Protein Synthesis by Membrane-Bound and Free Ribosomes of the Developing Rat Cerebral Cortex

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1. Rates of RNA and protein synthesis were measured in rat cerebral-cortex slices, and compared with amino acid incorporation into protein by membrane-bound and free ribosomes from the same tissue, in the first 3 weeks of life. 2. A rapid age-dependent decline in the incorporation of labelled precursors into both RNA and protein was observed, which was more marked for amino acid incorporation into protein. 3. Although membrane-bound ribosomes comprise only a small fraction of total ribosomes, they were more active in incorporating amino acids into protein than were free ribosomes, especially immediately after birth. The decline in activity with age was more marked in the membrane-bound fraction than in free ribosomes. This loss of activity was largely independent of alterations in soluble factors or endogenous mRNA content and appeared to involve some alteration of the function of the ribosome itself, with relatively small alterations in the ratio of membrane-bound to free ribosomes. 4. Thyroidectomy, performed soon after birth, had no effect on the incorporation of radioactive precursors into RNA or protein by either slices or the cell-free preparations during the first 3–4 weeks of life.

In previous papers (Andrews & Tata, 1968, 1971) we have shown that there exists a major difference in vectorial release of nascent protein synthesized *in vivo* and *in vitro* on membrane-bound ribosomes from a predominantly protein-secreting tissue such as the liver and a predominantly non-secretory tissue such as cerebral cortex or skeletal muscle. The attachment of ribosomes to membranes of the endoplasmic reticulum plays a major role in facilitating the export of some proteins from secretory cells (Palade, 1966; Campbell, 1970), but it is not yet clear what special functions may reside in similar ribosome-membrane complexes found in non-secretory cells. It was decided, as an initial approach to this problem, that it would be worthwhile to compare the activity of membrane-bound and free polyribosomes from the cerebral cortex, as a model for a predominantly non-secretory tissue, under different physiological conditions. First, we studied, both *in vivo* and *in vitro*, the well known but unexplained decline in the rate of incorporation of amino acids into brain protein that occurs in rodents during the first 3 weeks of life (Gaitonde & Richter, 1955; Murthy & Rappoport, 1965; Johnson & Belytschko, 1969; Lerner & Johnson, 1970). Second, we examined the smaller changes in brain protein synthesis that have been reported

to accompany quite well-defined behavioural, intellectual and developmental changes after neonatal thyroidectomy (see Eayrs, 1966; Balazs, Kovacs, Teichgraber, Cocks & Eayrs, 1968; Geel & Timiras, 1967; Geel, Valcana & Timiras, 1967; Dainat, Rebière & Legrand, 1970).

In the present paper we describe experiments with cerebral-cortex slices and cell-free preparations which show that although membrane-bound ribosomes comprise only a small fraction of total ribosomes they account for a substantial part of protein synthesis during the first week of life. The subsequent decline in overall brain protein synthesis is largely due to a decrease in the activity of attached ribosomes, with relatively smaller alteration in the ratio of membrane-bound to free ribosomes, whereas neonatal thyroidectomy had no influence.

EXPERIMENTAL

Animals and treatment. Sprague-Dawley rats from a strain inbred at the National Institute for Medical Research were used for all experiments. Radiothyroidectomy was performed by injecting 100 μ Ci of [131 I]iodide intraperitoneally in 0.05 ml of sterile 0.15 M-NaCl to one-half of each of two litters (of both sexes) within 4–8 h of birth, the remainder being reared as littermate controls. Injected and control animals were returned to separate nests with lactating rats until they were used between the ages of 3 and 28 days. Completeness of thyroidectomy was established on the basis of impaired growth and development,

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lowered basal metabolic rate, loss of ability to concentrate radioiodine and histological examination of the thyroid region.

