authors to explain differences in the action spectra of one- and two-stranded bacteriophages.

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*PURIFICATION OF VALINE TRANSFER RIBONUCLEIC ACID BY COMBINED CHROMATOGRAPHIC AND CHEMICAL PROCEDURES**

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The biological synthesis of proteins poses three distinct questions: first, how the energy for condensation of amino acids to form a long peptide chain is funneled into this endergonic process; second, how the intricate ordering of amino acids to form the sequence characteristic of a protein species is accomplished; and third, how the precise folding and cross-linking of a chain or chains of peptides is arranged to create biologically active protein molecules.

A fairly satisfactory answer may now be given to the first question,[1-3] and interest has therefore shifted to the second and third, whose solutions still lie ahead. Evidence (cf. refs. 4 and 5) indicates that the ribosome serves as the site for sequence arrangement of the amino acids, which may be accomplished by appropriate hydrogen-bond pairing of bases of the ribonucleic acid of the ribosomes with short-chain ribonucleic acids (S-RNA or transfer-RNA molecules)[6, 7] to

which activated amino acids have been esterified. These low-molecular weight transfer-RNA molecules thus serve as intermediate carriers of activated amino acids, with each amino acid bound to a specific transfer-RNA molecule.[8, 9] The amino acid is attached to the terminal adenosyl unit of transfer-RNA[10] by ester linkage to the 3′ or 2′ hydroxyl group of the ribosyl moiety.[11–13] This viewpoint on the sequence arrangement gives heightened interest to the effort to isolate an individual transfer-RNA molecule, coded to a particular amino acid by what may be regarded as the translation signature of the gene. The present communication reports a procedure to isolate individual transfer-RNA molecules.

Two general types of fractionation methods for transfer-RNA molecules have been used: (1) physicochemical separations[8, 14–21] employing adsorption-elution from chromatographic columns, electrophoresis, selective precipitation, or countercurrent distribution, and (2) chemical methods[22–26] depending on covalent combination of a large molecule with a particular amino acid bound to the RNA or with the oxidized vicinal 2′, 3′-hydroxyl groups of the terminal ribosyl moiety of transfer-RNAs to which no amino acid is attached. Complete isolation of a single aminoacyl-RNA has not been claimed by either type of procedure.

The present method combines a physical separation method, using DEAE-dextrans (DEAE-Sephadex, Pharmacia, Uppsala, Sweden)[27–29] under specified conditions and the chemical dye procedure previously described.[24] The calculated degree of purification of valyl-RNA approximates 65–80 per cent. Uncertainty as to the precise degree of purification obtained is related largely to choice of the molecular-weight figure used in the calculation.

Methods.—Preparation of S-RNA: Soluble RNA is prepared from intact yeast cells according to a slight modification of the procedure of Monier *et al.*[23,30,31] Instead of treatment with methoxyethanol to remove polysaccharide material, the RNA (from the first alcohol precipitation) is chromatographed on DEAE-cellulose[32] prior to treatment with activated charcoal. About 400 mg of S-RNA are dissolved in 300 ml of 0.1 *M* Tris-HCl buffer at pH 7.5 and are chromatographed on a 2 × 20 cm column of DEAE-cellulose (N,N-diethylaminoethylcellulose, Eastman Organic Chemicals). 1,300 ml of 0.1 *M* Tris-HCl buffer are run through and discarded, then the RNA is eluted with 250 ml of 1.0 *M* NaCl in 0.1 *M* Tris-HCl buffer, pH 7.5. The RNA solution is concentrated by lyophilization, dialyzed, and treated with acid-washed charcoal (Norit A, Fisher Scientific Co.) to remove traces of yellow impurities.[23]

Amino acids esterified to the S-RNA are removed by treatment for 30 min at 37°C with 0.1 *M* sodium carbonate adjusted to pH 10, followed by overnight dialysis against distilled water. The S-RNA solution is stored at −20°C until used.

