Studies on the Isolation and Biosynthesis of Macromolecular Ribonucleic Acid in the Brain

By J. G. NIEVEL and K. S. KIRBY

*Institute of Neurology, Department of Chemical Pathology,
The National Hospitals for Nervous Diseases, London, W.C.1, and Chester Beatty Research Institute,
Institute of Cancer Research: Royal Cancer Hospital, London, S.W.3*

(Received 1 July 1966)

Although the hypothesis that the molecular processes concerning memory and the higher nervous functions may be related to the transfer of genetic information (Hyden, 1960; Hyden & Egyhazi, 1962, 1963) has led to an accumulation of data on the total nucleic acid content of the brain under various conditions, relatively little study has been made on the isolation of ribonucleic acids with good secondary structure.

In mouse brain the ribosomal RNA was reported to be not significantly labelled in 2 hr. (Barondes & Jarvik, 1964). Kimberlin & Hunter (1965) found

only a single radioactive peak on sedimentation of brain RNA in a sucrose gradient and this material persisted after 66 hr., when labelled with [^{32}P]phosphate or [^3H]uridine. Fractionation of RNA from mouse brain on a column of methylated albumin on kieselguhr yielded rapidly labelled RNA associated with the fractions of higher molecular weight (Hadllov & Tencheva, 1965).

The present results have shown that rapidly labelled RNA is biosynthesized in the brain 20 min. after injection of [^3H]uridine and biosynthesis of ribosomal RNA begins after about 90 min.

Fig. 1. Centrifugation patterns in sucrose density gradients (5–20% sucrose in 0.1 M-sodium acetate, pH 6.0) of RNA from rat brain. Centrifugation was at 24000 rev./min. for 16 hr. in the Spinco model L centrifuge (SW 25.1 rotor). (a)–(e) Ribosomal RNA isolated with rapidly labelled RNA; (f)–(h) ribosomal RNA only. —, $E_{280\text{m}\mu}$; ----, radioactivity. (a) Three rats, 600 $\mu\text{C}/30 \mu\text{C}$, 20 min.; (b) three rats, 300 $\mu\text{C}/30 \mu\text{C}$, 40 min.; (c) four rats, 50 $\mu\text{C}/30 \mu\text{C}$, 60 min.; (d) three rats, 100 $\mu\text{C}/30 \mu\text{C}$, 2 hr.; (e) four rats, 50 $\mu\text{C}/30 \mu\text{C}$, 3 hr.; (f) six rats, 600 $\mu\text{C}/30 \mu\text{C}$, 10 min.; (g) six rats, 300 $\mu\text{C}/30 \mu\text{C}$, 30 min.; (h) six rats, 200 $\mu\text{C}/30 \mu\text{C}$, 90 min. The first amount of radioactivity is that injected intraperitoneally, and the second that injected into the sub-arachnoid space. In the radioactivity ordinate each division is equal to (a) 40, (b) 100, (c) 20, (d) 200, (e) 400, (f) 40, (g) 100 and (h) 200 counts/min.

Ten-day-old male rats (Chester Beatty Research Institute) were used since at this period the most rapid structural development occurs in the brain, with growth of neuronal extensions and the beginning of myelination. [^3H]Uridine was injected intraperitoneally and into the sub-arachnoid space at fixed times before decapitation and removal of the brain. The brains were immediately cooled and subjected to extraction procedures that yielded only ribosomal RNA or ribosomal RNA with rapidly labelled RNA (Kirby, 1965). The original method was modified in that there is relatively more DNA in brain than in liver and the procedure described has been devised to eliminate difficulties in the separation.

Extraction of ribosomal RNA with rapidly labelled RNA. The brains were broken down in 15 vol. of a mixture of 4-aminosalicylate (6%), NaCl (1%) and an equal volume of the phenol-cresol mixture. The double-extraction procedure (Kirby, 1965) was followed but, after precipitation, the sediment was dissolved in 0.1M-sodium acetate, made 3M with respect to NaCl, and kept at -5° overnight. The precipitate was centrifuged off, washed twice with 3M-sodium acetate, pH 6.0, then with ethanol-water-NaCl (75:25:1, v/v/w) and finally with ethanol and dried in a vacuum desiccator. DNA, polysaccharide and soluble RNA remained soluble.

