two or more guanylic acid in RNA. In TMV-RNA, this value is larger than in yeast RNA, and both deviate significantly from the random distribution. In these RNA's guanine nucleotide tends to be next to guanine nucleotide. The RNase T_1 attacked the highly polymerized RNA as well as low-molecular-weight RNA.

Further studies on the nucleotide distribution in RNA with the combination of the use of RNase T_1, pancreatic RNase I, and other methods are now in progress.

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Isolation of an enzyme catalyzing the transfer of amino acids from soluble RNA to microsomal ribonucleoprotein

In the course of incorporation of amino acids into microsomal RNP, recent investigations have demonstrated that activated amino acids formed a complex with a soluble RNA of relatively low molecular weight, and that the amino acids so bound to s-RNA were subsequently transferred to RNP[1,2]. Although it has been assumed that the latter transfer reaction is catalyzed by an enzyme which might be called "transferring enzyme" present in the cytoplasmic supernatant[2,3], isolation of such an enzyme has not yet been accomplished.

An approx. 15 % rat-liver homogenate was prepared in 0.25 *M* sucrose, 0.025 *M* KCl, 0.005 *M* $MgCl_2$, and 0.05 *M* Tris buffer, pH 7.6, and was centrifuged for 2 h at 105,000 × *g*. The supernatant fluid thus obtained was removed and its pH was lowered to 4.8–5.0 by 1 *N* acetic acid. The resulting precipitate was removed by a low-speed centrifugation (pH-5 fraction), and the pH of the supernatant was immediately adjusted again to pH 7.6 (pH-5 supernatant). Since the pH-5 supernatant showed a higher transferring activity than the pH-5 fraction and it does not catalyse activation of amino acids or overall incorporation of amino acids into RNP[2–4], the pH-5 supernatant was used for the isolation of the transferring enzyme. The method of purification of RNP from the microsome was the same as described in the preceding paper[5],

Abbreviations: RNA, ribonucleic acid; s-RNA, soluble RNA; RNP, ribonucleoprotein; GTP, ATP, CTP, and UTP, triphosphates of guanosine, adenosine, cytidine, and uridine; Tris, tris-(hydroxymethyl)aminomethane; DEAE-cellulose, diethyaminoethyl-cellulose.

and s-RNA–[^{14}C]leucine was prepared from the pH-5 fraction by the method of HOAGLAND *et al.*[1].

The pH-5 supernatant, without dialysis, was poured onto an DEAE-cellulose column, 1 cm in diameter and 8 cm in height, and the column was washed with 0.03 *M* KCl containing 0.02 *M* Tris buffer, pH 7.6. Approx. 20 % of the protein was adsorbed on the column, forming a yellow band, while the bulk of the protein contained in the original pH-5 supernatant passed through the column. The adsorbed protein can be eluted from the column with a concentration of KCl between 0.08 and 0.3 *M*. In a preliminary experiment, the adsorbed protein was eluted in two steps by using 0.15 *M* KCl and 0.3 *M* KCl each containing 0.02 *M* Tris buffer, pH 7.6, after washing the column with 0.08 *M* KCl–0.02 *M* Tris buffer, pH 7.6.

Transferring activity was observed only with the protein adsorbed on DEAE-cellulose, and was highest in the fraction eluted between 0.15–0.3 *M* KCl, as shown in Table I. The active fraction gave an absorption maximum at 280 mμ and a minimum at 250 mμ, showing a typical spectrum of a protein. When the fraction was boiled and added instead of the "native" fraction, no transfer occurred. The enzyme preparation has a considerable stability and could be kept at least one week in a refrigerator without loss of activity, whereas the original pH-5 supernatant quickly lost its activity. It was also found that the enzyme fraction does not catalyse the incorporation of free [^{14}C]leucine into RNP or s-RNA.