Incubation of tissue slices. Slices of cerebral cortex were incubated by the technique of Orrego & Lipmann (1967). After decapitation of the rats the cerebral hemispheres were removed and washed in ice-cold Krebs-Ringer buffer [freshly gassed with $O_2 + CO_2$ (95:5)] of the following composition: 118 mM-NaCl, 4.7 mM-KCl, 1.25 mM- $CaCl_2$, 1.18 mM- $MgSO_4$, 16 mM-sodium phosphate buffer, pH 7.4, and 10 mM-glucose. The cerebral hemispheres were separated and slices (0.2–0.5 mm thick) were cut free-hand from the cortex until white matter became visible. Slices from several cortices were pooled, washed in Krebs-Ringer buffer and blotted dry before being weighed out into batches (200–300 mg) into 25 ml conical flasks containing 2.5 ml of the buffer. Duplicate flasks were incubated in a water bath at 37°C, under $O_2 + CO_2$ (95:5), and shaken at 60 cycles/min. After a 5 min pre-warming period, the radioactive amino acid or nucleotide was added in 0.5 ml of the buffer and incubation continued for up to 60 min. Incorporation of the radioactive precursor was stopped by washing the slices in a nylon tea-strainer with a large volume of ice-cold medium. For experiments in which the incorporation into total tissue protein or RNA was studied, the contents of each flask were homogenized in 2.5 ml of the medium in a 15 ml Teflon-glass homogenizer and the suspension was processed for the determination of RNA, DNA, protein and radioactivity.

Subcellular fractionation. Membrane-bound and free ribosomes and cell sap were prepared from whole cortices by methods described in detail by Andrews & Tata (1971).

Determination of radioactivity. Labelled RNA or protein was precipitated at 0°C with 5% (w/v) trichloroacetic acid containing 0.1% of the unlabelled precursor. The precipitate was washed four times with ice-cold 5% trichloroacetic acid followed by two washes with chloroform-methanol (3:1, v/v) at 25°C and two at 45°C. With radioactive protein, an additional wash with 5% trichloroacetic acid at 75–80°C was included before the organic-solvent washing. Where radioactivity and chemical determinations of RNA, DNA and phospholipid were required on a single sample, the method described by Tata (1967) was followed. Radioactivity was finally determined by heating the precipitate at 85°C in 0.5 ml of 5% trichloroacetic acid and dissolving it in 10 ml of a scintillation fluid containing 180 g of nuclear-grade naphthalene, 4.0 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis-(4-methyl-2-phenyloxazol-2-yl)benzene in 1 litre of dioxan. Radioactivity was measured in a Packard Tri-Carb liquid-scintillation spectrometer with counting efficiencies of 32 and 75% for 3H and ^{14}C respectively.

Chemical determinations. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) with crystalline bovine serum albumin as the standard. RNA and DNA were measured by the methods of Fleck & Munro (1962) and Burton (1956) respectively.

Materials. All chemicals were of analytical grade and purchased from Sigma (London) Chemical Co., London S.W. 6, U.K., or C. F. Boehringer, London W5 2TZ, U.K. ATP, GTP and phosphoenolpyruvate were converted into their potassium salts before use. L-[U- ^{14}C]Leucine (311 mCi/mmol), U- ^{14}C -labelled amino acid mixture (54 mCi/mg-atom of carbon), L-[U- ^{14}C]phenylalanine

(504 mCi/mmol) and [5- 3H]orotic acid (6.1 Ci/mmol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K.

RESULTS AND DISCUSSION

Tissue concentrations of protein, RNA and DNA. Measurements of protein, RNA and DNA content were made on cerebral cortex and repeated at various stages of development between 2 and 47 days after birth. In line with the findings of other workers (Balazs *et al.* 1968; Geel & Timiras, 1967), there was a steady decline in the amount of DNA/g wet wt. of tissue. This is due to a combination of a progressive dehydration (Brante, 1949) as well as growth of the cells as reflected by the rise in protein and RNA content. Table 1 illustrates this trend with only two developmental stages (day 4 and day 21 after birth).

Our failure to observe a marked effect of neonatal thyroidectomy on the concentrations of protein, RNA and DNA in the brain during development is, however, in contrast with the observations of Geel & Timiras (1967) and Balazs *et al.* (1968). There are many possible reasons for this discrepancy but it may arise from our making the measurements on cortical slices rather than whole brain. It could also be that the cortical cells in our strain of rats do not grow substantially after birth. The lack of effect of thyroidectomy on overall composition is reflected in all the studies described below on protein synthesis. This was not due to incomplete hormone withdrawal since at 8–12 days after birth our neonatally thyroidectomized rats showed marked impairment of growth and development and depressed basal metabolic rate.

Protein and RNA synthesis by cortical slices from developing and thyroidectomized rats. The rate of protein synthesis by slices of cerebral cortex fell dramatically with increasing age of the rats used (Fig. 1) and by the age of 3 weeks had reached a value near that observed in slices from young adult animals.