Preparation of enzyme: In order to label S-RNA with only one amino acid, a partially purified enzyme, free of RNA and amino acids, is prepared from yeast in the following manner: 50 gm of packed yeast cake are homogenized in a Virtis "45" homogenizer with 200 gm of acid-washed glass beads and 50 ml of ice-cold medium consisting of 0.05 *M* Tris-HCl buffer, pH 7.5, 0.005 *M* $MgCl_2$. All procedures are carried out near 0°C. The iced homogenizer is run at top speed for two 4-min intervals, with a 3-min interval between runs to prevent the homogenate from warming up. The glass beads are rinsed free of the homogenate with an additional 200 ml of medium, and the combined homogenate plus washings are centrifuged at 15,000 × *g* for 15 min. The pH of the supernatant, usually about 6.8, is adjusted to pH 7.5 with KOH.

Most of the RNA is precipitated by the dropwise addition of one-tenth volume of 1 *M* $BaCl_2$. The enzyme solution, containing 0.1 *M* $BaCl_2$, is allowed to stand at 0°C for 10 min to allow the ribonucleoprotein precipitate to form. This fairly large precipitate is removed by centrifugation at 15,000 × *g* for 10 min. The optimal amount of $BaCl_2$ had been determined for several batches of enzyme, and, under the conditions described, a final concentration of 0.1 *M* $BaCl_2$ is sufficient to precipitate most of the ribonucleic acid. More concentrated enzyme solutions require a greater concentration of $BaCl_2$.

The small amount of RNA remaining in the 15,000 × *g* supernatant fraction is precipitated by the addition of streptomycin sulfate (Parke-Davis). The dry powder is dissolved in a minimum of medium and this concentrated solution of streptomycin sulfate is added dropwise, with constant stirring, to the enzyme solution until a final concentration of 20 mg per ml is obtained. After 10 min at 0°C the mixed precipitate of $BaSO_4$ and streptomycin-RNA complex is removed by centrifugation at 15,000 × *g* for 10 min. The supernatant solution is clear pale yellow.

This solution is concentrated to about one third the volume by lyophilization (from 250 to 85 ml). When these solutions are frozen and subsequently thawed, very often the pH has dropped and a further precipitate has formed. The pH is readjusted to 7.5, and any remaining precipitate is removed by centrifugation. The concentrated enzyme solution is then applied to a cross-linked dextran column (Sephadex-G-25, coarse grade) to remove amino acids and other small molecules. The protein is in the early effluent from the column, while the amino acids are retarded.[33] A 5-by-15 cm column is used, with 0.01 *M* Tris-HCl buffer pH 7.5 and 0.001 *M* $MgCl_2$ as the eluting fluid. 50 ml of enzyme solution are put on the column along with a marker of free C^{14}-valine to indicate where the free amino acids emerge. Aliquots are plated on flat planchets for counting of C^{14}-valine to locate the amino acids, and other aliquots are taken for determination of protein by the colorimetric method of Lowry *et al.*[34] All tubes containing protein, free of amino acids, are pooled, and the enzyme solution is lyophilized to about one quarter of the volume. The final protein concentration is about 6 mg per ml. There is little loss of enzyme activity by freezing, as measured by ability to label RNA with C^{14}-valine. Prolonged storage at −20°C or repeated freezing and thawing, however, results in some loss of activity.

Crude yeast enzymes prepared in this way contain valine-activating enzyme and, to a much lesser extent, leucine- and tyrosine-activating enzymes. Enzymes for other amino acids have not been assayed.

Preparation of C^{14}-valyl-RNA: The incubation mixture previously described contains the following in 1 ml:[24] 10 μmoles of adenosine triphosphate (sodium, magnesium salt), 0.5 μmole of cytidine triphosphate, 10 μmoles of phosphoenolpyruvate, 10 μg of pyruvate kinase, yeast enzyme solution, 25 μmoles of Tris-HCl buffer at pH 7.5, 2.5 μmoles of $MgCl_2$, 0.1 μmole of C^{14}-L-valine (1.75×10^6 cpm/μmole), and about 2 mg of S-RNA containing 1.4 μmoles of $MgCl_2$ per mg RNA. The RNA is added last in order to obtain the highest specific activity of valyl-RNA. Incubation is for 30 min at 37°C.

