed significantly for different states of the HeLa cell.

In view of the general limitations of the non-quantitative elution[52,83] and poor resolution of the mammalian rRNAs[83,100,109-111], ELLEM AND RHODE[91] introduced a new concentration gradient of guanidine thiocyanate as a substitute for the sodium chloride gradient in order to achieve a better than 90% elution of the tenaciously bound DNA-like RNA (D-RNA) and to give sharply defined peaks. This procedure furnished distinct profiles of D-RNA from different cell types.

(3) Plants

Wheat embryo rRNA has been analyzed by MAK column chromatography[91]; three peaks corresponding to 18S, an intermediate, and 28S fractions were observed, as against 18S and 29S fractions obtained by linear sucrose density gradient centrifugation. The intermediate peak, after characterization, was found to be 28S RNA in a different conformation. However, it could be converted into a regular 28S peak, with the degree of conversion being influenced by Mg^{2+} concentration. Protein contamination up to 2%, concentration of the adsorbate, and variation in temperature between 4–37° had no effect on the elution profiles. A quantitatively altered elution profile was obtained at a different pH of 5.2–6.6, possibly implying conformational differences. It was a rather odd observation that Na^+ concentration had an effect equivalent to that of Mg^{2+} concentration.

D. mRNA fractionation

(1) Bacteria

The mRNAs were fractionated from different morphogenetic phases of the life cycle (*viz.*, sporulation, germination, and stepdown transition—passage from rapid to slow growth) of *B. subtilis* and were studied[112] for their hybrid forming ability. These studies indicated that these messengers are derived from different genetic loci. To find common clusters of sequences, total RNA preparations from sporulating and log phase cells of *B. subtilis* and *B. cereus* were fractionated with a linear gradient of 0.3–1.2 *M* sodium chloride[113]. With pulse-labeled RNA for hybridization studies, a maximum of only 1–6% hybridization was noted with the heterologous (RNA of one species with DNA of the other) system compared to the hybridization with the homologous (RNA and DNA from the same species) system. Although efficiency was low, the results were reproducible. Thus, a small number of identical sequences among DNA and RNA of the heterologous system and complementarity in the homologous system were detected[113].

The ability of a MAK column to fractionate sharply RNA fractions of different molecular sizes (in range of sedimentation coefficients from 8.5S to 30S) has been clearly demonstrated in the resolution of *E. coli* pulse-labeled RNA components into a number of species[114]. The fractions obtained had a nucleotide composition similar to DNA and, thus, are probably mRNA.

RNA samples having different labels isolated from a "diploid" *E. coli* culture 200 PS strain (F^-Lac^+) at the end of the glucose phase and at the end of the diauxie lag phase, have been resolved using MAK column chromatography[115]. Marked differences were noted in elution profiles and in the percentage distribution of RNA. The profiles

gave sharp fractions of 4S, 16S, and 23S RNA; the latter fraction was believed to correspond to the presence of specific lactose mRNA in the induced culture. It is suggested that this chromatographic fractionation of RNA extracted at the end of the diauxie lag phase from cells growing in a glucose–lactose medium should provide a very useful means of isolating specific "lactose" mRNA in a relatively pure form.

The MAK column has been effectively employed to compare the molecular and metabolic properties of mRNA isolated from *E. coli* before and after infection by phage T_2 (refs. 32–34). By employing a double-labeling technique, significant differences in mRNAs at different time intervals of phage T_2 infection in *E. coli* BB strain were observed by following the ^3H- and ^{14}C-incorporated-RNA elution profiles[33,74]. RNAs isolated from *E. coli* K-12 "Lac" deletion mutant with and without inducer, from an induced and non-induced wild strain *E. coli* BB and from both these sources under different experimental conditions, were also resolved[35]. The non-infected and infected (with Phage P1*dl*) cultures of *E. coli* were labeled with [^{14}C]- and [^3H]-uridine, and the total RNA was subjected to chromatographic studies to detect phage P1*dl* specific RNA by hybridization studies. Phage P1*dl* was chosen in this work since it consistently yielded high titers of transducing phage. Similar studies were also performed with phage $\Phi X174$, and the hybrid formed appeared as a distinct peak between RNA and phage $\Phi X174$ DNA. Thus, MAK column chromatography[116,117] provided conclusive data that one of the strands of the DNA duplex is the source of translatable genetic information.