The rates of incorporation for slices made from animals taken at all developmental stages were linear for up to 2.5 h. Neonatal thyroidectomy did not affect the age-dependent fall in protein-synthetic activity, as illustrated for the 10th day after birth in Fig. 1; moreover, at no age could we demonstrate a significant difference in the rate of protein synthesis between hypothyroid and normal rats. Fig. 2 shows in more detail the progressive loss of ability of cortical slices to incorporate [^{14}C]leucine into protein. This pattern is in good agreement with that reported by Orrego & Lipmann (1967). Other workers (see Eayrs, 1966; Balazs & Gaitonde, 1968; Geel *et al.* 1967) have reported a decrease in amino acid incorporation *in vivo* in 3–5 week-old rats after

Table 1. *Effect of age and neonatal thyroidectomy on the relative concentrations of RNA, DNA and protein in developing rat brain*

Each value is the mean of five to eight determinations on tissue pooled from four to six rats, the variation being within $\pm 5\%$.

Rats	Age (days)	DNA (mg/g wet wt. of tissue)	RNA/DNA ratio	Protein/DNA ratio
Normal	4	1.25	2.15	12.70
Thyroidectomized	4	1.20	2.30	11.95
Normal	21	0.90	4.10	33.50
Thyroidectomized	21	1.10	3.85	31.50

Fig. 1. Time-course of incorporation of [^{14}C]leucine (311 mCi/mmol) into protein by slices of rat cerebral cortex. In each of the four experiments shown here four flasks were incubated in duplicate each containing 200 mg of tissue in 3 ml of medium. Duplicate flasks were incubated for 15, 30, 60 and 90 min and the amount incorporated was determined at each time-interval, so that each point on the graph represents the mean of two determinations. $\circ$, Control, 2 days; Δ , control, 10 days; $\triangle$, thyroidectomized, 10 days; $\bullet$, control, 21 days.

neonatal thyroidectomy, but these differences could be due to amino acid-transport effects. For example, it is known that cerebral blood flow is decreased by as much as 25% after thyroidectomy (see Pitt-Rivers & Tata, 1959).

With regard to RNA synthesis, a fall in the rate of incorporation of [^3H]orotic acid was observed with age, but one which was less substantial than that of [^{14}C]leucine incorporation into total slice

Fig. 2. Incorporation of [^{14}C]leucine into protein by slices of rat cerebral cortex: influence of age and neonatal thyroidectomy. Slices were pooled from two to four animals in each group depending on age and brain weight, and 200–300 mg wet wt. of tissue was incubated in 3 ml of medium. Duplicates contained equal weights of tissue, ± 10 –15%. Incubation was continued for 1 h at 37°C after which the slices were homogenized and the radioactivity of protein was determined. Each point on the graph represents the mean of duplicate determinations and the results are combined from three experiments comparing hypothyroid with normal animals in three or four age groups simultaneously. Blank incubations (minus [^{14}C]leucine) were performed with portions of brain from the thyroidectomized animals and correction was made for the residual ^{131}I (used for radiothyroidectomy) appearing in the ^{14}C channel of the scintillation counter. $\circ$, Untreated controls; Δ , thyroidectomized (indicated by vertical arrow).

protein (Fig. 3). Incorporation of [^3H]orotic acid into RNA was found to be linear for 2 h and at no time of incubation was there any consistent effect of thyroidectomy. Balazs *et al.* (1968) found that, after administration of [^{14}C]orotic acid *in vivo* (subarachnoidally), the specific radioactivities of UMP in RNA and in the acid-soluble fraction were closely correlated in the normal and hypothyroid rats. Geel & Timiras (1967) have also found that changes in specific radioactivities of brain RNA after thyroidectomy and thyroid-hormone replacement are largely a consequence of fluctuations in the

Fig. 3. Effects of age and thyroidectomy on incorporation of [^{14}C]leucine into protein (Δ , $\blacktriangle$) and [^3H]orotic acid into total RNA ($\circ$, $\bullet$) by infant-rat brain slices. Normal and thyroidectomized animals were used at 8, 22 and 47 days of age. Cortical slices were pooled from four or six animals in each group and 200–250 mg of tissue was incubated at 37°C for 1 h in the presence of $10\text{ }\mu\text{Ci}$ of [^3H]orotic acid (6.1 Ci/mmol). A second series of slices from the same brains were similarly incubated with $0.05\text{ }\mu\text{Ci}$ of [^{14}C]leucine (311 mCi/mmol) in each flask. At the end of the incubation the tissue was homogenized and radioactivity in RNA or protein was determined, and each value is the mean of duplicate incubations. ----, Thyroidectomized; —, normal control.