Prior to labeling a large amount of RNA with valine, preliminary experiments are carried out to establish the optimal enzyme and RNA concentration, so that the enzyme is in sufficient concentration to catalyse the labeling of RNA to the fullest extent. These small incubations are carried out in a total volume of 0.1 or 1.0 ml, and the RNA is isolated for counting according either to a membrane filtration technique (Scott, J. F., personal communication) or to a previously described procedure.[13] Generally 2 mg of RNA and about 1 mg of enzyme protein per ml provide optimal labeling of the largest amount of RNA. Higher concentrations of RNA cause an inhibition of the reaction. Many yeast enzyme S-RNA labeling preparations do not show a requirement for phosphoenolpyruvate, pyruvate kinase, or CTP.

In large-scale experiments 400 mg or S-RNA are incubated in a total volume of 200 ml of incubation mixture, after which the RNA is reisolated from the mixture using a phenol extraction procedure.[24] About 20 per cent of the RNA is lost during this procedure. The 200 ml of incubation mixture is shell-frozen and concentrated by lyophilization to about 70 ml. An equal volume of 90 per cent phenol is added, and the mixture is stirred for 15 min at room temperature. Following this, it is centrifuged for 15 min at 15,000 × *g* in polyethylene tubes. The slightly turbid upper layer containing the RNA is removed, and the lower layer is stirred for 10 min with 70 ml of water saturated with phenol. The mixture is centrifuged as before and the supernatants are combined. One-tenth volume of 2 *M* potassium acetate at pH 5 plus 2.5 volumes of cold ethanol are added to precipitate the RNA. After standing at least 2 hr at −20°C, the precipitate of RNA plus ATP is recovered by centrifugation. The precipitate, drained well of alcohol, is dissolved in a minimal amount of cold distilled water (about 80 ml), and the solution is dialyzed at 4°C for at least 8 hr against several changes of water to free the preparation of ATP and free amino acids. Following dialysis, the A_{260}/A_{280} ratio of about 2.1 still indicates the presence of small amounts of nucleotide material. This is removed by treatment with charcoal, as follows: about 100 ml of the RNA solution is stirred for 10 min at 0°C with 1 gm of acid-washed charcoal. The mixture is centri-

fuged at 15,000 × g for 15 min and the A_{260}/A_{280} ratio of the clear, slightly bluish supernatant is determined. If the ratio remains over 2, further treatment with charcoal may be necessary. Aliquots are plated for counting and the amount of RNA is determined spectrophotometrically, using an absorbency index of 21.4 cm^2mg^{-1}. Dr. Jesse F. Scott has calculated absorbency indices for yeast S-RNA based on phosphate determinations of the S-RNA. These are 26.2 and 21.4 cm^2mg^{-1} at 260 mμ. The former refers to S-RNA dissolved in distilled water, the latter to S-RNA dissolved in dilute salt solution, i.e., 0.14 M NaCl, 0.014 M sodium citrate. These values on unhydrolyzed S-RNA differ from those obtained by Scott *et al.*[35,36] on RNA hydrolyzed in either acid or alkali. The preparations of C^{14}-valyl-RNA used for the fractionations described in this paper did not show this difference of absorbency in water and salt solution, however, but behaved as though salt were present. This behavior is possibly related to previous exposure to Mg^{++} during the labeling procedure. For this reason, the absorbency index of 21.4 is used to calculate the concentration of RNA present in all the samples. The solution of C^{14}-valyl-RNA is stored frozen at −20°C. Large batches of S-RNA prepared in this manner usually contain 1,800–2,500 cpm (around 1.1 mμmoles of valine) per mg of S-RNA.

