Isolation of "soluble RNA" without an ultracentrifuge

Three things distinguish the RNA found in the soluble fraction of cell homogenates fractionated by differential centrifugation: (a) small size, (b) high content of 5-ribosyl uracil monophosphate[1], and (c) ability to accept specific amino acids[2] and transfer them under enzymic control to microsomal protein[3]. CRESTFIELD, SMITH AND ALLEN[4] found that a portion of yeast RNA was not precipitable by 1 *M* NaCl at 0°. DAVIS AND ALLEN[5] found that a new nucleotide (now identified as 5-ribosyl uracil monophosphate[6,7]) was present in high concentration in the fraction not precipitated by NaCl. OTAKA, HOTTA AND OSAWA[8] have recently equated the yeast RNA fraction which is not precipitable in NaCl with that fraction which is not sedimented at 105,000 × *g* for 90 min (soluble RNA of yeast) on the basis of their similar nucleotide composition and more specifically their high content of 5-ribosyl uracil monophosphate. DUNN[1] has shown that in mammalian cells soluble RNA is distinguished from microsomal RNA by its high content of 5-ribosyl uracil monophosphate. The present paper reports that the soluble RNA of mammalian cells as prepared by differential centrifugation is very similar or identical with the RNA which remains soluble in 1 *M* NaCl, as judged by its ability to accept amino acids under enzymic control.

Activating enzymes (pH 5 enzymes) were prepared from calf thymus and freed of RNA by treatment with protamine. After the respective cell fractions were separated by differential centrifugation, the RNA was isolated by extraction with phenol[9]. Part of the RNA was then further treated with cold 1 *M* NaCl[4]. The assay for the incorporation of amino acids into RNA was performed[10] using [^{14}C]algal-protein hydrolysate as the amino acid source.

TABLE I

INCORPORATION OF AMINO ACIDS BY VARIOUS RNA FRACTIONS FROM CALF THYMUS

RNA sample	*Specific activity**	
	No treatment	*RNA soluble after 1 M NaCl treatment*
Soluble RNA (small-scale preparation)	393**	—
Soluble RNA (large-scale preparation)	175	249
Non-nuclear RNA	134	578
Whole-cell RNA	88	430

* Counts/min/unit of absorbancy at 260 mμ.
** Average of duplicate assays.

Two samples of soluble RNA were studied. The large scale preparation was distinguished from the small scale preparation in that the 105,000 × *g* supernatant from the former preparation stood for several hours longer at 4° before extraction with phenol. The non-nuclear RNA was isolated from the cell fraction remaining after centrifugation at 3,500 × *g* for 10 min.

It is felt that the lowered ability of the bulk preparation of soluble RNA to take up amino acids as compared with the small-scale preparation (Table I) is probably due to enzymic degradation of the former sample because of a necessary delay in

Abbreviation: RNA, ribonucleic acid.

mixing the sample with phenol. The bulk preparation, however, did show an increase in activity after the removal of material insoluble in 1 *M* NaCl from the sample. The RNA remaining after removing the RNA insoluble in 1 *M* NaCl from the non-nuclear and whole-cell samples of RNA showed a higher ability to take up amino acids than our best sample of soluble RNA obtained by differential centrifugation. This is probably a reflection of the fact that much less time elapsed before these samples were treated with phenol to stop enzymic action.

These results will allow the easy bulk preparation of these biologically active ribonucleic acids without the necessity of the slow ultracentrifugation technique which can result in the isolation of a partially degraded product.

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The isolation of androsterone and aetiocholanolone from the urine of oophorectomised-adrenalectomised women

There have been several reports that patients with metastatic mammary cancer may continue to excrete various steroid hormones or their metabolites in the urine after the removal of the ovaries and the adrenal glands (for reviews, see ref. 1–3). The amounts of steroids found in the urine of such patients are very small and evidence for the identity of the compounds in question has generally been based on their chromatographic properties or, in the case of the oestrogens, on their biological activity[4].

Rigorous proof is needed for the identity of androsterone and aetiocholanolone since Δ 9–(11) artefacts produced by the dehydration of cortisone metabolites are very difficult to separate from androsterone and aetiocholanolone unless chemical modification is carried out[5]. PLANTIN *et al.*[6] found only Δ 9–(11) aetiocholenolone in urine obtained from adrenalectomized patients, a result which did not agree with the findings of KELLIE AND WADE[7], BULBROOK, GREENWOOD AND THOMAS[8] and HOBKIRK[9,10] that such patients continue to excrete androsterone and aetiocholanolone.

An attempt was therefore made to isolate and identify the compounds in the