The classical work of MANDELL AND HERSHEY[3] and that of other workers[32–35,116,117] in the fractionation of phage-induced nucleic acids laid down the following principles on which MAK column chromatographic separations are based. The MAK column fractionates RNA according to differences in both base composition and chain-length, the smaller fragments eluting at a lower electrolyte concentration. Although these two processes occur simultaneously, the effects of base composition are readily detectable because a high molarity of electrolyte is required for the elution of a high adenine uracil (A-U) containing RNA. The efficiency of the fractionating ability of the column can be further increased with respect to differences in base composition by prior fractionation with respect to size in linear sucrose gradients.

Broad fractionation of stable RNA from a tenaciously bound DNA-like RNA (D-RNA) from *S. cerevisiae* was achieved[118] by eluting with sodium dodecyl sulfate (SDS), and the fractions were characterized for physico-chemical properties. The binding of D-RNA with the adsorbent was so tenacious that SDS could not be substituted by any other eluting agent. It is believed that the SDS may denature the protein associated with D-RNA, thus making the elution easier and quantitative.

(2) Fungi

A broad separation of ^{32}P-labeled, unidentified polyphosphate and nucleic acids from plasmodia, *Physarum polycephalum*, has been obtained using a MAK column[110]. The fraction eluted at a 0.5 *M* sodium chloride concentration has the high molecular weight and characteristic properties applicable to a polyphosphate. An extract of purified nuclei of *Physarum polycephalum* also yielded a similar fraction, indicating its site of origin as nuclear. No fractionation of RNA species was, however, achieved under the conditions used for chromatography.

(3) Birds

The fractionation of ^{32}P-labeled RNA of immature duck erythroid cells revealed the presence of two types of mRNA on the basis of base composition and S values also[120]. SCHERRER *et al.*[120] feel that one of them is functional mRNA since it carries the fraction of genetic code actually expressed in a given cell, while the other is a nascent mRNA which is a chromosomal transcription product corresponding to the active fraction of the genome. It is further suggested that these two types may differ from each other qualitatively and quantitatively.

(4) Mammals

A MAK column was employed to resolve different species of RNA and purify them from cultured FL cells from human amnion[96,108]. It is claimed[96] that a fraction having a 50S value is a homogeneous mRNA corresponding to an entire operon comprised of many cistrons[66]. ELLEM AND SHERIDAN[59] have reported the clearcut separation of rapidly labeled nucleic acids from L-cells and noted that a large part of rapidly labeled RNA was tenaciously bound on to the column. It could not be eluted with the usual gradient of sodium chloride and seemed to represent the bulk of the messenger or D-RNA. Similar studies by KUBINSKY AND KOCH[121] revealed that RNA from the stationary phase is apparently similar to DNA in its properties, while the bulk of RNA from logarithmically growing cells has rRNA-like base composition.

LICHTENSTEIN *et al.*[90] raised the resolution power of the MAK column while fractionating nRNAs from Zajdela ascites hepatoma and rat liver. The improved elution procedure consisted of using a temperature gradient from 35° to 95°, and 1.5 *M* sodium chloride as an eluting agent, after the elution of RNA by the usual salt concentration gradient (0.5–1.5 *M*). It was noted that the chromatographic profiles of nRNAs from the sources studied differed significantly and were reproducible. The possibility of the occurrence of certain alterations in the molecular characteristics of nRNA during elution due to progressively rising temperature was examined by rechromatographing the same fraction; this proved successful. Of course, whether the use of a temperature gradient to the extent of 95° is feasible for all problems remains questionable in view of contradictory data[96,100,111] in the past. Successful rechromatography of nRNA after elevating it to such a high temperature is also unconvincing. Even though LICHTENSTEIN *et al.*[90] claim it was successful for nRNA (possibly due to complementary base composition), it may not work with tRNA or rRNA.

