Biochimica et Biophysica Acta, 1000 (1989) 99
Elsevier

Commentary by

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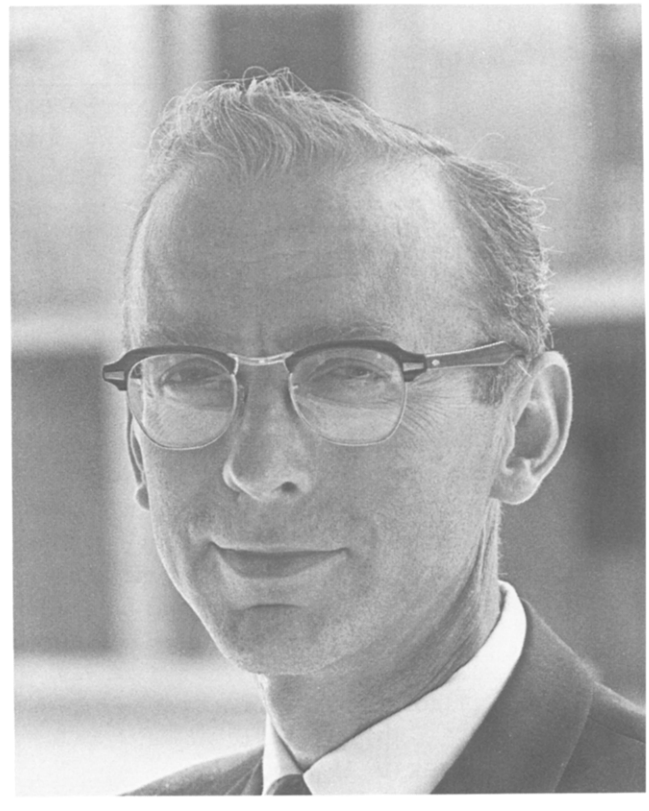
on 'Countercurrent distribution of rat-liver "soluble"-fraction ribonucleic acids'
by R.W. Holley, B.P. Doctor, S.H. Merrill and F.M. Saad
Biochim. Biophys. Acta 35 (1959) 272–273

This article described the first separation of different amino-acid-specific tRNAs by countercurrent distribution.

Publication of this article changed attitudes about the separation of tRNAs. At the time of its publication, the prevailing view was that different amino-acid-acceptor tRNAs would be too similar to be separated by any standard fractionation technique. It was thought that there would be only minor variations in nucleotide sequence between different tRNAs, and that any differences in physical properties would be negligible. The results of the countercurrent distribution showed otherwise. Countercurrent distribution was a particularly useful technique to demonstrate differences between tRNAs because the experimental results are essentially free from adsorption artifacts and show a close fit to calculated theoretical distribution curves [1,2]. The experimental results in this article demonstrated that different amino-acid-specific tRNAs partitioned differently between two immiscible solvents, suggesting that the tRNAs could be separated by this technique.

Countercurrent distribution was subsequently used to obtain the first pure species of amino acid-acceptor tRNAs [3], and this made possible the first determination of the nucleotide sequence of an RNA [4].

The history of this work highlights the importance of maintaining an open mind about unpopular research approaches. In particular, there is a need for peer reviewers to be very cautious in their criticism of unconventional research proposals.



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Countercurrent distribution of rat-liver, "soluble"-fraction ribonucleic acids

It has been reported[1] that the ribonucleic acids isolated from the soluble fraction of rat-liver homogenate can be fractionated by countercurrent distribution. These ribonucleic acids are of interest as acceptors of enzymically activated amino acids and are believed to take part in intermediate stages of protein synthesis. In this note we wish to report that countercurrent distribution for 250 transfers gives almost complete separation of the alanine-active ribonucleic acid from the tyrosine-active ribonucleic acid.

Starting with 60 mg of the rat-liver ribonucleic acid preparation, countercurrent distribution was carried out as previously described[1]. After 100 transfers, the contents of tubes 1–40 and 71–100 of the apparatus were emptied (removing approximately 40 % of the ultraviolet-absorbing material), and the empty tubes were refilled with fresh solvents. The apparatus was arranged to recycle, and the distribution was continued for 150 more transfers. As shown in Fig. 1, the material that remained in tubes 41–70 at the end of 100 transfers was spread over approximately 80 tubes.

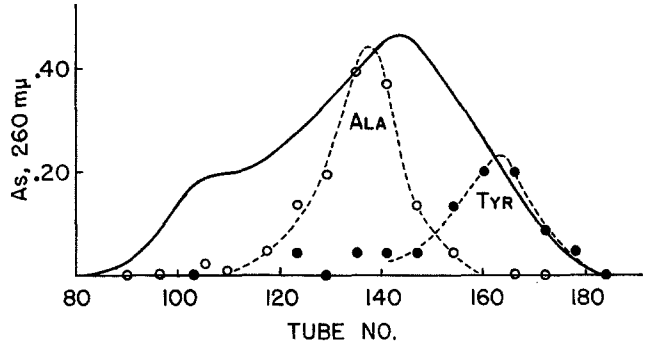

Fig. 1. 250-Transfer countercurrent disribution of rat-liver, "soluble"-fraction ribonucleic acid: ———, absorbance of fractions, measured in the Beckman DU spectrophotometer; —○—, activity for alanine; —●—, activity for tyrosine (acivities in arbitrary units).

The ribonucleic acids present in the fractions were reisolated by dialysis and lyophilization. Assays for amino acid-acceptor activity demonstrated that the peak of activity for alanine was separated by approximately 25 tubes from the peak of activity for tyrosine, with relatively little overlapping of the two activity curves (Fig. 1). (Alanine activity was determined by alanine-dependent adenosine monophosphate incorporation[2,3], and tyrosine activity by incorporation of tyrosine into the ribonucleic acid fractions[4]). However, the recovery of activity was low (approx. 40 %), due in part to the high pH of the solvent system. As a result, the increase in specific activities of the reisolated active fractions was only approximately 2-fold.

Experiments are underway to improve and extend these results.

This work was supported in part by grants from the National Science Foundation and the Research Corporation.

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Received June 15th, 1959