labelling of precursors of RNA. Perhaps experiments with slices, and almost certainly those with subcellular systems described below, obviate the complications imposed by cerebral permeability barriers or the dynamics of precursor-pool equilibration *in vivo*.

Amino acid incorporation in vitro by isolated microsomal and submicrosomal preparations: effect of developmental stage and thyroidectomy. In previous papers (Andrews & Tata, 1968, 1971) we have compared the amino acid-incorporating activity *in vitro* of cerebral-cortical rough microsomal membranes with that of free ribosomes. As shown in Fig. 4, at 2 days after birth the activity of membrane-bound ribosomes is 6–7-fold higher than that of free ribosomes, with the crude microsomal fraction representing the average activity of the mixture of the two fractions. A rapid fall in the rate of amino acid incorporation during the first 2 weeks of life was detected in all fractions, but by far the greatest effect was observed in the rough membrane fractions. Other workers have also described a substantial loss in activity of total microsomal fraction or polyribosomes derived by detergent treatment during the first 2–3 weeks of postnatal development of the rat and mouse (Murthy & Rappoport, 1965; Johnson, 1968; Johnson & Belytschko, 1969; Lerner & Johnson, 1970).

If one were to extrapolate the above observations on activity in subcellular fractions to the intact cell, and take account of the fact that some 20% of the total cytoplasmic RNA is recovered in the membrane-bound fraction (Andrews & Tata, 1971;

Merits, Cain, Rdzok & Minard, 1969), then over half the protein synthesis in cerebral cortex of a newborn rat may occur on ribosomes bound to endoplasmic-reticulum membranes. In the liver during hormone-induced growth it is also the rough microsomal fraction that reflects most dramatically the response to withdrawal or replacement of growth stimuli (Tata & Williams-Ashman, 1967; Tata, 1970). Since the attached ribosomes of cerebral cortex also exhibited the greatest relative change during development, we examined in further work the response of these rough membranes to other physiological alterations.

Fig. 5 illustrates the time-course of incorporation of [^{14}C]leucine into protein by membrane-bound ribosomes from 2-day-old and 21-day-old rats. Neonatal thyroidectomy did not alter the higher protein-synthetic rate *in vitro* of rough membranes from cerebral cortex of 2-day-old or from 21-day-old rats. Similarly no effect was observed in the free-polyribosome fraction (not shown in Fig. 5).

An obvious explanation for the rather abrupt postnatal decline in ribosomal activity is that there may be a rapid loss of mRNA from the polyribosomes. This possibility was explored indirectly by measuring the incorporation of [^{14}C]phenylalanine in the presence and absence of poly(U). Although poly(U) is not comparable with natural mRNA, its use in these experiments to indicate the relative endogenous template activity of the polyribosomal preparations was based on the work of Hunter & Korner (1968). These workers had shown that the stimulation by poly(U) of phenylalanine incorpora-

Fig. 4. Incorporation of [^{14}C]leucine into protein *in vitro* by unfractionated microsomal preparation, free ribosomes and membrane-bound ribosomes from the brains of normal rats in the neonatal period. The three subfractions were prepared from portions of the same homogenates of brain from all four age groups on the same day. Some 50–100 μg of rRNA was used in 1 ml of incubation suspension and each preparation was incubated in the presence of homologous cell sap. The values given are the means of duplicate determinations. Other conditions are as those described for Table 2. ○, Unfractionated microsomal preparation; Δ, membrane-bound ribosomes; □, free ribosomes.

