# Separation and Analysis of Rat Brain RNA by Rate-zonal Ultracentrifugation

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Cold phenol extracted rat brain RNA from trained and untrained rats has been subjected to rate-zonal ultracentrifugation. The separation was achieved by using 3 to 18 % (w/w) isokinetic sucrose gradient and a 5 h centrifugation at 46 000 rpm. It was found that the RNA preparations contain at least five main components having sedimentation coefficients of 4, 13, 15, 18, and 27 S, respectively. In rats which had received a daily dose of $^{32}$P labelled phosphate over 14 days the labelled phosphate was found mainly in the 4 and in the 27 S components.

It has been shown that RNA extracted from the brains of trained rats contains, in comparison with untrained rats, a facilitating principle, which on transfer to naive rats can modify their behaviour on training to the same situation. The active principle is contained in a protein-free cytoplasmatic RNA fraction, extractable by a cold phenol method from brains of trained rats.[1-3]

The present paper deals with the rate-zonal ultracentrifugation technique for separation and analysis of cold phenol extracted RNA from the brains of trained and untrained rats, and of the occurrence of $^{32}$P-labelled compounds in different fractions after prolonged administration of $^{32}$P-labelled phosphate.

Rats were bred in the stables of the institute from white Wistar rats, originally delivered by Mr. K. Gøtsche, Copenhagen. The rats were used at a weight of 200 g (corresponding to about 100 days of age). Rats for training and control groups were chosen randomly. The training was carried out in a two alley runway as described previously.[4] The conditioned stimulus was light, and the reward was water.

$^{32}$P-labelled orthophosphate was obtained from Dansk Atomenergi Kommission, Risø. The phosphate was diluted with 0.5 M tris buffer pH 7.2 (25°C) to 100 ml. 10 $\mu$C was injected intraperitonally daily 15 min before the daily training session. The control animals were left one by one in their home cages after injection of phosphate and not subjected to the training procedure.

The brains were removed by operation 24 h after the last training session under ether narcosis, and immediately frozen on solid carbon dioxide for storage until all the rats in the same group had reached the same predetermined level of training. The control animals received the same number of injections as did the trained animals.

RNA preparations from rat brains were prepared as described previously.[4] A preparation of RNA was regarded as protein-free when the ratio between the absorbances at 260 and 230 nm, and between 260 and 280 nm, respectively, was about 2.

For rate-zonal ultracentrifugation we have used a Superspeed 65 Mk. II ultracentrifuge with a B-XIV titanium zonal rotor [5] and a force-time integrator (Measuring and Scientific Equipment Co., London). Sucrose gradients were formed in an automatic variable gradient former. For these separations isokinetic sucrose gradients, *i.e.* gradients which give the sedimenting particles constant velocities, were used. Isokinetic sucrose gradients were constructed as described by Steensgaard.[6] The profile of the gradients is shown in Fig. 1. The stock solutions consisted of 3 and 23 % (w/w) sucrose in 0.05 M tris buffer at pH 8.0. The sample volume was 2 ml (containing approximately 5 mg RNA). 100 ml tris buffer was used as overlay. The duration of the run was 300 min at 46 000 rev./min, corresponding to $\int \omega^2 dt = 4.5 \times 10^{11}$ rad$^2$ sec at a tempera-
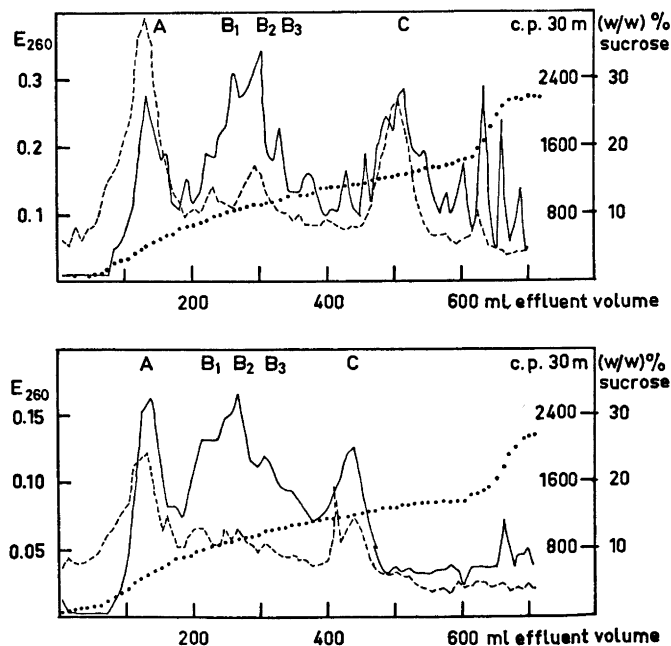


*Fig. 1.* Separation and analysis of rat brain RNA by rate-zonal ultracentrifugation from trained (lower part) and from untrained (upper part) animals. The solid line is the absorption at 260 nm. The dashed line represents $^{32}$P radioactivity, and the dotted line the sucrose concentration in weight per cent. The abscissa is the effluent volume in ml.

ture of 8°C. During unloading 700 ml of 30 % (w/w) surcrose were pumped through the rotor edge feed. Fractions of approximately 9.4 ml were collected on a time schedule.