(5) Plants

The pattern of mRNA synthesis at different stages of peanut cotyledon development was studied by following the MAK column profiles[72]. It was found that relatively more mRNA is synthesized in 14-day-old cotyledons than in 2-day-old ones.

E. Alternatives to the MAK column

Denatured proteins[122] and batchwise adsorption[123] have been tried as possible alternatives to the MAK column. The resolution obtained is not superior to that on the MAK column, and the good flow-rate provided by Kieselguhr[124] is absent. Further, the limitations of batchwise adsorption seem evident. A more fruitful approach

may be the modification of the MAK column to provide distinct resolution of different tRNAs in a single chromatographic run, rather than minor alterations[122,123].

F. Advantages of the MAK column

Because of its flexibility and resolving power, MAK column chromatography is a useful tool for rapid analysis of nucleic acids[125]. Suitable adjustment of the sodium chloride gradient can magnify any region of the chromatographic profiles. The column is relatively simple and convenient as elimination of clogging allows a high flow-rate. It can sharply distinguish different types of RNAs (tRNA, rRNA, and mRNA) from DNA and can resolve isoaccepting species of tRNA, viral, and phage specific RNA.

G. Disadvantages of the MAK column

(1) Limited capacity

A major drawback of the column is its limited capacity—about 1 mg of RNA per 10 ml of the column volume[2] (ten times less than the capacity of DEAE–cellulose), and this is particularly a problem in the fractionation of tRNA since only a small fraction of total tRNA is specific for any given amino acid.

Partial loss in acceptor activity of seryl-tRNA after passage through the column has also been reported[40], with increased loss of activity in preparative separations which employ bigger columns. Separation is clearcut only if a long column is used or if small quantities of adsorbate are loaded on the column[9]. Any modification which reduces the flow-rate and capacity of the column is not feasible. Therefore, MAK column chromatography is not used for preparative work.

(2) Tailing

The commonly used column, as devised by MANDELL AND HERSHEY[2], does not result in a homogeneous species of RNA with respect to one biological expression since contamination of a fraction with the nucleic acid appearing in the preceding fraction (tailing) is usually observed[60]. Various workers[96,100,111] have observed that mammalian rRNAs, in particular , are not clearly resolved into the 18S and 31S components, while bacterial rRNAs are separated more clearly[87,102,108]. This discrepancy appears to be a characteristic of the MAK column with respect to mammalian rRNAs, and is not due to technical errors[126].

That the column preparation and the handling of material is critical has been reported by BROWN *et al.*[71]. Pre-wash of the column with 1.0% versene solution was required to obtain reproducible results since heavy metal ions apparently interfere with RNA adsorption, and the profiles of rRNA can change dramatically. It was found that the elution pattern depends on a finite concentration of Mg^{2+} ions[61,127].

(3) Effect of temperature

Controversy has raged for a long time over the use of a temperature gradient[90] for the fractionation of tRNA. Elution at a higher temperature often gives better resolution, but perhaps at the cost of other characteristics. Furthermore, the operational advantages of the MAK column at 35°, over those at 15° (ref. 78) in RNA fractionation have been questioned in STAEHELIN's laboratory[111].

(4) Incomplete recovery

Chromatography of nucleic acids on MAK columns using a 0.3–1.2 *M* salt gradient is a universally recognized method. However, ELLEM AND SHERIDAN[52] found that even 2.0 *M* sodium chloride could not quantitatively elute HeLa cell RNA, as about 10% RNA still remained tenaciously bound to the adsorbent. Others have also observed incomplete recovery on the MAK column[83,86]. The remaining RNA could be eluted with either 1.5 *M* ammonium hydroxide or 1.5 *M* sodium chloride at an elevated temperature of 90°. It should be noted that both the eluting agents and the high temperature conditions are drastic: 1.5 *M* ammonium hydroxide will probably degrade RNA with concomitant loss of biological expression and an elevated temperature approaching T_m (temperature of melting) will do the same.

