A SIMPLIFIED PROCEDURE FOR THE PREPARATION OF "SOLUBLE" RNA FROM RAT LIVER[1/]

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A simplified procedure that would give good yields of "soluble" RNA from mammalian tissue was essential because of the current studies on the purification and composition of different amino acid-acceptor "soluble" RNAs. Such a procedure, involving direct homogenization of the tissue with phenol, is described in this communication. The isolation of a mixture of the high molecular weight RNA and "soluble" RNA after homogenization of rat liver with phenol was reported recently (Sakar, 1962).

White rats were stunned and decapitated. The livers were removed and immediately placed into an ice bath. The following operations were carried out in a cold room (4° C). One hundred grams of liver were homogenized in a Waring Blendor with 150 ml of phenol (Mallinckrodt, Analytical Reagent, 88%) saturated with water (15:3) and 150 ml of 1.0 M NaCl, 0.005 M EDTA in 0.1 M Tris-chloride[2/] buffer, pH 7.5. The homogenate was spun for ten minutes at top speed in the International clinical centrifuge and the upper layer was carefully decanted off. To this aqueous layer, three volumes of 95% ethanol were added. The resultant precipitate was spun down at top speed in the

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2/ The abbreviations used are: Tris, tris (hydroxymethyl) amino methane; EDTA, ethylenediaminetetraacetic acid and DEAE-cellulose, diethylaminoethyl-cellulose.

International clinical centrifuge and resuspended in 250 ml of 0.1 M Tris-chloride buffer, pH 7.5. This solution was added (flow rate of 15-20 drops per minute) to a column (2 x 10 cm) of 2 g of DEAE-cellulose previously equilibrated with cold 0.1 M Tris-chloride buffer, pH 7.5. The column was then washed with 1 L of Tris-chloride buffer, pH 7.5 and the RNA eluted with 1.0 M NaCl in 0.1 M Tris-chloride buffer, pH 7.5. The first 10 ml of NaCl solution were discarded as "hold-up." Sufficient salt solution (60-80 ml) was then collected until the optical density of the effluent was less than three at 260 mμ. This solution was extracted twice with an equal volume of phenol saturated with water and twice with ether. To the aqueous solution containing the RNA, three volumes of 95% ethanol were added and the solution was allowed to stand overnight in the cold. The precipitate was spun down and washed first with 80% and then twice with 95% ethanol and dried in a vacuum.

Approximately 60 mg of "soluble" RNA were obtained from a 100-g lot of rat liver. The absorbancy at 260 mμ of a 0.1 mg per ml solution was 2.0. The 240/260 ratio was 0.6 while the 260/280 ratio was 1.7. The amino acid-acceptor activity was assayed by the usual procedure (Holley, et al., 1961). A comparison of the activities of the "soluble" RNA prepared by this method, and RNA recovered from the pH 5 enzymes (Holley and Goldstein, 1959), is shown in Table 1. The activity of the two preparations is equivalent; however, the yield from the present method is approximatly three times greater.

References

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Table 1

AMINO ACID ACCEPTOR ACTIVITY OF "SOLUBLE" RNA*

Amino Acid	Rat Liver RNA†	
	Present Method	From pH 5 Enzymes
Lysine	3000	2800
Tyrosine	4900	4600
Valine	1850	2250

*The activity is expressed as counts/minute/mg RNA.

The specific activities of lysine, tyrosine and valine were 4.5, 22.9 and 6.5 mC/mM, respectively.

The amino acid activating enzymes were prepared according to the procedure for the histidine-activating enzyme (Doctor, et al., 1961), except that only one precipitation at pH 5.0 preceded application of the enzymes to the DEAE-cellulose column.

†An active RNA from fresh beef liver (kindly supplied by Dr. G. H. Wellington) was also obtained with this procedure. The yield from one pound was approximately 200 mg.