Extraction of ribosomal RNA. Rat brains were broken down in 0.15M-NaCl containing 4 μg . of polyvinyl sulphate/ml. (Scherrer & Darnell, 1962). An equal volume of the phenol-cresol mixture was added, the mixture was shaken for 20 min. and the phases were separated by centrifugation. The aqueous phase was removed, made 2.5% with respect to sodium dodecyl sulphate (specially purified; British Drug Houses Ltd., Poole, Dorset) and re-extracted with 0.5 vol. of the phenol-cresol mixture. After centrifugation the aqueous phase was again separated, and NaCl (0.3 g.), sodium benzoate (2 g.) and *m*-cresol (1 ml.) were added to each 10 ml. of aqueous phase. The mixture was kept at 2° and the gelatinous precipitate centrifuged off. The sediment was washed twice (20 ml.) with a mixture made from water (100 ml.), NaCl (3 g.), sodium benzoate (20 g.) and *m*-cresol (10 ml.). The ribosomal RNA was then washed with ethanol-water-NaCl (75:25:1, v/v/w), then with ethanol and dried. The specific activity of the pyrimidine nucleo-

tides was followed by hydrolysing with N-HCl at 100° (Markham & Smith, 1949) and separation by paper chromatography (Kirby, 1956). If, after the initial homogenization in 0.15M-NaCl and polyvinyl sulphate, solid 4-aminosalicylate (6%) was added to the mixture the extraction procedure described for isolation of ribosomal RNA with rapidly labelled RNA could be followed, so that if the extracts were divided into two portions it was possible to study ribosomal RNA with and without rapidly labelled RNA from the same tissue.

The ribonucleic acids were examined by centrifugation in sucrose density gradients [5–20% (w/v) sucrose in 0.1M-sodium acetate], $E_{260\text{m}\mu}$ being measured and radioactivity determined by extraction with diethyldihexylamine (Kidson, Kirby & Ralph, 1963) followed by liquid-scintillation spectrometry. The results are shown in Fig. 1. RNA with good secondary structure was obtained and the rate of biosynthesis of RNA in the brain is not intrinsically different from that in other tissues (cf. Hiatt, 1962).

We thank Sir Alexander Haddow, F.R.S. for permitting J.G.N. to work at the Chester Beatty Research Institute. This investigation has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council and the British Empire Cancer Campaign for Research, and by the Public Health Service Research Grant no. CA-03188-08 from the National Cancer Institute, U.S. Public Health Service.

- Barondes, S. H. & Jarvik, M. E. (1964). *J. Neurochem.* **11**, 187.
 Hadolov, A. A. & Tencheva, Z. S. (1965). *Abstr. 2nd Meet. Fed. Europ. biochem. Soc., Vienna*, p. 372.
 Hiatt, H. H. (1962). *J. molec. Biol.* **5**, 217.
 Hyden, H. (1960). In *The Cell*, vol. 4, p. 215. Ed. by Brachet, J. & Mirsky, A. E. New York: Academic Press Inc.
 Hyden, H. & Egyhazi, E. (1962). *Proc. nat. Acad. Sci., Wash.*, **47**, 1366.
 Hyden, H. & Egyhazi, E. (1963). *Proc. nat. Acad. Sci., Wash.*, **49**, 618.
 Kidson, C., Kirby, K. S. & Ralph, R. K. (1963). *J. molec. Biol.* **7**, 313.
 Kimberlin, R. H. & Hunter, G. D. (1965). *Abstr. 2nd Meet. Fed. Europ. biochem. Soc., Vienna*, p. 328.
 Kirby, K. S. (1956). *Biochem. J.* **64**, 405.
 Kirby, K. S. (1965). *Biochem. J.* **96**, 266.
 Markham, R. & Smith, J. D. (1949). *Biochem. J.* **45**, 297.
 Scherrer, K. & Darnell, J. E. (1962). *Biochem. biophys. Res. Commun.* **7**, 486.