Addition of dye to S-RNA molecules containing no amino acid: The procedure described by us earlier[24] is used to add dye to the S-RNA molecules containing no amino acid. Briefly, the free C2′ and -3′ hydroxyl groups on the terminal ribosyl moiety of the S-RNA molecules containing no amino acid are oxidized to the dialdehyde by periodate. A hydrazone is formed by reaction of the aldehyde with 2-hydroxy, 3-naphthoic acid hydrazide. This colorless hydrazone is then reacted with tetrazotized o-dianisidine, with the formation of a deep blue dye, bound to the RNA. The presence of amino acid esterified to the C2′ or -3′ hydroxyl group prevents the periodate oxidation. Thus, aminoacyl-RNAs present in the mixture do not form dye-bound complexes, and their physical properties differ sufficiently from those of the dye-RNA to make partial separation possible.

Chromatography on DEAE-dextran columns: DEAE-dextran gel was prepared in the following manner. 50 gm of DEAE-dextran (DEAE-Sephadex-A-25, coarse grade), supplied in the hydrochloride form, are suspended in an excess of water. The fine grains are removed by decantation after standing 10 min, with resuspension and repetition of this process about six times. The DEAE-dextran is washed onto a Buchner funnel with 1,000 ml of 0.5 N HCl. About 1,000 ml of distilled water are washed through the filter, and the DEAE-dextran is suspended in 1,000 ml of 0.5 N NaOH for about 10 min at room temperature. The suspension is filtered as before and washed with 2,500 ml of water, followed by 1,500 ml of 0.1 N HCl to neutralize the NaOH. The gel is washed with 2,000 ml of water and finally with roughly 500 ml of 0.1 M Tris-HCl buffer at pH 6.0. The DEAE-dextran gel is stored in suspension in the 0.1 M Tris-HCl buffer at 4°C.

Favorable conditions for separation of valyl-RNA from the total S-RNA fraction have been evolved as a result of experiments with some thirty columns using DEAE-dextran. These details are given for the experiments described in Figure 1 and are as follows: Columns (1.2 x 55 cm) are prepared by allowing the DEAE-dextran gel to settle by gravity. Such columns are operated at a flow rate of approximately 100 ml per hour. In order to elute the valyl-RNA prior to the bulk of the unlabeled or dye-RNA, a very slight linear gradient of NaCl in 0.1 M Tris-HCl buffer, pH 6.0 (0.35 to 0.40 M NaCl) is used. After the emergence of the valyl-RNA, the NaCl gradient is increased abruptly to 1.0 M NaCl to elute much of the remaining RNA. Most of the dye-bound RNA still remains on the column at this salt concentration.

In each of the experiments shown in Figure 1, the RNA solution (21 mg) was put on the column in 25 ml of 0.1 M Tris-HCl buffer pH 6.0, 0.35 M NaCl. A linear salt gradient was established, with 1,000 ml of 0.1 M Tris-HCl buffer pH 6.0 and 0.35 M NaCl in the mixer and 1,000 ml of 0.1 M Tris-HCl buffer pH 6.0 and 0.40 M NaCl in the reservoir. The chromatography was carried out in a cold room at 4°C and about 7.0 ml fractions were collected. After the above volume of elution fluid had passed through the column, the NaCl gradient was increased to elute the remaining RNA. In the mixer there were 250 ml of 0.1 M Tris-HCl buffer pH 6.0 and 0.4 M NaCl, and 250 ml of 0.1 M Tris-HCl buffer pH 6.0 and 1.0 M NaCl were placed in the reservoir, following which the collections were continued as before. 83 per cent of RNA and 47 per cent of the RNA sample which had been treated with dye were eluted under these conditions. The absorbency at 260, 280, and 560 mμ (the last-mentioned for the dye-RNA) was determined on the fractions collected. The mg of RNA in each tube were calculated from the A_{260} using the absorbency index

of 21.4 cm^2mg^{-1}. Because of the low concentrations of RNA in the fractions eluted very early from the columns, there may be as much as 15 per cent error involved in the readings. This type of error would decrease with use of larger amounts of RNA for fractionation.