(5) Channeling and reproducibility

MANDELL AND HERSHEY[2] found that channeling is a serious drawback and that competition among nucleic acids for adsorption sites seriously limits the resolution power of closely related species. In addition, the distribution pattern is often not quantitatively reproducible because of variations in the quality of batches of methylated bovine serum albumin. Thus, lack of reproducibility poses difficulties in clearcut interpretation[81].

Finally, MAK column chromatography can be used as a screening technique only to indicate whether differences exist or not; it cannot furnish qualitative or quantitative relationships.

IV. PROTAMINE–KIESELGUHR COLUMN

Protamine coated on Hyflo-Supercel (Kieselguhr) has been recommended as an adsorbent for the fractionation of nucleic acids[128]. The column gives a different distribution pattern to that obtained with the MAK column. Although bacterial RNA and DNA could be eluted with a salt gradient, rat liver RNA could not be eluted even at 4.0 *M* sodium chloride or 1.0 *M* ammonia concentration. Its tenacious binding onto the column required a low concentration of SDS, possibly to denature the protamine, and to effect elution of the rat liver RNA in a single peak, in contrast to a 0.3 *M* sodium chloride concentration which yielded two sharp peaks of yeast RNA. BROWN *et al.*[120] also used a protamine–Kieselguhr column for a similar purpose.

The protamine–Kieselguhr column has a higher capacity than the MAK column. However, it furnishes sharp resolution only below 40% capacity; it also separates RNA from DNA more easily than the MAK column and can fractionate yeast tRNA, unlike the histone-coated Kieselguhr column[8]. Protamine, in contrast to methylated serum albumin and histone, is a stable material with constant properties. Use of both the columns—MAK and protamine–Kieselguhr—in conjunction may well improve the separations.

V. METHYLATED SERUM ALBUMIN–SILICIC ACID COLUMN

Use of silicic acid in the partition chromatographic separation of tRNAs was first reported by EVERETT *et al.*[130]; the specific activities of different tRNAs were considerably increased, encouraging the hope that this procedure could be developed

to subfractionate different tRNAs. Although the method was simple and furnished considerably enriched tRNAs, the operational losses were heavy, as much as 60%. For this reason, the procedure was not developed further.

The need for a rapid and simple alternative to the MAK column, preferably improved, prompted OKAMOTO AND KAWADE[131] to develop the methylated serum albumin–silicic acid (MASA) column as a promising preparative tool for the resolution of tRNAs from brewer's yeast and rRNA from *E. coli*. REVEL AND LITTAUER[132] used both MAK and MASA columns to compare the fractionation of *E. coli* methyl-deficient phenylalanyl-tRNA and its normal counterpart. The fractions isolated gave different responses with different triplets. It was also noted that unacylated-, methyl-deficient-, and normal-phenylalanyl-tRNA behaved differently on the MASA column than on the MAK column. Further studies showed[133,134] that four peaks of phenylalanyl-tRNA were resolved using pre-charged methyl-deficient tRNA; the coding properties of these fractions responded differently to different templates. The phenylalanyl-tRNA species, thus obtained, were 50% pure. That multiple species of several aminoacyl-tRNAs failed to be resolved on the MASA column in the absence of aminoacylation presumably implied that the free amino group of the attached amino acid is responsible for the distinct elution pattern of aminoacylated tRNAs[133]. That the differences in profiles reflect differences in conformation is yet to be established.

The MASA column was also employed[135,136] to compare the chromatographic profiles of methionyl-tRNA before and after formylation. Considerable differences in profiles and acceptor activity were reflected. Furthermore, transfer of methionine and recognition of the A_pU_pG codon were completely lost after formylation. This is possibly due to a change in the secondary structure, resulting in changed chromatographic behavior. It has been emphasized by earlier workers[131–134] that changed secondary structure reflected a change in chromatographic behavior on both MASA and MAK columns.