tion by rat liver polyribosomes largely indicated relative amount of active but non-programmed monomeric ribosomes. In other experiments (T. M. Andrews & J. R. Tata, unpublished work), we had established that the amount of poly(U) added to brain polyribosomal preparations was a saturating one and that the time-course of incorporation was only slightly prolonged. This suggested that the increase in phenylalanine incorporation was not largely due to a protection of natural mRNA by the addition of poly(U). Table 2 shows that phenylalanine incorporation by membrane-bound ribosomes was increased by 80–90% in the presence of 200 μg of poly(U). The magnitude of the poly(U) effect was similar in 8- and 21-day-old animals so that the age-related decrease in activity of cortical ribosomes cannot be explained in terms of a progressive decline in the content of endogenous mRNA. There must be some intrinsic alteration in the capacity of ribosomes to synthesize protein. A similar situation is observed in liver ribosomes after hypophysectomy or thyroidectomy (Tata & Williams-Ashman, 1967; Korner, 1970), in uterine ribosomes after ovariectomy (Teng & Hamilton, 1967) and in muscle ribosomes from diabetic mammals (Wool *et al.* 1968). Johnson & Belytschko (1969) and Lerner & Johnson (1970), working with crude microsomal preparations

Fig. 5. Time-course of incorporation of [^{14}C]leucine *in vitro* by membrane-bound ribosomes from the brains of normal (○, Δ) and hypothyroid (●, ▲) rats at 2 and 21 days after birth. Incubation conditions were as described in the text and 50–150 μg of microsomal RNA was incubated in 1 ml of medium. Each preparation was incubated with 3 mg of homologous cell-sap protein. Values given are the means of duplicate determinations and are normalized for 100 μg of microsomal RNA.

and deoxycholate-prepared ribosomes from whole rat brain, also demonstrated that the age-related decline in protein-synthetic activity was not associated with any change in polyribosome profiles or ribonuclease activity in the brain. Table 2 also shows that neonatal thyroidectomy had no significant effect on the response to poly(U), confirming the findings of Nievel, Robinson & Eayrs (1968), working with 'heavy' and 'light' microsomes (Campbell, Lowe & Serck-Hanssen, 1967) from developing rat brain.

In all the experiments on rough microsomal membranes described above, the preparations were incubated in the presence of freshly prepared Sephadex-treated homologous cell sap. It was considered possible that in the brain of neonatally thyroidectomized rats declining intrinsic activity of ribosomes might be compensated for by a simultaneous increase in the concentration of some rate-limiting factor in the cell sap. To test this possibility various combinations of 'cross-over' experiments with heterologous cell sap and ribosomal preparations were performed. The results of a typical experiment in which 4- and 21-day-old (normal and thyroidectomized) rats were used are summarized in Table 3.

They show that the age-related effect is intrinsic to the ribosomes and that thyroidectomy does not alter the capacity of cortical cell sap to support protein synthesis by isolated brain ribosomes. These

Table 2. Incorporation of [^{14}C]phenylalanine into protein by membrane-bound ribosomes from normal and hypothyroid rats: effect of age and poly(U)

Incubation (15 min) conditions were as described by Andrews & Tata (1971). Each incubation tube contained in a final volume of 1 ml: 50 μmol of tris-HCl buffer, pH 7.4 at 20°C; 100 μmol of KCl; 10 μmol of MgCl_2 ; 10 μmol of 2-mercaptoethanol; 3 μmol of ATP; 1 μmol of GTP; 5 μmol of sodium phosphoenolpyruvate; 10 μg of pyruvate kinase; 0.1 μmol of each of 20 non-radioactive L-amino acids, excluding the radioactive amino acid used; 0.1–1.0 μCi of the radioactive amino acid and 200 μg of poly(U) were added where indicated. Each value represents the mean of duplicate determinations. Between 50 and 100 μg of rRNA was used in each 1 ml of incubation medium. All preparations of rough microsomal fraction were made simultaneously and each was incubated at 37°C in the presence of Sephadex-treated cell sap prepared from its own post-microsomal supernatant by using 3 mg of cell-sap protein/ml of suspension. Poly(U) effect is the additional [^{14}C]phenylalanine incorporated over the control values in the presence of 200 μg of poly(U).

