ELECTROPHORETIC SEPARATION OF RNA FROM SNAIL EMBRYOS

TSANEV[1] claimed that agar-gel electrophoresis would clearly separate RNA of various S values. Later Dessev *et al.*[2] reported separation of ribosomes by ion-agar electrophoresis.

We have now succeeded in obtaining reasonably clear separation of embryonic RNA from *Limnæa* with the help of ion-agar electrophoresis and compared the results with sucrose-density-gradient. It seems that though in many respects ultracentrifugation is a superior method, considerable information can be obtained with this very simple technique of electrophoresis and at least in one respect it is more valuable than density-gradient.

Limnæa eggs and embryos collected from the pond or from vessels in the laboratory (maintained on dry lettuce) were fed with ^{32}P (Trombay) isotope solution at different developmental stages and the RNA was extracted by shaking with hot phenol.[3] Marker RNA, *i.e.*, non-radioactive RNA was likewise extracted from *E. coli* and mixed with snail RNA and precipitated by cold alcohol. After centrifugation the RNA pellet was dissolved in 0·1 ml. 20% potassium acetate and 0·1 ml. NaCl (final molarity 0·05). 1·25% ion-agar (dissolved in citrate-phosphate buffer[4]) was melted and 4–5 ml. of this fluid was allowed to set on a microscope slide of usual dimensions. About an hour or so after preparing this layer a groove was cut on the slide and the RNA solution was introduced into the groove and allowed to soak in. After this the two ends of the slide were connected to the baths containing the citrate-phosphate buffer (pH about 8) and the electrophoretic run was allowed to last for 60–75 minutes at a voltage of 350 and constant current of 29 mA.

After this the slide was put in a mixture of phosphate buffer and toludine blue (final concentration of toludine blue being 0·1%) in order to stain the RNA bands and wash out the free ^{32}P. After 30–40 minutes of staining and 10–20 minutes of washing in running water the three marker RNA bands (*i.e.*, *E. coli* RNA of 23S, 16S and 4S fractions) would be visible. According to earlier findings[1] the heaviest RNA fraction lies nearest to the origin and as such the individual marker bands can be recognized. Under good conditions the separation between the bands would be as much as 4–5 mm. In such cases, not only the bands but the intermediate regions could be cut out. More often because of diffusion, etc., the clear separation space would be much less. As it has already been found that the γ RNA of *Limnæa* embryos have very nearly the same S values as those of the bacterial γ RNA,[3] it was now attempted to cut out the three bands in order to test their relative radioactivity. The cut pieces were washed again overnight in usual (non-radioactive) phosphate buffer in order to remove the ^{32}P. This was followed by another washing next day In order to be sure that the major part of free ^{32}P has been removed, a piece larger than the bands was cut out either in the pre-23S or in the post-4S region and its radioactivity was tested. This fraction gave low counts.

TABLE I

The relative values of biosynthesis of different RNA fractions in different stages of development of Limnæa *embryos*

Stage	Bands	cpm
Morula	23 S	331
	16 S + 16–4 S	252
	4 S	162
Trochophore	23 S	532
	16 S + 16–4 S	1700
	4 S	335
Veliger	23 S	2644
	16 S + 16–4 S	3200
	4 S	152

Counts were taken after dissolving the pieces of agar in planchettes by heating with 1 ml. of water. The gel in planchettes was then allowed to set uniformly or the planchettes were altogether dried.

In this manner it was found that the counts in the middle region, that is 16S and 16–4S were highest at the trochophore stage (Table I). This result is also in general agreement with density-gradient profiles which clearly show that the 16S and 16–4S peaks together are about three times the value of 23S.[3] However, as ultracentrifugation is unable to distinguish between 5S, 4S and free ^{32}P, density-gradient cannot give a true picture of the synthesis of the lighter RNA fraction. Thus from our earlier results[3] it could not be said whether the comparatively large peak of 4S (much larger than 23S) represented real transcription or was only due to free ^{32}P. The present method of electrophoretic separation shows clearly that in all the three stages 4S constitutes the smallest fraction. Thus it has been possible to shed light on the transcription of 4S RNA.

Unit of Embryology, R. L. BRAHMACHARY.
Indian Statistical P. K. TAPASWI.
Institute,
Calcutta-35, *July* 8, 1969.

1. Tsanev, R. G., *Biochimica Biophysica Acta*, 1965, 103, 374.
2. Dessev, G. N., Venllov, C. D. and Tsanev, R. G. *European J. Biochem* 1969, 1, 280.
3. Brahmachary, R. L., Banerjee, K. P. and Basu, T. K., *Experimental Cell Research*, 1968, 51, 177.
4. Colowick, S. P. and Kaplan, N. O., *Methods in Enzymology*, 1955, 1, 141.