Studies on MASA, as well as a polyacrylamide gel column, for the chromatographic separation of *E. coli* tRNA showed that phenylalanyl- and methionyl-tRNAs are eluted earlier from the MASA column than their aminoacylated counterparts[137]. Phenylalanyl-tRNA is further subfractionated into four species out of the total tRNA isolated from a relaxed methionine-requiring mutant of *E. coli* grown in a methyl-deficient medium. Polyacrylamide column chromatography showed that N-blocked tRNAs, such as N-acetyl-phenylalanyl-tRNA and N-formyl-methionyl-tRNA, are excluded more than their aminoacylated or unacylated counterparts, a finding consistent with a more extended configuration for N-blocked species.

MASA has been used to separate ^{32}P-labeled nucleic acids of phage MI2 (ref. 138). At a finite electrolyte concentration, infectious single-stranded phage RNA was adsorbed on to MASA and was removed by centrifugation, while the infectious RF remained in solution. By following the band sedimentation of the MASA fractions, two distinct types of RF were obtained: one sedimenting at 15S, the other between 17 and 27S (22S). 15S RF has been shown to behave chromatographically like DNA, whereas the 22S RF fraction found in the supernatant is like single-stranded phage RNA. It may be mentioned here that the procedure developed[138] does not look like a column procedure, but more or less like a batchwise adsorption.

It was noted that the MASA column has 100-fold greater adsorptive capacity and higher resolution power for tRNA than the MAK column[16,134]. However, the

flow-rate is painfully slow. The MASA column is able to differentiate between unacylated-, aminoacylated-, and N-blocked aminoacylated-tRNA species. These studies revealed that the three above-mentioned types of tRNA have three different conformations which permit the separation. It seems that, like the MAK column, the MASA column separates species of tRNA upon differences in secondary structure.

VI. POLYAMINO ACID–KIESELGUHR COLUMNS

A. Poly-L-lysine–Kieselguhr column

AYAD AND BLAMIRE[139] have ushered in a new era of chromatography by introducing the use of the synthetic polypeptide, poly-L-lysine, in the fractionation of DNA. The principle behind this column technique is the association of poly-L-lysine, like other basic proteins, with DNA or RNA and the discriminating dissociation of the complex (between RNA and poly-L-lysine) with an increasing concentration gradient of salt or suitable eluting agent. It has been shown that the column can resolve tRNA from DNA and that protein contamination in the nucleic acid preparation did not have any effect on the elution profiles. The column furnished better separations than the MAK column.

A column of poly-L-lysine Kieselguhr (PLK) gave a composite peak[140] between 1.6 and 1.8 *M* sodium chloride which revealed the presence of large amounts of RNA and some contaminating DNA and proteins. It has been further shown[141] that the column provides a distinct separation of DNA from RNA, that it enables partial resolution of genes corresponding to the synthesis of histidine and tryptophan and that the fractionation is mainly a function of base composition and, like other Kieselguhr columns, to some extent of secondary structure[2,131]. After studying the chromatographic profiles of *E. coli* and yeast RNA[142,143], it is claimed that RNA fractionation depends in part on secondary or tertiary structure (which affects the binding of RNA to PLK) and that the nature of the binding determines the concentration of salt required for elution.

The PLK column seems to be a quick, reliable method of ascertaining the type of nucleic acid (depending on the position of a particular fraction on the chromatogram) and, also, to what degree each component is homogeneous. The tentative mechanism proposed for binding to the column seems reasonable from an understanding of the MAK and MASA columns. However, one has to consider the size of the molecule and, also, hydrogen bonding before arriving at a final conclusion.

B. Poly-L-ornithine- and poly-L-arginine–Kieselguhr columns

LOESER *et al.*[144] introduced some new columns—Kieselguhr columns coated with any of the following polyamino acids: poly-L-lysine, poly-L-arginine, or poly-L-ornithine. The poly-L-lysine coated Kieselguhr column has been reported previously[139–143], and the introduction of a poly-L-arginine column was anticipated[135].