Rats	Age (days)	[^{14}C]Phenylalanine incorporated (c.p.m./mg of RNA)			
		-poly(U)	+poly(U)	Poly(U) effect	
				(c.p.m./mg)	(% Stimulation)
Control	8	9650	18250	8600	89
	21	4400	8200	3800	86
Thyroidectomized	8	10150	19950	9800	85
	21	4350	8250	3900	91

results agree with those of Johnson & Belytschko (1969) who demonstrated, using crude microsomes from mouse brain, that tRNA-charging activity was not diminished with increasing age. However, Lerner & Johnson (1970) have suggested that there may be an age-related decline in concentration of some soluble factor required to support protein synthesis by brain ribosomes. The work of Yamagami, Fritz & Rappoport (1966) showed that a sharp and unexplained decrease in the effectiveness of pH 5 enzyme to support amino acid incorporation *in vitro* occurred at the age of 50 days. Our experiments were mainly designed to explore the early postnatal changes and rarely extended beyond 3–4 weeks after birth.

Conclusions

We have extended the findings of other workers (Johnson, 1968; Johnson & Belytschko, 1969; Orrego & Lipmann, 1967; Lerner & Johnson, 1970) on the rapid perinatal decline in brain protein-synthetic activity to studying this notable developmental phenomenon at the level of membrane-bound and free polyribosomes. Bearing in mind the low rate of cell proliferation and the slow turnover of brain ribosomes in the neonatal period we would suggest that the age-related decline in cortical protein synthesis reflects a progressive loss of activity by all or part of the ribosome population. The reason for this rapid decline remains unknown except that it seems unlikely to be due to a change in the activity of cell-sap factors or merely an abrupt loss of mRNA. In this respect the situation is very reminiscent of the decreased activity of ribosomes from liver, muscle and uterus which

follows the experimental arrest of growth by hypophysectomy, thyroidectomy or castration (Korner, 1970; Tata, 1970; Wool *et al.* 1968; Teng & Hamilton, 1967).

A noteworthy but negative result is our finding that neonatal thyroidectomy failed to alter significantly the normal course of early postnatal changes in amino acid incorporation by cortical ribosomes, which is apparently incompatible with earlier reports of decreased amino acid incorporation *in vivo* in brain after thyroidectomy (Balazs & Gaitonde, 1968; Dainat *et al.* 1970). This was also unexpected in view of the profound behavioural and developmental disorders after neonatal thyroidectomy (see Pitt-Rivers & Tata, 1959; Eayrs, 1966). We have no explanation for the difference between our findings *in vitro* and the results of the other workers *in vivo*, but it is worth recalling the findings of Geel & Timiras (1967) and Balazs *et al.* (1968) that the presence or absence of thyroid hormones affects the uptake and distribution of amino acids and nucleotides in the brain *in vivo*.

An important feature of this work was our demonstration of the much greater activity of membrane-bound as compared with free ribosomes in neonatal brain. The overall decline in brain protein synthesis with age could be due largely to falling activity of the membrane-bound fraction of ribosomes (see Fig. 4). This focuses attention on the role of attachment of ribosomes to membranes of the endoplasmic reticulum in non-secretory tissues. In an earlier paper we have discussed in detail the marked difference in vectorial discharge of nascent polypeptide from membrane-bound ribosomes from secretory and non-secretory tissues (Andrews & Tata, 1971). One has to consider the possibility of

Table 3. *Effect of cell sap on incorporation of [¹⁴C]leucine by membrane-bound ribosomes from infant rat brain*

Cell-sap protein (3 mg) or rat liver pH 5 enzyme (2.5 mg) was added to each 1 ml of incubation mixture. Rough microsomal fractions were incubated in homologous and heterologous cell-sap preparations for 15 min at 37°C, 60–120 µg of microsomal RNA being used in each case. The results are the means of duplicate determinations. Other conditions were as described for Table 2.

Rats	Age (days)	[¹⁴ C]Leucine incorporated (c.p.m./mg of RNA)			
		+pH 5 enzyme (normal rats)	+4-day cell sap (normal rats)	+21-day cell sap (normal rats)	+21-day cell sap (thyroidectomized rat)
Normal	4	8510	8460	8270	—
Normal	21	5500	5720	5550	5480
Thyroidectomized	21	—	—	5780	5920

another role for the attachment, besides secretion (see Palade, 1966; Campbell, 1970), in both secretory and non-secretory tissues. From observations on the co-ordinated proliferation of endoplasmic-reticulum membranes and ribosomes during rapid growth and development Tata (1968, 1970) has proposed that the attachment may serve to segregate or 'compartmentalize' ribosomes engaged in synthesizing different classes of pre-developmental and post-developmental proteins. It would therefore be of great interest to study the proliferation of membranes to which cerebral ribosomes are attached just before and after birth.

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