

The infrared spectrum of I shows two strong bands in the metal carbonyl stretching region at 5.09 and 5.31 microns.

The carbonyl (I) also can be prepared by treatment of II with two equivalents of butyllithium and then reaction with carbon monoxide, or by reaction of titanium tetrachloride in benzene with four equivalents of cyclopentadienyl sodium with subsequent reaction with carbon monoxide. Heating bis-(cyclopentadienyl)-titanium dichloride or diphenyl bis-(cyclopentadienyl)-titanium with carbon monoxide resulted in the formation of little or no I.

Details of this work and some reactions of I will be reported at a later date.

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### COUNTERCURRENT DISTRIBUTION OF AN ACTIVE RIBONUCLEIC ACID

Sir:

The enzymatic transfer of activated amino acids to ribonucleic acid (RNA), suggested by the discovery of an alanine-dependent, ribonuclease-inhibited incorporation of AMP into ATP,<sup>1</sup> and subsequently established by the work of Hoagland, *et al.*,<sup>2</sup> Ogata and Nohara,<sup>3</sup> Berg and Ofengand,<sup>4</sup> Lipmann, *et al.*,<sup>5</sup> Schweet, *et al.*,<sup>6</sup> and Zamecnik, *et al.*,<sup>7</sup> is of interest as a possible intermediate reaction in protein synthesis.

This communication presents the results of countercurrent distribution of RNA isolated from the "soluble" fraction of rat liver homogenate.<sup>8</sup> The solvent system described by Warner and Vaimberg<sup>9</sup> was used with slight modification, and the distribution was carried out in the 100-tube countercurrent apparatus described by Raymond.<sup>10</sup>

As shown in Fig. 1, countercurrent distribution of the RNA gives a broad distribution pattern, measured by absorption at 260 m $\mu$ . Redistribution (Fig. 2) of material from different parts of the first

(1) R. W. Holley, Abstracts, 130th Meeting, American Chemical Society, Atlantic City, N. J., Sept. 1956, p. 43C; R. W. Holley, *THIS JOURNAL*, **79**, 658 (1957).

(2) M. B. Hoagland, P. C. Zamecnik and M. L. Stephenson, *Biochim. et Biophys. Acta*, **24**, 215 (1957); M. B. Hoagland, M. L. Stephenson, J. F. Scott, L. I. Hecht and P. C. Zamecnik, *J. Biol. Chem.*, **231**, 241 (1958).

(3) K. Ogata and H. Nohara, *Biochim. et Biophys. Acta*, **25**, 659 (1957).

(4) P. Berg and E. J. Ofengand, *Proc. Natl. Acad. Sci.*, **44**, 78 (1958).

(5) F. Lipmann, *ibid.*, **44**, 67 (1958); S. B. Weiss, G. Acs and F. Lipmann, *ibid.*, **44**, 189 (1958); J. Mager and F. Lipmann, *ibid.*, **44**, 305 (1958); H. G. Zachau, G. Acs and F. Lipmann, *ibid.*, **44**, 885 (1958).

(6) R. S. Schweet, F. C. Bovard, E. Allen and E. Glassman, *ibid.*, **44**, 173 (1958); E. Glassman, E. H. Allen and R. S. Schweet, *THIS JOURNAL*, **80**, 4427 (1958).

(7) P. C. Zamecnik, M. L. Stephenson and L. I. Hecht, *Proc. Natl. Acad. Sci.*, **44**, 73 (1958); L. I. Hecht, M. L. Stephenson and P. C. Zamecnik, *Biochim. et Biophys. Acta*, **29**, 460 (1958).

(8) Isolation of the RNA after phenol treatment, and assay of RNA by alanine-dependent AMP incorporation using purified alanine-activating enzyme will be described in detail by R. W. Holley and J. Goldstein, manuscript in preparation.

(9) R. C. Warner and P. Vaimberg, *Fed. Proc.*, **17**, 331 (1958). The amount of formamide was increased 10% in these experiments. By varying the concentrations of 2-propanol and formamide, the gross partition coefficient of the rat liver RNA can be varied at least from 0.1 to 1.5.

(10) S. Raymond, *Anal. Chem.*, **30**, 1214 (1958).

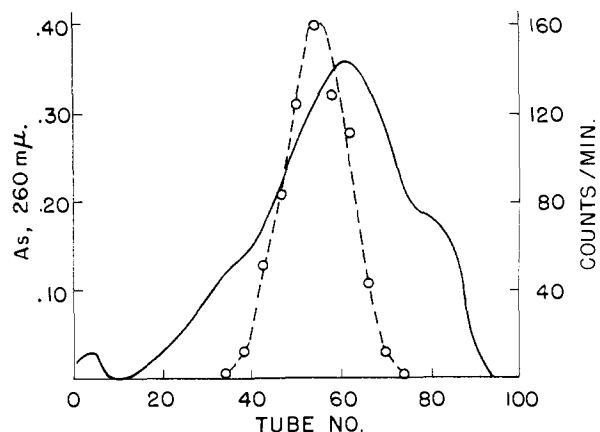


Fig. 1.—Countercurrent distribution of 20 mg. of RNA isolated from the "soluble" fraction of rat liver homogenate: —, absorbance of fractions, measured in the Beckman DU spectrophotometer; —○—, activity of fractions, after dialysis and lyophilization, given as counts/min. observed in Ba<sub>2</sub>ATP obtained from assay for activity in the alanine-dependent incorporation of radioactive AMP into ATP (blank value of 5 counts/min. subtracted).

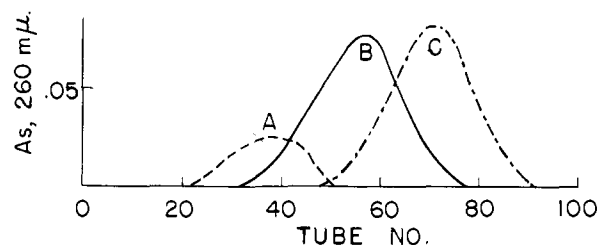


Fig. 2.—Redistribution of fractions of the RNA: the contents of these tubes from the distribution shown in Fig. 1 were placed in tubes 1-6 or 7 of the apparatus at the start of the redistribution: A, tubes 25, 27, 29, 31, 33, 35, 37; B, tubes 45, 47, 49, 51, 53, 55; C, tubes 65, 67, 69, 71, 73, 75.

distribution pattern establishes that there has been actual fractionation of the RNA.

Using alanine-dependent AMP incorporation<sup>1,8</sup> to assay for the alanine-active RNA, activity is found significantly displaced from the peak of ultraviolet absorption and separated from much of the original RNA (Fig. 1). The location of activity for amino acids other than alanine remains to be determined.

Because of the possibility that different molecular species of "soluble" fraction RNA act in protein synthesis by transferring specific amino acids to the "template," and the possibility that amino acid specificity is carried in nucleotide sequences which may be related to nucleotide sequences in DNA, the isolation of these different molecular species of RNA is a problem of great significance. The above results indicate that countercurrent distribution furnishes a promising approach to this problem.

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