Aliquots of the RNA were precipitated in 5 per cent trichloroacetic acid and were filtered on membrane filters for counting. The amount of RNA filtered ranged from 0.01 to 0.1 mg. A Nuclear-Chicago gas flow counter with low background (2 cpm) and counting efficiency of approximately 35 per cent was used to count the samples.

Results.—Figure 1-*A* shows the type of fractionation obtained using RNA labeled with C^{14}-valine. Valyl-RNA emerges prior to the bulk of the RNA. As indicated by the sharp drop in specific activity in the initial part of the curve, however, the valyl-RNA is being diluted by other species of RNA molecules. Almost all of the radioactivity from C^{14}-valine appears early in the elution. The

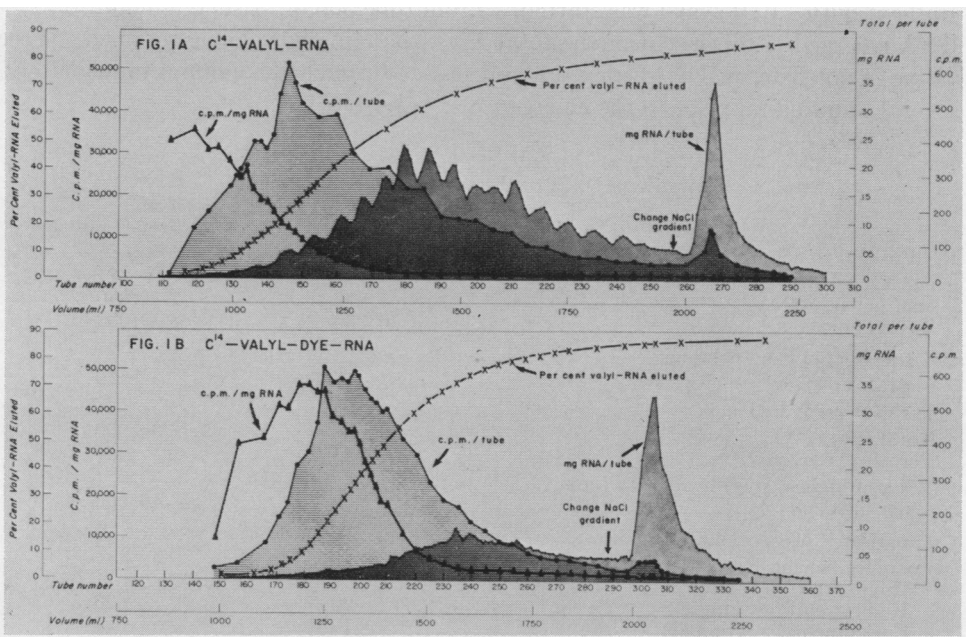

FIG. 1.—1 *A:* Fractionation of Valyl-RNA using DEAE-dextran, 21.25 mg valyl-RNA, 1,890 cpm per mg (1.08 mμmoles valine per mg) was chromatographed as described in text. 6.7 ml aliquots were collected.

1 *B:* Fractionation of Valyl-RNA treated with dye (valyl-dye-RNA) using DEAE-dextran. 21.70 mg valyl-dye-RNA, 1,840 cpm per mg (1.05 mμmoles valine per mg) was chromatographed as described in text. 7.7 ml aliquots were collected. This dye-RNA had a ratio of $A_{560}/A_{260} \times 100$ of 6.5.

cumulative per cent of the total C^{14}-valine added indicates that when 50 per cent of the valyl-RNA is eluted, only 9 per cent of the total RNA has emerged. The specific activity is increased from 1,890 cpm per mg in the original mixed sample to 35,900 cpm per mg in the most highly enriched sample. The amount of RNA in the enriched fractions is, however, extremely small.