TABLE I

FRACTIONATION OF THE pH-5 SUPERNATANT FROM RAT-LIVER CYTOPLASM BY DEAE-CELLULOSE COLUMN

Incubation mixture contained, in a final volume of 1.0 ml per tube, 2.5 mg purified RNP; s-RNA–[^{14}C]leucine, 180 counts/min (A_{260} = 1.6); 0.5 μmole ATP; 0.5 μmole GTP; 10 μmoles phosphocreatine, 0.05 mg creatine kinase; 5 μmoles $MgCl_2$; 30 μmoles KCl; 50 μmoles Tris buffer, pH 7.6. 0.1 ml of each fraction was added and the mixture incubated for 15 min at 37°. The reaction was stopped by 0.5 *N* $HClO_4$, washed once with cold 0.5 *N* $HClO_4$, and then the RNA was hydrolyzed by incubating for 15 min at 70° in 0.5 *N* $HClO_4$. The remaining protein fraction was washed twice with cold 0.2 *N* $HClO_4$ and mounted on a filter paper. The ^{14}C activity was counted by a low-background gas-flow counter (background count lower than 1 count/min).

Fractions	*Protein (mg)*	*Activity transferred (counts/min)*	*Protein recovery (mg)*
Whole pH-5 supernatant	0.73	18	
	2.90	92	279
DEAE-cellulose			
non-adsorbed fraction	0.60	9	210
0.08–0.15*M* KCl eluate	0.61	72	24
0.15–0.3 *M* KCl eluate	0.55	94	17

When the effect of the other cofactors on the transfer reaction was studied, it was found that only GTP is essential for the transfer, and that ATP or any other cofactor tested has no effect (Table II). Addition of cell dialyzate inhibits the reaction. An observation that ATP and an ATP-generating system is essential in this transfer[3] may be accounted for the fact that the whole pH-5 supernatant was used as the enzyme fraction.

TABLE II

THE COFACTOR REQUIREMENTS FOR THE TRANSFER REACTION

Incubation mixture contained, in a final volume of 1.0 ml per tube, 2.5 mg RNP; s-RNA–[^{14}C]leucine 180 counts/min (A_{260} = 1.6); 0.55 mg enzyme fraction; 5 μmoles $MgCl_2$; 30 μmoles KCl; 50 μmoles Tris buffer, pH 7.6. After incubation for 15 min at 37°, the reaction was stopped by 0.5 *N* $HClO_4$ and counts in protein were determined according to the method described in Table I. ATP, GTP, UTP, and phosphocreatine were supplied by the Sigma Chemical Co.

Incubation conditions	*Activity transferred (counts/min)*
Incubation mixture	0
+ 0.3 μmole GTP	97
+ 0.3 μmole ATP	6
+ 0.3 μmole CTP	7
+ 0.3 μmole UTP	5
+ GTP, ATP, CTP, UTP, each 0.3 μmole	104
+ 0.3 μmole GTP, 10 μmoles phosphocreatine, 0.05 mg creatine kinase	102
+ 0.3 μmole GTP, 0.3 μmole ATP, 10 μmoles phosphocreatine, 0.05 mg creatine kinase	98
+ 0.3 μmole GTP, cell dialyzate	75

Further purification and characterization of the transferring enzyme are in progress.

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Isolation of free acid-soluble nucleotide peptides from normal rat liver

It has been shown by WEINSTEIN *et al.*[1] that normal rat liver contains nucleotides of adenine, cytosine and uracil associated with amino acids or peptides. WILKEN AND HANSEN[2] also identified two adenine nucleotide peptides from bovine liver.

During our investigation of nucleotide peptides from normal rat liver we have been able to isolate three different peptides associated with an adenine–free acid-soluble nucleotide.

25 Wistar rats fed on stock laboratory diet have been utilised for this purpose. The animals, divided in groups A, B and C, were killed by exsanguination, the livers

Abbreviations: ADP, adenosine 5′-diphosphate; UDPAG, uridine 5′-diphosphate acetyl glucosamine; UDPG, uridine 5′-diphosphate glucose; DPN, diphosphopyridine nucleotide; UMP, uridine 5′-monophosphate; CMP, cytidine 5′-monophosphate; and TMP, thymidine 5′-monophosphate.