Hentriacontane.—On elution with petroleum ether, it was obtained as colourless shining plates (900 mg.), m.p. 66–68°. It was optically inactive and the I.R. spectrum in KBr showed absorption at 720 cm.$^{-1}$ (alkane). It analysed for $C_{31}H_{64}$ and gave no colouration with tetranitromethane. Mixed m.p. with an authentic sample of hentriacontane remained undepressed.

β-sitosterol was eluted with a mixture of petroleum ether and benzene (4 : 1). It crystallized from acetone as colourless needles (450 mg.), m.p. 135–37° and identified as above.

Meso-Inositol.—Petroleum ether exhausted roots were subsequently extracted with ethanol in cold. The ethanolic extract (6 × 4 L) was concentrated to 500 ml. and kept at room temperature for 48 hours. A crystalline residue was deposited on the walls of the container. The residue was filtered and on crystallization from methanol (charcoal) afforded colourless crystals, m.p. 221–23° (Found. C, 40·32; H, 6·38; $C_6H_{12}O_6$ requires C, 40·0; H, 6·66%). It was freely soluble in water and was optically inactive. It showed no depression in m.p. with an authentic sample of meso-inositol. The identity was further established by paper chromatography employing butanol, acetic acid and water (4 : 1 : 5), (upper layer) as solvent and aniline hydrogen phthalate as spray giving only one spot (R_f 0·12) identical to meso-inositol. Acetylation yielded inositol hexa-acetate, m.p. and mixed m.p. 215–16° (Found C, 49·76; H, 5·82; $C_{18}H_{24}O_{12}$ requires C, 50·00; H, 5·55%).

The authors are highly thankful to the Director, Indian Veterinary Research Institute, Izatnagar, for his keen interest in these investigations.

I.V.R.I., Izatnagar (U.P.), *June* 16, 1969.

SURESH CHANDRA.
V. S. BISARIA.
R. K. GUPTA.

1. Chopra, I. C., Handa, K. L. and Kapur, L. D., *Indigenous Drugs of India*, 2nd Ed., V. N. Dhur & Sons, Calcutta, 1958, p. 601.
2. Chopra, R. N., Nayar, S. L. and Chopra, K. C., *Glossary of Indian Medicinal Plants*, Council of Scientific and Industrial Research, New Delhi, 1956, p. 221.

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

cold (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	1796
	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,
Indian Statistical
Institute,
Calcutta-35, *July* 8, 1969.

R. L. BRAHMACHARY.
P. K. TAPASWI.

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. I., 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.

RHIZOSPHERE MICROFLORA OF COFFEE PLANTS AFFECTED BY DECLINE DISEASE

A SEVERE decline disease of coffee is prevalent in Cannon Cadu Estate of the Consolidated Coffee Estates, Pollibetta, Coorg. This disease is characterized by sudden yellowing of the foliage, severe leaf shedding followed by reduced leaf size and chlorosis of new flush, poor bearing, and die-back of the twigs. Usually plants above three years of age, which bear very heavily during one or two successive crop seasons, seem to succumb to the disease. After the early disease symptoms set in the plants may take one to three years to wilt and die. The disease first appears sporadically on a few plants in the field and later spreads rapidly to cover the entire field during the course of three or four years. It is stated that various experts have examined the plants and carried out experimental studies to establish the causal agent. Though involvement of fungal and/or nematode pathogens is suspected no conclusive evidence in this regard has so far been obtained.

It was therefore considered worthwhile to approach the problem from the point of view of interaction of micro-organisms with the plant roots in the given ecosystem. Even though the micro-organisms are small they exert large effects—neutral, beneficial or antagonistic on macro-ecosystem. Hence, the activity in the rhizosphere of coffee plants as influenced by the quantity and quality of micro-organisms present therein was examined.

The last two authors visited the diseased area during September 1968 and collected soil and rhizosphere samples from healthy and diseased plants showing typical symptoms. Also, trials were laid out to examine the effect of heavy liming of the soil on diseased and healthy plants. The root zone soil of the healthy and affected plants were carefully dug out upto a depth of about 12 inch, with least damage to the roots, and 2 lb. of slaked lime powder and 2 lb. of ammonium sulphate were added per plant to cover the entire radius of plant-spread. Then the same unearthed soil was replaced and pressed. After an interval of six months representative samples of soil and rhizosphere from the healthy, diseased and treated-diseased plants were collected.

The soil and rhizosphere samples were analysed for the bacterial, actinomycete and fungal populations by the dilution plate method using soil extract agar for bacteria,