An improvement in yield and somewhat in purity of high specific activity valyl-RNA is achieved by subjecting the RNA sample to the dye addition procedure before application to the column. The dye-bound RNA is more firmly bound to the DEAE-dextran than is non-dye-bound RNA, and even at a concentration of 1.0 *M* NaCl as eluant, only about 47 per cent is eluted from the column. Thus,

by holding back the bulk of the RNA under conditions where valyl-RNA emerges early, it is possible to obtain significant amounts of valyl-RNA which are relatively free of other RNA molecules. Figure 1-*B* indicates that several fractions (tubes 176–186) have the same high specific activity of about 46,500 cpm per mg. The specific activity of the valyl-RNA in the following tubes drops rather rapidly, indicating contamination with non-labeled RNA. The figure shows that the first 25 per cent of the valyl-RNA eluted has an average specific activity of over 40,000 cpm per mg. RNA. Half of the dye-RNA has remained bound to the DEAE-dextran, although 83 per cent of the untreated RNA is eluted under similar conditions (Fig. 1-*A*). A compilation of these and other details is given in Table 1. No material having an absorbence at 560 mμ (dye-RNA) was eluted until the gradient with 1.0 *M* NaCl was started. From tube 300 on, a small amount of dye-RNA emerged (7.6 per cent of the total A_{560}), the remainder being retained. Free valine is not held on DEAE-dextran and passes through the column immediately, several hundred ml prior to the emergence of valyl-RNA.

TABLE 1

SUMMARY OF DATA ON FRACTIONATION OF RNA

	C^{14}-Valyl-RNA	
	Untreated	Treated with dye
RNA added to columns:		
mg RNA	21.2	21.7
cpm per mg RNA	1,890	1,840
Total cpm	40,300	40,000
RNA recovered from columns:		
mg RNA	17.6	10.1
Per cent recovered	83	47
Total cpm	34,400	34,900
Per cent recovered	85	87
RNA of highest specific activity (cpm per mg)	35,900	46,500
Comparison of specific activities:		
mμmoles per mg RNA		
Original mixed sample	1.08	1.05
Highest elution sample	20.5	26.5
Enrichment ($\times$ original)	19	25
Per cent purity:		
If molecular weight 25,000	51	66
If molecular weight 30,000	62	80

In single experiments, both seryl-RNA and tyrosyl-RNA showed some enrichment upon fractionation on DEAE-dextran. Valyl-RNA is eluted at a lower salt concentration than either of the former and therefore is more readily separated from the bulk of the RNA. The peak of seryl-RNA was found to follow closely that of the valyl-RNA peak, while tyrosyl-RNA emerged at a somewhat higher salt concentration along with the bulk of the RNA.

The estimation of the purity of the valyl-RNA is dependent upon the choice of molecular weight of valyl-RNA, a value not known with precision. The molecular weight of the S-RNA family has been variously estimated as between 25,000 and 30,000, with the former figure being at present more generally accepted.[37–41] Added to this uncertainty are the combined errors of counting and of estimation of RNA values on small samples of RNA in effluent tubes. Bearing in mind these limitations, the following rough calculations may be made. From a starting ma-

terial containing 1.05 mμmoles of valine per mg RNA, we have obtained an RNA containing 26.5 mμmoles of valine per mg RNA after fractionation (cf. Table 1). If a molecular weight of 25,000 is used, the valyl-RNA would be 66 per cent pure, while a molecular weight of 30,000 for the RNA would result in a calculation of 80 per cent purity.

In several other experiments, using conditions similar to those outlined in Figure 1, we have obtained specific activities of valyl-RNA in the same range as those given in Figure 1-*B*. In a single experiment, the specific activity of several effluent tubes reached an estimated 80 per cent of purity, based on a molecular weight of 25,000.

In another single experiment, we have employed medium grade DEAE-dextran, as furnished by Pharmacia (DEAE-Sephadex-A-25, medium), rather than coarse grade for fractionation of RNA first labeled with C^{14}-valine, then subjected to the dye addition procedure. In five contiguous peak tubes, totaling 45 μg of RNA, the average specific activity was 63,000 cpm per mg RNA or 36 mμmoles of valine per mg RNA. This represents a purification in the range of 90 per cent, using 25,000 as a molecular weight for the RNA, or close to 100 per cent if the molecular weight is in fact somewhat higher.