The sucrose concentration in each cut was determined by measurements of the refractive index at 20°C. The sedimentation coefficient for each fraction was calculated by a Gier Algol 4 program based on the theoretical considerations of Bishop [7] and Barber [8] as described previously.[9] In the calculations the particle density was taken as 1.7.

$^{32}$P radioactivity was measured by a Friseke & Hoepfner low level $\beta$-counter. Each fraction was evaporated on an aluminium planchet by an infrared lamp to give an even distribution of the radioactive material.

Fig. 1 shows the results of rate-zonal ultracentrifugation of (a) untrained rat brain RNA, and (b) trained rat brain RNA, respectively. It appears from the absorbance curve that the RNA contains three main components, here tentatively named A, B, and C. The shape of peak B indicates that B comprises at least three minor components, $B_1$, $B_2$, and $B_3$. Based on 7 runs, the sedimentation coefficients were calculated to A, $3.7 \pm 0.1$; $B_1$, $12.7 \pm 0.2$; $B_2$, $15.3 \pm 0.2$; $B_3$, $17.9 \pm 0.3$; C, $27.4 \pm 0.3$ Svedberg units; the number following the average is the standard error of the mean.

Peak A having a sedimentation coefficient of just below 4 S, probably is soluble RNA. Peaks $B_1$, $B_2$, and $B_3$ with sedimentation coefficients of 13, 15, and 18 S, respectively, correspond to light ribosomal RNA fraction, and peak C with a sedimentation coefficient of 27 S corresponds to the heavy ribosomal RNA fraction. Components B and C probably consist of a mixture of ribosomal and messenger RNA.

The total picture of the components separated by rate-zonal ultracentrifugation resembles the results of separations of rat and mice brain RNA by methylated albumin kieselguhr column chromatography and sucrose density gradient centrifugation.[10] However, the rate-zonal ultracentrifugation technique only demonstrates the existence of more components in peak B, corresponding to the picture obtainable with polyacrylamide gel electrophoresis.[11] In addition the rate-zonal ultracentrifugation provides the advantage of permitting the computation of sedimentation coefficients for the separated fractions.

No reproducible differences between the RNA preparations from trained and untrained rats were detected.

The radioactivity curve in Fig. 1 shows the distribution of $^{32}$P in the separated RNA samples, the rats having been subjected to a course of injections with $^{32}$P-labelled phosphate over approximately a fortnight. It appears that the bulk of the radioactive material is located in the A and C peaks, in trained as well as in untrained RNA preparations. To our knowledge no other experiments with prolonged administration of $^{32}$P-labelled phosphate to rats during a long training period have been published. However, the present results show a striking similarity to the results of experiments with incorporation of $^{32}$P in the brains of rats 4 h after administration.[12] In all cases the highest radioactivity is found in the lightest and heaviest components, while the radioactivity in 13 to 20 S components were rather low. Although the activity in peak B is low, the shape of the radioactivity curves confirms that these components are more heterogeneous than previously supposed.

It is remarkable that the $^{32}$P curve does not follow the $E_{260}$ curve. This finding shows two important things, firstly, a very longlived pool of RNA is included in this preparation, and secondly, the heterogeneity of this preparation is not due to a partial degradation as the radioactivity then would be more evenly distributed.

## REFERENCES

1. Fjerdingstad, E. J., Nissen, T. and Røigaard-Petersen, H. *Scand. J. Psychol.* **6** (1965) 1.
2. Ungar, G. and Oceguera-Navarro, C. *Nature* **207** (1965) 301.
3. Babich, F. R., Jacobson, A. L., Bubash, S. and Jacobson, A. *Science* **149** (1965) 656.
4. Røigaard-Petersen, H., Nissen, T. and Fjerdingstad, E. J. *Scand. J. Psychol.* **9** (1968) 1.
5. Anderson, N. G., Waters, D. A., Fisher, W. D., Cline, G. B., Nunley, C. E. and Rankin, C. T. *Anal. Biochem.* **21** (1967) 235.
6. Steensgaard, J. *Eur. J. Biochem.* **16** (1970) 66.
7. Bishop, B. S. *Natl. Cancer Inst. Monograph* **21** (1966) 175.
8. Barber, E. J. *Natl. Cancer Inst. Monograph* **21** (1966) 219.
9. Steensgaard, J. and Hill, R. J. *Anal. Biochem.* **34** (1970) 485.
10. Mahler, H. R., Moore, W. J. and Thompson, R. J. *J. Biol. Chem.* **241** (1966) 1283.
11. Peacock, A. C. and Dingman, C. W. *Biochemistry* **6** (1967) 1818.
12. Jacob, M., Stevenin, J., Lund, R., Judes, C. and Mandel, P. *J. Neurochem.* **13** (1966) 619.