- Campbell, P. N., Cooper, C. & Hicks, M. (1964). *Biochem. J.* **92**, 225.
- Campbell, P. N. & Lowe, E. (1967). In *Regulation of Nucleic Acid and Protein Biosynthesis*, p. 373. Ed. by Koningsberger, V. V. & Bosch, L. Amsterdam: Elsevier Publishing Co.
- Campbell, P. N., Lowe, E. & Serck-Hanssen, G. (1967). *Biochem. J.* **103**, 280.
- Campbell, P. N., Serck-Hanssen, G. & Lowe, E. (1965). *Biochem. J.* **97**, 422.
- Ganosa, M. C. & Williams, C. A. (1969). *Proc. natn. Acad. Sci. U.S.A.* **63**, 1370.
- Korner, A. (1966). *Biochem. J.* **101**, 627.
- Layne, E. (1957). In *Methods in Enzymology*, vol. 3, p. 450. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Lawford, G. R., Langford, P. & Schachter, H. (1966). *J. biol. Chem.* **241**, 1835.
- Macdonald, R. I. & Korner, A. (1971). *FEBS Lett.* **13**, 62.
- Marsh, J. B., Drabkin, D. L., Braun, G. A. & Parks, J. S. (1966). *J. biol. Chem.* **241**, 4168.
- Mejbaum, W. (1939). *Hoppe-Seyler's Z. physiol. Chem.* **258**, 117.
- Munro, A. J., Jackson, R. J. & Korner, A. (1964). *Biochem. J.* **92**, 289.
- Porterfield, J. S. (1960). *Bull. Wld Hlth Org.* **22**, 373.
- Ragnotti, G., Lawford, G. R. & Campbell, P. N. (1969). *Biochem. J.* **112**, 139.
- Ragnotti, G., Cajone, F. & Bernelli-Zazzera, A. (1970). *Expl molec. Path.* **13**, 295.
- Wettstein, F. O., Staehelin, T. & Noll, H. (1963). *Nature, Lond.*, **197**, 430.

APPENDIX

A Rapid Method for the Determination of Ribonucleic Acid in Subcellular Fractions

By GIOVANNI RAGNOTTI

Institute of General Pathology, University of Milan, 20133 Milan, Italy

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The methods available for RNA determination (Mejbaum, 1939; Schneider, 1957; Scott, Fraccastoro & Taft, 1956; Fleck & Munro, 1962), though very accurate, are time consuming because of the

many steps involved. They are not suitable for the rapid determination of RNA in the samples to be assayed for protein-synthesizing activity. The present method has therefore been developed.

Table 1. *RNA content of liver subcellular fractions determined by methods A and B (see the text)*

Values represent the means $\pm$ s.e.m. of the number of experiments given in parentheses. The A/B ratio was calculated for every pair of RNA values and is given as the mean $\pm$ s.e.m. Regression equations were calculated by the program GZC 122 for the Olivetti Programma 101 desk-top computer. The range of RNA amounts (mg/ml) for which the methods were compared is given in brackets. In the regression equations ($y = a + bx$) x = mg of RNA/ml (method A) and y = mg of RNA/ml (method B).

	RNA content (mg/ml)				Ratio A/B
	Method A	$\frac{\text{Mean}}{\text{s.e.m.}} \times 100$	Method B	$\frac{\text{Mean}}{\text{s.e.m.}} \times 100$	
Post-mitochondrial supernatant (22)	4.57 $\pm$ 0.491 [0.94–9.16]	11	2.26 $\pm$ 0.240 [0.48–4.76]	11	2.03 $\pm$ 0.044
Unfractionated microsomes (40)	1.82 $\pm$ 0.307 [0.33–9.90]	17	1.35 $\pm$ 0.223 [0.25–7.57]	17	1.35 $\pm$ 0.022
Membrane-bound ribosomes (31)	2.14 $\pm$ 0.419 [0.44–9.85]	20	1.28 $\pm$ 0.234 [0.25–5.44]	18	1.67 $\pm$ 0.026
Membrane-free ribosomes (23)	2.26 $\pm$ 0.782 [0.29–16.36]	35	2.40 $\pm$ 0.820 [0.31–17.24]	34	0.93 $\pm$ 0.010
Detergent-treated polyribosomes (25)	4.07 $\pm$ 1.200 [0.18–19.48]	29	4.51 $\pm$ 1.352 [0.27–23.55]	30	0.90 $\pm$ 0.025
Fraction	Regression equation		<i>F</i>		
Post-mitochondrial supernatant	$y = 0.07081 + 0.4780 x$		334		
Unfractionated microsomes	$y = 0.01008 + 0.73615 x$		5180		
Membrane-bound ribosomes	$y = 0.08800 + 0.55848 x$		3116		
Membrane-free ribosomes	$y = 0.03536 + 1.04762 x$		32038		
Detergent-treated polyribosomes	$y = -0.04440 + 1.12080 x$		2342		

For preparations of isolated ribosomes, RNA may be accurately measured by its extinction at 260nm, by the equation:

$$\text{RNA (mg/ml)} = \frac{E_{260} - (E_{320} \times 1.59)}{20} \times \text{dilution}$$

(Loeb, Howell & Tomkins, 1967) where 20 is the extinction of 1mg of RNA/ml for a 1cm light-path cell and 1.59 is a factor to evaluate ferritin interference on the reading at 260nm. However, this equation could not be used directly for the determination of RNA in crude subcellular fractions (Attardi, Cravioto & Attardi, 1969).

To overcome this difficulty, the values obtained for RNA concentrations by using the formula of Loeb *et al.* (1967) (method A) were compared with those found for the same samples by using the orcinol method (Mejbaum, 1939) (method B). It was found (Table 1) that for samples from similar fractions, the correlation between the two sets of values is highly significant ($P < 0.001$), giving in each case a regression equation that is linear ($P < 0.001$). The high values found for F by analysis of variance (see Table 1) indicate that the experimental results lie on the theoretical regression line. Moreover, as the standard errors of the means of the RNA values determined for a given fraction, by methods A and B, represent the same percentage of the means, we may conclude that the two methods have the same precision.

On the basis of the results presented in Table 1, a conversion factor (A/B ratio) was calculated for each fraction so that the true RNA concentration (orcinol RNA) could be obtained from the value given by method A. We therefore suggest that, under well-defined conditions, method A may be taken as an alternative to the pre-existing ones for RNA determination in subcellular fractions, provided that the appropriate conversion factor is used. The validity of this approach has also been confirmed under different experimental conditions, namely with ischaemic livers (Ragnotti, Cajone & Bernelli-Zazzera, 1970).

REFERENCES

- Attardi, B., Cravioto, B. & Attardi, G. (1969). *J. molec. Biol.* **44**, 47.
- Fleck, A. & Munro, H. N. (1962). *Biochim. biophys. Acta*, **55**, 571.
- Loeb, J. N., Howell, R. R. & Tomkins, G. M. (1967). *J. biol. Chem.* **242**, 2069.
- Mejbaum, W. (1939). *Hoppe-Seyler's Z. physiol. Chem.* **258**, 117.
- Ragnotti, G., Cajone, F. & Bernelli-Zazzera, A. (1970). *Expt molec. Path.* **13**, 295.
- Schneider, W. C. (1957). In *Methods in Enzymology*, vol. 3, p. 680. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Scott, J. F., Fraccastoro, A. P. & Taft, E. B. (1956). *J. Histochem. Cytochem.* **4**, 1.