In addition to a very critical range of NaCl concentration, the concentration of the Tris-HCl buffer plays an important role in the pattern of elution of S-RNA. In one experiment, at a low concentration of Tris-HCl buffer (0.005 *M*), even in the presence of 0.5 *M* NaCl, 85 per cent of S-RNA failed to elute from the column. The chloride ion in the buffer undoubtedly contributes a great deal to this effect.

Cross-linked dextrans alone, without DEAE (Sephadex-G-25 and G-75), do not fractionate S-RNA under the conditions used. Both types of dextran, which differ only in the degree of cross-linking, fail to retard the passage of the RNA molecules, so that virtually all of the RNA emerges immediately. DEAE-cellulose, an anion exchanger, does give a three-fold enrichment of valyl-RNA (cf. also refs. 18 and 20) following elution of the RNA with increasing concentrations of NaCl in the presence of 0.1 *M* Tris-HCl buffer at pH 7.5.

Discussion.—The use of DEAE-dextran for isolation of valyl-RNA from the family of closely related S-RNA molecules provides a surprisingly effective means for achieving a separation. Neither DEAE-cellulose nor dextran alone is sufficient, and the combination is thus a synergistic one. It is interesting to note in Figure 1-*A* that roughly 20 peaks of RNA are eluted in the gradient between 0.35 and 0.40 *M* NaCl. Whether or not these peaks represent partial separations of amino acid–specific RNA molecules remains to be determined.

A further improvement is obtained when the DEAE-dextran procedure is combined with the dye-addition technique. The dye-RNA remains in large measure visibly close to the point of addition at the top of the column, while the valyl-RNA emerges early during the elution procedure. The effect of systematic variations of pH and salts has not been explored, nor has the use of substituents other than DEAE in the body of the dextran polymer. The particle size of the DEAE-dextran also plays a role in the elution pattern. In the single experiment using DEAE-Sephadex-A-25 medium grade instead of coarse grade, the fractionation of valyl-RNA was improved. Using the "fine" grade of this material, we have had difficulty maintaining satisfactory flow rates on columns of the dimen-

sions mentioned in the *Methods* section. The rate of flow maintained in the column plays a significant role in determining the point of emergence of RNA at a given salt concentration and grade of DEAE-dextran. Fractionation of valyl-RNA on a less highly cross-linked polymer (DEAE-Sephadex-A-50) was not quite so good as with the DEAE-Sephadex-A-25.

Further improvements in fractionation may also well be possible with more effective chemical procedures[25, 26] prior to use of the DEAE-dextran column. A favorable feature of the present fractionation is the stability of the valyl-RNA bond under these conditions, an 85 per cent recovery of valyl-RNA being obtained, as indicated in Table 1.

It would be important to ascertain whether the presence of amino acid esterified to the RNA influences the rate of migration of this molecule through the column. We have not yet answered this question. That not all species of aminoacyl-RNA emerge before the bulk of the non–amino acid RNA is already clear from the experiments with tyrosyl-RNA and seryl-RNA. It is also tempting to consider that individual variations in secondary structure of S-RNA molecules as well as variations in nucleotide composition may play a large role in the ability of the column to separate them. This situation has a parallel in the separability of proteins on the basis of properties conferred on them by the individuality of their space-filling configurations and total net charges rather than as a sole consequence of their amino acid composition in the extended peptide chain.

Summary.—The combined use of a dye-addition method followed by DEAE-dextran column chromatography has been employed in an effort to isolate valyl-RNA, and a purification of the order of 65–80 per cent has been obtained. Further improvements in technique seem possible and a single experiment has given a purification in the vicinity of 90 per cent.

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*THE OPTICAL ROTATORY DISPERSION OF RIGHT-HANDED α-HELICES IN SPERM WHALE MYOGLOBIN**

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Optical rotatory dispersion studies indicate that most synthetic polypeptides known to be helical in solution have an identical screw sense. In terms of the dispersion properties of these substances, this method moreover provides evidence that many proteins contain helical regions of varying extent characterized by the same handedness.[1] Although several lines of argument imply that this prevailing