Nucleic acids binding on to poly-L-arginine-Kieselguhr (PAK) and poly-L-ornithine-Kieselguhr (POK) columns could be dissociated by increasing the salt concentration. It was noted[144] that in the case of the PAK and the PLK columns an additional pH gradient was necessary to bring about optimum resolution. The pattern

of elution varied between one polyamino acid column and another. On all three columns, *E. coli* 5S rRNA was completely resolved from tRNA. However, the PAK column furnished superior resolution of 5S rRNA over the other columns studied. Quick preparation, rapid flow-rate, and use of the columns several times without loss of reproducibility are some of the operational advantages[144].

In the near future, it may be possible to introduce poly-L-citrulline- and poly-L-histidine-Kieselguhr columns; these polyamino acids are similar in physico-chemical properties to those now reported[130,144]. Of course, it may be difficult to introduce the poly-L-histidine column; the procedure of polymerization with histidine may pose difficulties due to the presence of the heterocyclic ring in the molecule.

VII. POLYNUCLEOTIDE–KIESELGUHR COLUMNS

The MAK column as introduced by MANDELL AND HERSHEY[8], has undergone a series of modifications, including the introduction of silicic acid in place of Kieselguhr[131], the introduction of several polyamino acids in place of methylated serum albumin[130,144] and other minor modifications[122,123,128]. Recently, LIN[145] developed yet another modification for fractionation of RNAs. The ability of polynucleotides to hybridize with each other to varying degrees under suitable annealing conditions has been exploited to form the basis of the separation.

The method[145] consists of precipitation of the desired polynucleotide, natural or synthetic, from its aqueous solution by hexamine cobalt chloride in the presence of an inert porous support, such as Kieselguhr; adsorption of the RNA in the presence of dioxane; and the development of the column using the standard procedure[8]. It was rather interesting to note that retention of RNA on the column did not necessarily require any complementarity in base sequences. Other requirements for annealing (*viz.*, low temperature and low ionic strength) were, however, obligatory.

Chromatography of RNA on Kieselguhr coated with pig liver denatured DNA permitted partial fractionation of tRNA from ^{14}C-labeled rRNA, of high- and low-molecular-weight RNA and also of [^{14}C]-valyl-tRNA from the bulk of the tRNAs, all from microbial sources. This column seems basically similar to the DNA–cellulose column[146], the DNA–agar column[147], and the polynucleotide–cellulose column[148].

Although the novel ability of dioxane to promote polynucleotide interaction has been exploited to introduce an alternate adsorbent for RNA fractionation[145], the column has many potential limitations. For example, dioxan itself is a corrosive solvent and liberates heat when mixed with aqueous media. For this reason dioxan has been a failure in the extraction of enzymes in the past. With such a drastic inherent property, dioxane is likely to inactivate the acceptor activities of the tRNAs to be fractionated. Moreover, none of the separations attempted were complete; contamination of DNA with poly-I was obvious. Further studies using the column may throw more light on the problem.

VIII. CONCLUSION

Of the different Kieselguhr columns impregnated with proteins, polyamino acids, and polynucleotides, the MAK and the MASA columns appear to be superior. They provide a clearcut and reproducible method for the fractionation of RNA into

tRNA, rRNA and mRNA and for further subfractionation of tRNA into isoaccepting species. They are also simpler than polyamino– or polynucleotide–Kieselguhr columns. The availability and low cost of methylated serum albumin in comparison with costly polyamino acids or polynucleotides makes it a universally applicable tool. The MASA column has higher capacity than the MAK column; however, the latter affords rapidity, having an appreciably higher flow-rate than the MASA column. The use of the MASA in conjunction with the MAK column may have both high capacity and good flow-rate, thus being a potential tool in the preparative fractionation of tRNA. With the meagre data available on the performance of polyamino acid– and polynucleotide–Kieselguhr columns, it may, however, be premature to evaluate their ability to fractionate nucleic acids at this stage.

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